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RESEARCH ARTICLE

Microsatellite analyses for evaluation of genetic diversity among Sicilian grapevine cultivars

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Abstract A total of 82 grapevine genotypes were sampled from several areas of the Italian region of Sicily where vineyards are widely spread. The grapevines were characterized using six microsatellite markers (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79) to evaluate genetic diversity. Thirty-seven of the 82 cultivars sampled had their names quoted in historical and literary sources, while 45 cultivars from old vineyards did not have their names reported in ancient literature. According to their genetic profiles at SSR loci, 70 different cultivars were found, while interesting cases of synonymies (Regina and Moscato bianco, Alicante and Dolcetta or among different clones of Zibibbo and Catarratto) and cases of homonymy (Frappato and Nerello Mascalese) were discovered. Several genetic parameters were calculated to assess the efficacy of the loci chosen in this work. Pairwise genetic distances between all cultivars were calculated. A dendrogram representing the genetic similarities among cultivars was depicted using the UPGMA method to investigate their relationships, explaining them from a historical point of view. The cluster distribution of cultivars clearly does not reflect their current geographical distribution, suggesting successive introductions of cultivars in Sicily from different areas of origin.

Keywords Local genotypes · Simple sequence repeat · Synonymies and homonymies · *Vitis vinifera* L.

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Introduction

The grapevine (*Vitis vinifera* L.) is a clonally propagated, highly polymorphic crop, and more than 7,000 cultivars of grapevine are believed to exist worldwide. This important fruit crop is also one of the most widespread plants in Italy, with about 800,000 ha cultivated, unequally distributed in all the regions of the country. In the survey of Italian viticulture, Sicily is one of the most important regions in Italy, as in Europe, with a long and rich tradition of viticulture practices.

Sicily is the largest island in the Mediterranean, and has 128,000 ha of vineyards more than any other Italian region (Anderson 2006). Paleobotanical findings of *Vitis* in Sicily show that grape has been eaten by the native populations since the Protohistoric Age (Collesano 1998). Macroremains of fruit have been found in Grotta dell'Uzzo (province of

Trapani), Morgantina (province of Enna) and Mucu-lufa (province of Agrigento) (Costantini 1989; Leighton 1993, 1999). In Sicily, the large complexity of local cultivars may be ascribed to different reasons: varied environmental conditions within the island, cultivation of new genotypes, introduced by human migrations (Dangl et al. 2001) and modified by centuries of mutations, and cross-pollination with wild populations or by the domestication of wild cultivars (Snoussi et al. 2004; Arroyo-Garcìa et al. 2006).

The variability of the grapevine can be observed in terms of both morphology and quality (Alleweldt and Possingham 1988). Ampelography (from the Greek *ampelos*, grapevine, and *graphos*, description), i.e., the analysis and comparison of the morphological character of leaves, shoot tips, fruit clusters, and berries, is useful in the identification of grape cultivars (Galet 1979, 1991; IPGRI, UPOV, OIV 1997). Unfortunately, skill in ampelography is now limited to a declining number of specialists. Moreover, these analyses are based on characteristics that can be influenced by environmental conditions (Levadoux 1956; Tessier et al. 1999). Finally, genetically related cultivars are frequently so similar morphologically that they cannot be distinguished by visual comparison (Aradhya et al. 2003); on the other hand, clones of the same cultivar sometimes differ in their phenotype, even though they present similar or identical DNA profiles (Franks et al. 2002; Riaz et al. 2002).

The use of molecular markers is a useful methodology that complements ampelography to detect similarities, and to define genetic relationships among grapevine cultivars (Sefc et al. 1999, 2001). Methods based on genetic variation have been used with varying degrees of success, depending on the genetic relationships between samples and the number of markers employed. Molecular markers, such as RAPD, RFLP, SSR and AFLP, have been used on *Vitis vinifera* in several studies in order to distinguish between grape cultivars (Thomas et al. 1993; Botta et al. 1995; Bowers and Meredith 1997; Scott et al. 2000).

Microsatellites or simple sequence repeats (SSRs) consist of tandemly repeated simple sequence motifs with a high variation in repeat number among individuals. This high level of polymorphism has made SSRs invaluable molecular markers for organisms in which other marker types are no more informative (Morgante and Olivieri 1993). Applications of microsatellite markers include individual or cultivar identification (Hokanson et al. 1998), parentage testing (Kirst et al. 2005), pedigree reconstruction (Sefc et al. 1998b) and studies of population structure (Tollefsrud et al. 2009).

In grapevines, one of the major applications of microsatellite markers is the identification and discrimination of cultivars in order to assist the management of cultivar collections and control the trade of plant material. Vitis SSR primers have been so far developed mainly by three research groups (Thomas and Scott 1993; Bowers et al. 1996; Sefc et al. 1999). The usefulness of these markers has been assessed in samples of grapevine cultivars cultivated in the vine-growing regions of Australia, California and Central Europe (Lopes et al. 1999).

In the present paper, we chose six polymorphic nuclear SSRs among the published microsatellite sequences, and compared their usefulness for the genotyping of grapevines from seven different vine-growing provinces of Sicily. A broad representation of the autochthonous grapevine cultivars of the region is presented. The existing synonymies and homonymies together with the presence of rare genotypes are explored in order to establish the genetic relationships among cultivars.

Materials and methods

Plant material

The grapevine germplasm collection was obtained from the experimental station of the Institute of Plant Genetics, Palermo—CNR. In the present study, 82 grapevine putative cultivars (Table 1) collected from different provinces (Fig. 1) were analysed. Five putative different clones of each cultivar of regional interest from different sites (Catarratto bianco comune, Frappato, Grecanico, Grillo, Inzolia, Nerello Mascalese, Nero d'Avola, Perricone and Zibibbo), including commercial plant material, were sampled. Furthermore, in the cases of Catarratto, Inzolia and Zibibbo other presumed similar genotypes (e.g., Zibibbo carricante, see Table 1) were analysed in order to identify possible homonyms. Only one plant for each cultivar of local and minor interest was analysed to identify synonymies.

Table 1 List of grapevine cultivars analysed

	Cultivar name	Zone of sampling	Berry colour	Diffusion and interest
1	Albanello*	SR	W	L
2	Alicante, Licante	CT	N	L
3	Alzano	CT	W	M
4	Austina bianca	PA	W	M
5	Barbarossa*	PA	R	M
6	Bracaù	CT	N	M
7	Bruntisi nero	SR	N	M
8	Calabrese*	CT	N	M
9	Canina*	ME	W	M
10	Carnuffino	ME	N	M
11	Carricante*	CT	W	L
12	Catanese nero*	ME	N	M
13	Catarratto bianco comune*	TP	W	R
14	Catarratto bianco lucido*	AG	W	R
15	Catarratto bianco extralucido	TP	W	R
16	Catarratto nero	PA	N	M
17	Cessalà	SR	W	M
18	Cirrinciò	AG	R	M
19	Coda di volpe	ME	W	M
20	Corinto*	SI	N	L
21	Diretta bianca	PA	W	M
22	Diretta nera	PA	N	M
23	Dolcetta	RG	N	M
24	Francisi, Uva di Francia	PA	W	M
25	Frappato*	SR	N	R
26	Fumusa*	SR	W	M
27	Gamay	SR	N	M
28	Grecanico*, Grecanico dorato	AG	W	R
29	Grecaù*	CT	W	M
30	Grillo*	TP	W	R
31	Inzolia*	TP	W	R
32	Inzolia imperiale*	PA	W	M
33	Inzolia nera*	TP	N	M
34	Inzuccarato	CT	W	M
35	Inzuccarato di Noto	SR	W	M
36	Jala bianca*	PA	W	M
37	Lacrima di Maria*	AG	W	M
38	Leanfurtisi	SR	N	M
39	Lorisi	PA	N	M
40	Lucignola	ME	W	M
41	Malvagia*	SR	W	M
42	Malvasia*	SI	W	L
43	Malvasia di Lipari*	SI	W	L
44	Marsigliana*	AG	N	M

Table 1 continued

	Cultivar name	Zone of sampling	Berry colour	Diffusion and interest
45	Minna di vacca	PA	W	M
46	Minnavacchina*	ME	W	M
47	Minnella bianca*, Passulana	SR	W	L
48	Monteleone	SR	N	M
49	Moscato bianco*	SR	W	L
50	Muscatedda*	SR	W	M
51	Muscatidduni	SR	W	M
52	Nerello Mascalese*	CT	N	R
53	Nero d'Avola*	TP	N	R
54	Nero grosso*	PA	N	M
55	Nivureddu*	ME	N	M
56	Nivuro Bronte	CT	N	M
57	Nzuccarato	SR	W	M
58	Oriddru	ME	W	M
59	Orisi	ME	N	M
60	Osso nivuro	SR	N	M
61	Perricone*	TP	N	R
62	Pizzutella*	PA	W	M
63	Precoce	SR	W	M
64	Primintia, Giugnettina	PA	W	M
65	Racignola bianca	SR	W	M
66	Racignola nera	SR	N	M
67	Racinedda	CT	N	M
68	Recunu	SR	W	M
69	Regina*	SR	W	M
70	Rucignola	ME	W	M
71	Russetto	SR	N	M
72	Sultanina	SI	R	M
73	Tintorè, Ibisu	AG	N	M
74	Triboti nera*	PA	N	M
75	Tripolina bianca	PA	W	M
76	Visparola*	CT	W	M
77	Vitraruolo	ME	N	M
78	Zibibbo*	SI	W	R
79	Zibibbo augustano	SR	W	M
80	Zibibbo carricante	SI	W	M
81	Zibibbo grosso	SI	W	M
82	Zibibbo nero	ME	R	M

The colour of the berries is indicated as N = noir—blue berries; B = white—green—yellow berries; R = red berries

Diffusion and interest: *R* regional, *L* local, *M* minor interest

Provenience: *AG* Agrigento, *CT* Catania, *ME* Messina, *PA* Palermo, *SR* Syracuse, *TP* Trapani, *RG* Ragusa, *SI* Small Island

Cultivar names followed by * were mentioned in the ancient literature

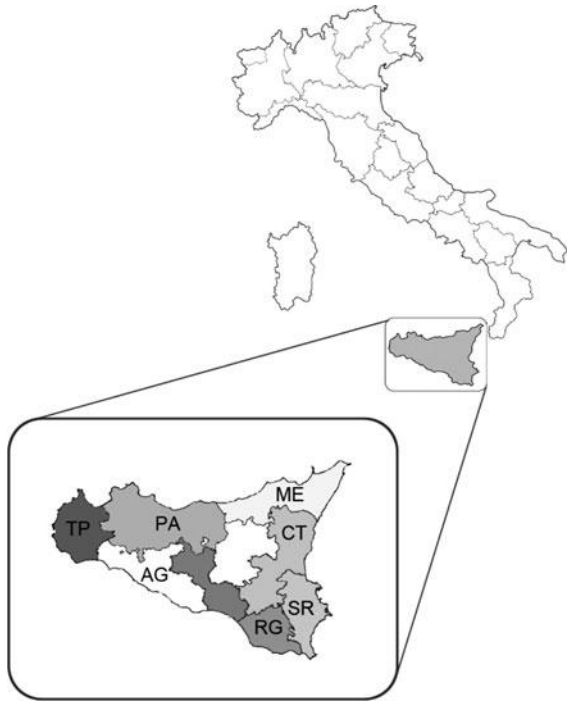

Fig. 1 The Sicily region and its provinces where the grapevine germplasm was collected: *AG* Agrigento, *CT* Catania, *ME* Messina, *PA* Palermo, *SR* Syracuse, *TP* Trapani, *RG* Ragusa

DNA extraction

DNA was extracted from young, fresh leaves collected in the field and maintained in liquid nitrogen before extraction, DNA after lyophilization was stored at 80°C until used. The extraction was carried out by the Doyle and Doyle (1990) CTAB method.

Microsatellites analyses

The samples were analysed at six microsatellite loci as proposed by the GENRES 081 Project (European Vitis Database, www.genres.de/vitis/vitis.htm): VVS2 (Thomas and Scott 1993), VVMD5, VVMD7 and VVMD27 (Bowers et al. 1996), VrZAG62, and VrZAG79 (Sefc et al. 1999). This set of microsatellite markers was selected to allow the comparison of the resultant profiles with the available databases. PCR amplification was performed using the Quiagen multiplex PCR kit, and two different annealing temperatures (52°C for the loci VVS2, VVMD5 and VVMD27 and 50°C for the loci VVMD7, VrZAG62 and VrZAG79). One of the primers of each pair was fluorescently labelled with FAM, JOE or TAMRA.

The fragments were separated by capillary electrophoresis and genotyped with an ABI PRISM 310 Genetic Analyzer. As a result of the analysis, each genotype was characterized for the studied loci, outputting a data matrix based on the presence or absence of each specific allele. The genetic distances were calculated from SSR data for all possible pairs of cultivars using Simple Matching coefficient (SM) for co-dominant and multiallelic data and cluster analysis was performed using UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) by using the Numerical Taxonomy and Multivariate Analysis System software (NTSYS-pc computer package version 2.02; Rohlf 1998).

Data analysis

Several parameters for the evaluation of the microsatellite loci were studied. The number of alleles (n) per locus and their frequency were estimated. The usefulness of microsatellites for differentiating among grapevine genotypes was evaluated calculating the observed (H_o) and expected (H_e) heterozygosities from the data obtained in the set of 70 genotypes that showed different genetic profiles. H_e values were estimated using the formula (Nei 1973):

$$H_e = 1 - \sum p_i^2$$

where p_i is the frequency of the ith allele.

Microsatellite screening ability (MSA) was also based on the probability of identity (PI) (Paetkau et al. 1995) and the polymorphic information content (PIC) (Weber 1990) derived as follows:

$$\mathrm{PI} = \sum_{i=1}^{n} p_i^4 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

$$\mathrm{PIC} = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

where p_i and p_j are the frequencies of the ith and jth allele and n is the number of alleles.

The above-mentioned indices range from 0.0 to 1.0 and provide information of the effectiveness to differentiate among genotypes. Thus, a more effective SSR in discriminating among genotypes has a high level of H_o, and higher PIC and DI values along with lower PI values. Allele frequencies, expected (H_e) and observed (H_o) heterozygosity, estimated

frequency of null alleles (r), and PI were calculated with the software IDENTITY (Wagner and Sefc 1999 version 1.0; Centre for Applied Genetics, University of Agricultural Sciences, Vienna); the PIC was directly calculated starting from H_e and PI. Finally, the discrimination power (D_j) of each microsatellite was directly calculated according to Tessier et al. (1999):

$$D_j = 1 - C_j = 1 - \sum_{i=1}^{I} c_i = 1 - \sum_{i=1}^{I} p_i \frac{(Np_i - 1)}{N - 1}$$

where c_i and C_j are the confusion probability for the ith genotype of the given jth microsatellite and the confusion probability for the jth microsatellite, respectively; p_i is the ith genotype frequency; N is the number of individuals analysed.

Based on the SSR data obtained the Simple Matching (SM) coefficients were estimated using the SIMQUAL program from NTSYS-pc ver. 2.02j package (Exeter Software, Setauket, NY). Only data coming from polymorphic microsatellites were employed. A dendrogram was generated by using SM coefficient and the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis of the similarity using the SAHN-clustering and TREE program of the NTSYS-pc ver. 2.02j package (Rohlf 1998). The relationship between the groupings obtained and the geographical origin was studied.

Results and discussion

The 82 cultivars analysed at 6 SSR loci showed 70 different genetic profiles (Table 2); in our genotypes, the probability to find different plants with the same profile at all loci appeared to be low (PI = 2.93×10^{-6}), and therefore identical profiles/genotypes can be considered as synonyms (Table 3). Starting from 82 putative cultivars, nine cases of suspected and unsuspected synonymies among or between couples of cultivars were found, based on six SSR loci (Table 3). In contrast, considering different clones or biotypes of the same cultivars five cases of homonymies were found (Table 3). Frappato and Nerello Mascalese showed different genetic profiles among some of the five plants sampled representative of the cultivar, indicating two cases of homonymies (Table 3). Beside, suspected (Inzolia and Inzolia nera, Catarratto and Catarratto nero) and unsuspected (Malvasia, Malvagia and Malvasia di Lipari) homonymies were also found in the cultivars Inzolia, Catarratto and Malvasia. Number of alleles (n), allele size range for each locus are listed in Table 4, whereas the allele frequencies are reported in Fig. 2. Seventeen of 51 alleles detected in the present study had a frequency lower than 5.0%. Total number of alleles per locus ranged from 7 to 10, with an average of 8.5, in agreement with previous analyses (Bowers et al. 1996, 1999; Sefc et al. 1999, 2000). The main genetic parameters such as expected (H_e) and observed (H_o) heterozygosity, estimated frequencies of null alleles (r), probability of identity (PI), polymorphic information content (PIC), discrimination power (D_j), percentage of homozygosity and number of heterozygotic profiles are reported in Table 4. Starting from the cultivars showing the 70 different profiles, heterozygosity values were high (ranging from 0.743 to 0.857). The mean of observed heterozygosity (H_o) over the six loci (0.814) was comparable with prior studies (Costantini et al. 2005). Estimated frequency of null alleles (r) was negative for 5 out of 6 loci, and thus the positive r value for VVMD5 locus only indicates a possibility of the presence of null alleles. PI ranged from 0.092 to 0.167, and higher values compared to the value at which a microsatellite is considered hyperpolymorphic in grapevine (Sefc et al. 2001).

Analyzing the ancient literature, in Sicily 121 different names of cultivars have been mentioned (Table 5), of which 37 names are still present in cultivation when the sampling for the present study were performed (Table 1). In contrast, 45 cultivars found and collected in old vineyards during sampling had names that were not mentioned in the historical literature analysed (Tables 1 and 5).

Catarratto is the most important wine cultivar of Sicily (35% of the vineyard cultivated area) where it has been cultivated for centuries. It was described by Cupani in 1696. The cultivar is widespread in the province of Trapani, where it is used for the production of Marsala DOC, a wine originated by English merchant traders two centuries ago. Different Catarratto biotypes are cultivated in Sicily (Collesano 1998); Pastena (1971) described four different Catarratto biotypes (Catarratto bianco comune, Catarratto bianco lucido, Catarratto bianco lucido spargolo and Catarratto bianco extralucido). The Catarratto bianco biotypes analysed in the present study (Catarratto comune, Catarratto lucido and Catarratto

Table 2 Genetic profiles of the cultivars at the six microsatellite loci studied

	Accession number	Cultivar name	VVS2	VVS2	VVMD5	VVMD5	VVMD7	VVMD7	VVMD27	VVMD27	VrZAG62	VrZAG62	VrZAG79	VrZAG79
1	VV-CNR-1	Albanello	131	137	220	222	237	251	178	182	181	195	248	256
2	VV-CNR-2	Alicante, Licante	131	139	220	234	237	241	190	190	183	183	254	254
3	VV-CNR-3	Alzano	139	139	226	234	237	237	176	186	183	191	238	238
4	VV-CNR-4	Austina bianca	127	137	222	230	237	245	182	186	189	199	248	256
5	VV-CNR-5	Barbarossa	129	141	226	232	237	247	180	190	187	199	244	254
6	VV-CNR-6	Bracaù	127	139	226	234	237	237	176	186	183	191	240	248
7	VV-CNR-8	Calabrese	137	145	220	234	237	241	176	178	183	197	248	254
8	VV-CNR-9	Canina	133	137	220	226	245	251	176	182	195	199	246	252
9	VV-CNR-10	Carnuffino	127	137	230	234	247	247	176	182	189	197	240	248
10	VV-CNR-11	Carricante	127	127	220	230	237	247	176	182	189	191	248	256
11	VV-CNR-13	Catanese nero	127	127	234	234	237	245	180	182	183	193	244	248
12	VV-CNR-14	Catarratto bianco comune	137	145	220	220	237	247	176	176	195	197	248	248
13	VV-CNR-18	Catarratto nero	127	129	222	230	237	247	180	182	183	197	248	254
14	VV-CNR-20	Cessalà	129	145	228	230	245	251	176	190	191	199	248	254
15	VV-CNR-21	Cirrinciò	127	145	222	230	237	247	190	190	183	199	254	256
16	VV-CNR-22	Coda di volpe	127	137	220	226	251	251	176	190	195	195	248	248
17	VV-CNR-24	Corinto	133	137	222	222	237	247	176	186	181	193	246	248
18	VV-CNR-26	Diretta bianca	127	129	230	232	247	249	182	186	181	189	236	258
19	VV-CNR-28	Diretta nera	127	139	220	220	241	247	178	186	183	199	256	258
20	VV-CNR-27	Francisi, Uva di Francia	129	131	234	234	247	247	186	186	197	199	240	248
21	VV-CNR-30	Frappato A	127	137	220	220	237	247	176	182	183	197	240	246
22	VV-CNR-31	Frappato B	127	137	220	220	237	237	176	182	189	191	240	246
23	VV-CNR-32	Frappato C	127	137	220	220	237	237	176	182	189	191	240	244
24	VV-CNR-37	Gamay	127	139	220	234	241	245	190	190	181	183	248	254
25	VV-CNR-38	Grecanico, Grecanico dorato	127	137	220	226	247	251	176	190	195	195	248	248
26	VV-CNR-41	Grecaù	127	127	220	220	241	245	176	178	183	199	252	256
27	VV-CNR-42	Grillo	133	137	220	226	245	251	176	182	195	199	244	252
28	VV-CNR-120	Inzolia	129	137	220	234	237	245	176	180	183	197	244	248
29	VV-CNR-48	Inzolia imperiale	129	129	220	230	237	247	176	178	181	183	252	256
30	VV-CNR-49	Inzolia nera	131	143	222	232	237	251	176	182	183	183	244	248
31	VV-CNR-50	Inzuccarato	129	129	230	234	247	249	182	190	181	189	252	254

Table 2 continued

	Accession number	Cultivar name	VVS2	VVS2	VVMD5	VVMD5	VVMD7	VVMD7	VVMD27	VVMD27	VrZAG62	VrZAG62	VrZAG79	VrZAG79
32	VV-CNR-51	Inzuccarato di Noto	139	147	220	228	237	241	176	190	183	199	248	254
33	VV-CNR-45	Jala bianca	137	143	220	222	237	241	176	190	183	197	248	252
34	VV-CNR-52	Lacrima di Maria	131	141	220	234	237	241	182	190	183	199	248	254
35	VV-CNR-53	Leanfurtisi	129	145	220	222	237	245	176	182	183	193	246	248
36	VV-CNR-55	Lucignola	127	137	220	220	237	247	176	182	189	197	240	248
37	VV-CNR-56	Malvagia	137	145	220	234	237	247	176	176	183	197	240	248
38	VV-CNR-57	Malvasia	137	139	220	220	241	247	176	180	183	197	238	244
39	VV-CNR-58	Malvasia di Lipari	137	139	222	234	241	247	176	190	183	197	248	252
40	VV-CNR-60	Marsigliana	127	131	234	234	237	237	180	182	183	183	248	254
41	VV-CNR-61	Minna di vacca	131	137	222	234	237	237	182	190	181	199	248	254
42	VV-CNR-62	Minnavacchina	137	137	234	234	237	247	188	190	183	197	244	248
43	VV-CNR-63	Minnella bianca, Passulana	131	131	222	232	231	237	182	190	181	199	248	254
44	VV-CNR-64	Monteleone	127	137	226	230	245	261	186	190	189	191	246	248
45	VV-CNR-68	Muscatedda	127	137	222	234	237	247	176	176	181	183	246	252
46	VV-CNR-69	Muscatidduni	137	145	220	228	237	247	188	190	181	197	244	254
47	VV-CNR-75	Nerello Mascalese A	127	137	220	230	237	247	176	176	183	197	246	256
48	VV-CNR-70	Nerello Mascalese B	127	137	220	230	237	247	176	176	191	197	248	256
49	VV-CNR-76	Nero d'Avola	137	145	220	234	237	247	176	178	181	197	248	248
50	VV-CNR-77	Nero grosso	129	143	226	234	237	237	182	190	183	183	248	254
51	VV-CNR-81	Nzuccarato	139	147	220	228	241	249	176	190	183	183	238	254
52	VV-CNR-82	Oriddru	127	137	220	226	237	249	176	182	197	199	248	248
53	VV-CNR-83	Orisi	127	139	220	234	245	261	176	176	189	191	240	256
54	VV-CNR-84	Osso nivuro	127	133	226	232	237	237	186	190	183	199	248	254
55	VV-CNR-85	Perricone	127	129	222	230	237	237	180	182	183	191	240	244
56	VV-CNR-86	Pizzutella	127	129	220	226	237	247	182	182	183	183	240	248
57	VV-CNR-87	Precoce	127	127	220	232	241	247	186	190	181	183	248	254
58	VV-CNR-92	Racignola nera	131	137	234	234	237	247	176	190	183	195	248	254
59	VV-CNR-94	Racinedda	133	137	222	222	237	241	176	186	181	183	248	254
60	VV-CNR-95	Recunu	127	137	220	220	245	251	182	190	181	195	248	248
61	VV-CNR-96	Regina	127	129	220	230	245	247	176	182	181	199	248	252
62	VV-CNR-97	Rucignola	137	139	220	234	247	247	182	188	195	197	240	244

Table 2 continued

	Accession number	Cultivar name	VVS2	VVS2	VVMD5	VVMD5	VVMD7	VVMD7	VVMD27	VVMD27	VrZAG62	VrZAG62	VrZAG79	VrZAG79
63	VV-CNR-98	Russetto	137	139	220	220	237	245	176	176	191	191	244	252
64	VV-CNR-101	Sultanina	127	145	222	230	237	247	190	190	183	199	252	256
65	VV-CNR-103	Tintorè, Ibisu	127	139	220	232	237	241	178	190	183	183	240	254
66	VV-CNR-104	Triboti nera	143	147	220	226	237	241	176	190	183	199	244	254
67	VV-CNR-105	Tripolina bianca	137	139	220	228	241	261	176	188	183	189	240	244
68	VV-CNR-107	Visparola	127	127	220	220	237	237	182	190	183	191	246	246
69	VV-CNR-108	Vitraruolo	127	137	220	226	237	249	182	182	197	197	248	248
70	VV-CNR-109	Zibibbo	127	143	222	226	247	249	176	190	181	199	244	252

extralucido) displayed identical SSR profiles at all loci analysed. Only Catarratto nero showed a rather different SSR profile from Catarratto bianco.

The presence of *Frappato* in Sicily is known since the eighteenth century (Nicosia 1735; Sestini 1812a). The cultivar is widespread in the provinces of Ragusa and Syracuse, where it is used for the production of the Cerasuolo di Vittoria DOCG. Three of five putative Frappato clones analysed (named Frappato A, Frappato B and Frappato C) displayed polymorphic SSR profiles (Table 2).

The presence of *Grecanico* in Sicily has been known since the seventeenth century (Cupani 1696). However, its presence is reported in several southern Italian regions (Bica 2007). Geremia (1835) described two different Grecanico biotypes (Grecanico a giriamoli and Grecanico a croce) based on bunch shape and density. The cultivar is mainly spread in the province of Trapani, where it used for the production of Bianco d'Alcamo wine. The Grecanico biotypes analysed displayed identical SSR profiles at all loci analysed.

The presence of *Grillo* in Sicily is known only since the end of the nineteenth century (Alagna Spanò 1873). It is cultivated mainly in the province of Trapani, where it was probably introduced from Apulia (Gagliano 2008) and is used for the production of Marsala wine. The different biotypes analysed displayed identical SSR profiles.

Inzolia is an ancient Sicilian cultivar noted by Roman authors such as Pliny (Gagliano 2008). Cupani (1696) described three different Inzolia biotypes; two with white and one with black berries. The cultivar is widespread in all of Sicily, especially in the provinces of Trapani, Agrigento and Palermo. It is mainly used in the province of Trapani for the production of Marsala and Bianco d'Alcamo DOC wines. Although the five putative clones of Inzolia did not show SSR polymorphism, Inzolia, Inzolia imperiale and Inzolia nera displayed different SSR profiles (Table 2).

The presence of *Malvasia* in Sicily is known since the seventeenth century (Cupani 1696). However, it was probably introduced in Sicily during the sixth century B.C. by the Greeks (Bica 2007). Malvasia is cultivated in the province of Messina, in particular in the volcanic Aeolian isles (Hammer and Laghetti 2006), where it is used for the production of the Malvasia di Lipari DOC dessert wine. The biotypes

Table 3 Cases of synonymies and homonymies found among grapevine cultivars

Synonymies	Homonymies
Albanello and Fumusa	Frappato A, Frappato B and Frappato C
Alicante, Bruntisi nero, Dolcetta and Nivuro Bronte	Catarratto bianco comune, Catarratto nero
Catarratto bianco comune, Catarratto bianco lucido and Catarratto bianco extralucido	Inzolia, Inzolia imperiale and Inzolia nera
Inzolia imperiale and Primintia/Giugnettina	Nerello Mascalese A and Nerello Mascalese B
Minnavacchina and Racignola bianca	Malvasia, Malvagia and Malvasia di Lipari
Regina and Moscato bianco	
Orisi and Lorisi	
Perricone and Nivureddu	
Zibibbo, Zibibbo augustano, Zibibbo carricante, Zibibbo grosso and Zibibbo nero	

Table 4 Genetic parameters at the 6 SSR loci analysed in the grapevine cultivars

Locus	No. alleles	Allele size range (bp)	H_e*	H_o*	r**	PI[#]	PIC	D_j*	Hom.[a]	No. Het.[b]
VVS2	10	127–147	0.817	0.857	−0.022	0.100	0.7279	0.9304	14.3	22
VVMD5	7	218–234	0.776	0.743	0.019	0.133	0.6663	0.9300	25.7	16
VVMD7	8	219–261	0.744	0.786	−0.024	0.167	0.6091	0.8861	27.4	13
VVMD27	7	172–190	0.773	0.800	−0.015	0.153	0.6383	0.9238	20.0	16
VrZAG62	9	181–199	0.821	0.843	−0.012	0.092	0.7411	0.9441	15.7	21
VrZAG79	10	226–258	0.814	0.857	−0.024	0.093	0.7362	0.9329	14.3	21
All Loci	51	–	–	–	–	2.93×10^{-6}	–	–		
Mean	8.5	–	0.791	0.814	–	0.123	0.6865	0.9245	19.57	18.17

*Expected (H_e) and observed (H_o) heterozygosity

**Estimated frequency of null alleles (r)

[#] Probability of identity (PI); polymorphic information content (PIC); discrimination power (D_j)

[a] Percentage of homozygosity in each locus

[b] Number of heterozygotic profiles

analysed in the present study (Malvagia, Malvasia and Malvasia di Lipari) displayed different SSR profiles (Table 2).

Nerello Mascalese is an ancient Sicilian cultivar noted by the Romans (Gagliano 2008); it belongs to the group of "Nigrelli" described by Sestini (1812b). Nerello Mascalese is present in the province of Catania. The name is derived from the town of Mascali, which is a small town at the bottom slopes of the volcano Etna, where the cultivar is used for the production of the Etna rosso wine. Two of the Nerello Mascalese biotypes analysed (named Nerello Mascalese A and Nerello Mascalese B) displayed different SSR profiles (Table 2).

Nero d'Avola was described by Cupani (1696) in the seventeenth century; however, its presence in Sicily is known since ancient times. It is the most important red wine cultivar in Sicily (15% of the vineyard cultivated area), where it is used for the production of several Nero d'Avola wines exported worldwide. The cultivar is widespread in Sicily and is used for the production of several DOC wines (Etna rosso and Cerasuolo di Vittoria). The Nero d'Avola biotypes analysed in the present study displayed identical SSR profiles at all loci analysed.

Zibibbo is widespread in the island of Pantelleria (Hammer and Laghetti 2006), where it is used for the production of the Passito or Zibibbo di Pantelleria

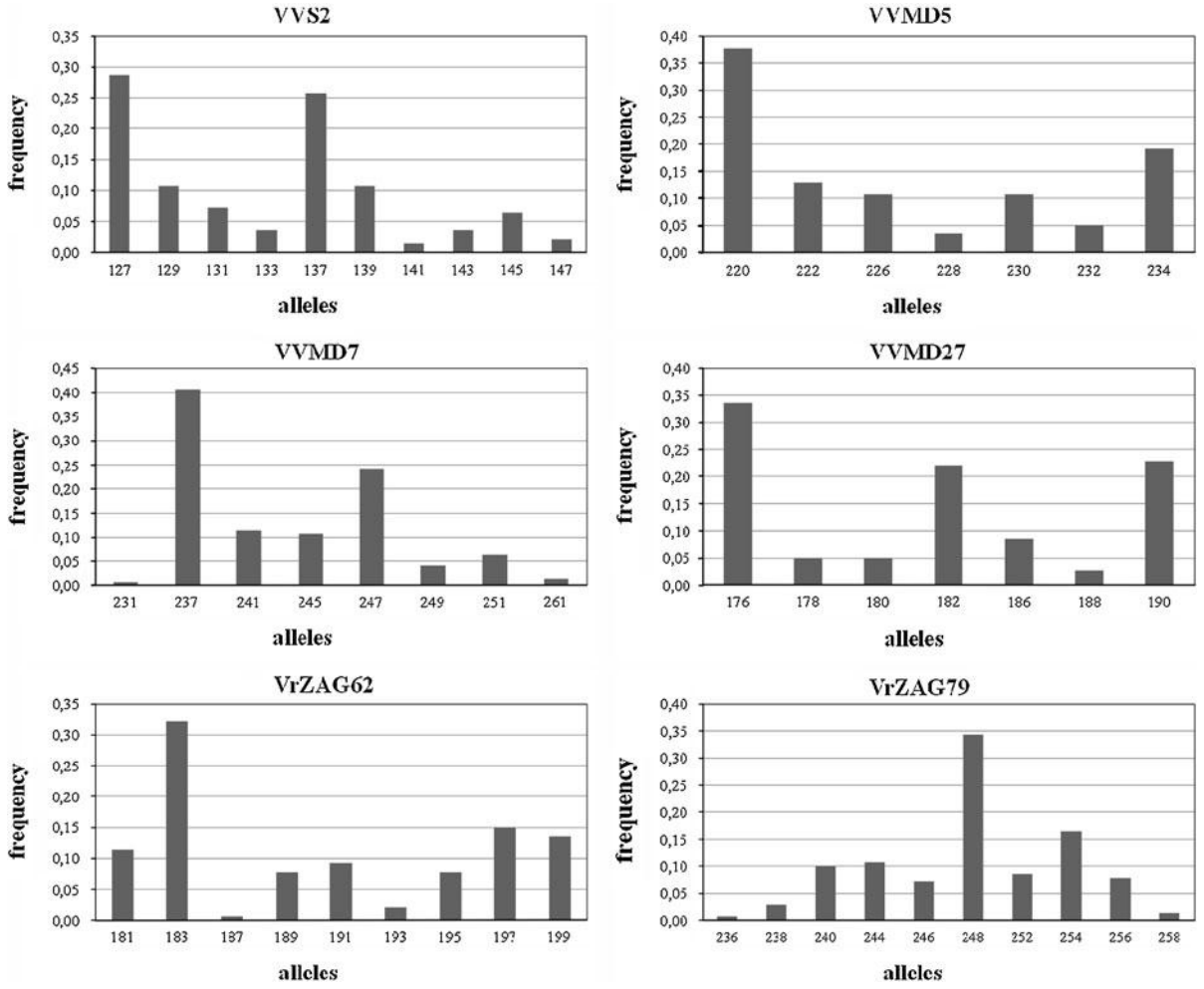

Fig. 2 Distribution and relative frequency of microsatellite alleles in 70 grapevine cultivars from Sicily analysed at 6 SSR loci

DOC wine (Collesano 1998), which is one of the richest and most esteemed Italian sweet wines (Anderson 2006). It was described by Cupani (1696) in the seventeenth century, although its presence in Sicily is known since ancient times. Zibibbo biotypes having minor differences in morphological traits and yield performances (Zibibbo augustano, Zibibbo carricante, Zibibbo grosso and Zibibbo nero) were analysed, showing all the same SSR profile. Therefore, it is possible to conclude that the different Zibibbo biotypes represent one single cultivar and that the other forms are morphological mutants. In particular Zibibbo nero is a berry colour mutant. Berry colour mutants, which show identical microsatellite profiles, have been already reported in other cases (Sefc et al. 1998a; Lopes et al. 1999).

Genetic relationships

A dendrogram based on SM coefficient and UPGMA is presented in Fig. 3. Six main groups were distinguished, indicating several origins for Sicilian grapevine germplasm. It was not always possible to correlate between clustering and the actual geographic distribution of cultivars, considering the sites where each genotype was sampled during the constitution of germplasm collection (for the site of sampling see Table 1). It is also evident that there were no relationships between clustering and berry colour (see Table 1). Instead, in Fig. 3 is it possible to distinguish at least five clusters (indicated from A to E) and five cultivars, reported in the last three small branches in the lower part of the figure. In

Table 5 List of Sicilian grapevine cultivars mentioned in the ancient literature during past centuries

	Cultivar name and synonyms	Reference
1	Ala	Minà Palumbo
2	Albanello	Nicosia
3	Amorosa	Minà Palumbo
4	Barbarossa, Barbarussa, Varva russa, Vitis uva barbata	Cupani, Sestini, Minà Palumbo
5	Bottone di Gallo, Buttuna di Gaddu, Curniola	Cupani, Sestini
6	Buxazzara	Cupani, Sestini
7	Caravella	Minà Palumbo
8	Carricante	Sestini, Geremia
9	Catanese bianco	Boll. Amp. 1883
10	Catanese nero	Boll. Amp. 1878
11	Catarratto bianco comune, Catarrattu vrancu	Cupani, Sestini
12	Catarratto bianco lucido	Boll. Amp. 1883
13	Catarrattu reusu, Uva Rhetica	Pliny, Cupani, Sestini
14	Cela	Minà Palumbo
15	Centorotoli nero	Geremia
16	Chitichiti biancu	Minà Palumbo
17	Chitichiti niura	Minà Palumbo
18	Ciminnita, Ciminnese, Ciminnutu, Cominnita	Fazello, Cupani
19	Citana bianca	Fazello
20	Corinto nero, Uva marina nera	Gallo
21	Corniola bianca, Corniola di Milazzo	Fazello, Cupani
22	Corniola nera, Curniola niura	Fazello, Sestini, Minà Palumbo
23	Corniola, Liparota, Vitis olivaria	Cupani, Sestini
24	Curnicchia	Minà Palumbo
25	Curriola	Geremia
26	Damaschino	Mendola
27	Duppia	Minà Palumbo
28	Duracina, Duraca	Cupani, Sestini
29	Duraina bianca	Minà Palumbo
30	Duraina niura	Minà Palumbo
31	Durignola	Minà Palumbo
32	Eppole	Sestini
33	Eugnea, Eugeniam Tauromenitani	Pliny
34	Frappato di Vittoria	Nicosia, Sestini
35	Fumusa russa	Cupani, Sestini
36	Fumusa vranca, Fumusa bianca, Famusa	Cupani, Sestini, Minà Palumbo
37	Fumusa vranca pilusa, Fumusa bianca pelosa	Cupani, Sestini
38	Gerusalemi, Gerosolemitana, Buttuna di Gatta, Zuccarina	Cupani, Sestini, Minà Palumbo
39	Giustulisa bianca, Giustulisi	Cupani
40	Greca	Fazello, Cupani, Minà Palumbo
41	Greca di Napoli, Neapoli graeca, Uva greca, Passule di Lipari, Regina	Pliny, Cupani, Sestini, Minà Palumbo
42	Greca di Palermo, Greca femminina	Cupani, Sestini
43	Greca tribota	Minà Palumbo
44	Grecanico	Cupani
45	Grecau, Gercu, Grecu masculinu	Cupani, Sestini
46	Grillo	Alagna Spanò
47	Gruppina	Minà Palumbo
48	Guarnaccia niuru, Guarnaccia nera	Cupani, Sestini
49	Guarnacciu vrancu, Guarnaccia bianca	Cupani, Sestini
50	Imperatrici	Minà Palumbo
51	Inzolia bianca, Inzolia vranca, Irziola, Zuolia bianca, Ansonica	Pliny, Cupani, Geremia, Minà Palumbo
52	Inzolia imperiale, Inzolia di Napuli	Cupani
53	Inzolia nera, Inzolia nigra, Zuolia niura	Cupani, Minà Palumbo
54	Isabella	Geremia
55	Lacrima di Madonna, Lacrimi di Madonna	Minà Palumbo
56	Lacrima di Maria di Termini Imerse	Acerbi
57	Maimuni	Minà Palumbo
58	Malvagia, Marvascia	Cupani, Sestini
59	Malvasia bianca	Minà Palumbo
60	Malvasia di Lipari	Mendola
61	Mantonicu fimmininu	Minà Palumbo
62	Marsigliana nera, Marsighiana, Olivedda	Cupani, Minà Palumbo
63	Maruascia	Cupani
64	Martellata	Minà Palumbo
65	Mennavacca nera	Cupani
66	Minnavacchina, Mannavacchina bianca	Minà Palumbo
67	Minnella bianca	Sestini, Geremia

Table 5 continued

	Cultivar name and synonyms	Reference
68	Montonico bianco, Mantonicu vrancu, Mantonicu masculu, Muntonicu	Venuti, Cupani, Sestini, Minà Palumbo
69	Montonico nero femminino, Mantonicu niuru fimmineddu	Venuti, Cupani, Sestini
70	Montonico nero, Mantonicu niuru	Cupani, Sestini
71	Moscatella bianca, Moscadella bianca, Muscateddu vrancu, Muscatedda bianca, Vitis Apiana	Cupani, Sestini, Minà Palumbo
72	Moscato di Siracusa, Muscato Orthygia	Cupani
73	Muscatedda curunata	Minà Palumbo
74	Muscatedda niura	Minà Palumbo
75	Narese	Fazello, Sestini
76	Nerello cappuccio di Catania	Mendola
77	Nerello Mascalese	Sestini, Geremia
78	Nero d'Avola, Calabrese, Calabrese di Vittoria, Calaurisi	Cupani, Sestini, Minà Palumbo, Mendola
79	Nero grosso	Acerbi
80	Nero tavola	Boll. Amp. 1878
81	Niureddu ammantiddatu	Minà Palumbo
82	Niuridduni, Nerellone	Cupani, Sestini
83	Nivureddu, Niurdda, Niureddu ordinario, Negrello ordinario	Cupani, Sestini, Minà Palumbo
84	Nocera nera di Milazzo	Cupani
85	Canina, Nuciddara	Cupani, Minà Palumbo
86	Olivella di Milazzo	Cupani
87	Olivetta	Minà Palumbo
88	Orsina	Minà Palumbo
89	Passolina di Lipari	Cupani
90	Perricone	Nicosia
91	Petrese bianca, Pitrisi vrancu, Pitrusa janca, Putrisi	Cupani, Sestini, Minà Palumbo
92	Petrese nera, Pitrisi niuru, Pitrusa niura	Cupani, Sestini
93	Pizzutello bianco, Pizzutella bianca	Cupani, Sestini
94	Prunara, Racina Prunara, Vitis ferax	Cupani, Sestini, Minà Palumbo
95	Racina indiana	Minà Palumbo
96	Rosa bianca	Minà Palumbo
97	Rotulaca, Rotulura	Cupani, Sestini
98	Rubinieddu	Minà Palumbo
99	Russella ruggia	Minà Palumbo
100	Salamitana	Minà Palumbo
101	San Leonardo	Minà Palumbo
102	Siturna	Minà Palumbo
103	Survana	Minà Palumbo
104	Survana niura	Minà Palumbo
105	Tasta e lassa	Minà Palumbo
106	Tomasina	Minà Palumbo
107	Triboti nera, Tribota nigra	Minà Palumbo
108	Triboto bianco, Triboti bianco, Trivoti, Prunesta, Pumestra, Vitis trifera	Cupani, Venuti
109	Tripedana	Sestini
110	Tripianu, Trupianu, Moscato nigro	Cupani, Sestini
111	Trivoti di sciorta	Cupani, Sestini
112	Tuccarineddu, Niuridduzzo, Passulina di lu nostru Regnu	Cupani, Sestini
113	Tuccarinu	Cupani, Sestini
114	Tuccarinu cu'cocci minuti	Cupani, Sestini
115	Tuccarinu cu'cocci grossi	Cupani, Sestini
116	Virdisi bianca, Virdusa	Cupani, Sestini, Minà Palumbo
117	Virdisi niura	Minà Palumbo
118	Visazzate	Sestini
119	Visparola	Cupani, Sestini
120	Zibibbo, Zibibu	Cupani, Sestini, Minà Palumbo
121	Zubidia, Frijstedda, Ducignola	Cupani, Sestini

Acerbi (1825), Alagna Spanò (1873), Bollettino Ampelografico (1878, 1883), Cupani (1696, 1697), Fazello (1558), Gallo (1595), Geremia (1834, 1835, 1836, 1839), Mendola (1868), Minà Palumbo (1853, 1891), Nicosia (1735), Sestini (1812a, b), Venuti (1516)

cluster A, many cultivars from the eastern part of Sicily are included (provinces of Messina, Catania and Syracuse), except the cultivars Grecanico, Grillo and Pizzutella that were sampled in the provinces of Agrigento, Trapani and Palermo, respectively. In cluster A, many important cultivars of ancient origin such as Frappato, Carricante, Nerello Mascalese, Grecanico and Regina are found. In this cluster, two important cases of homonymies (Frappato and Nerello Mascalese) are present, where the Frappato A clone tightly clusters with Lucignola (a cultivar of minor interest present in the province of Messina)

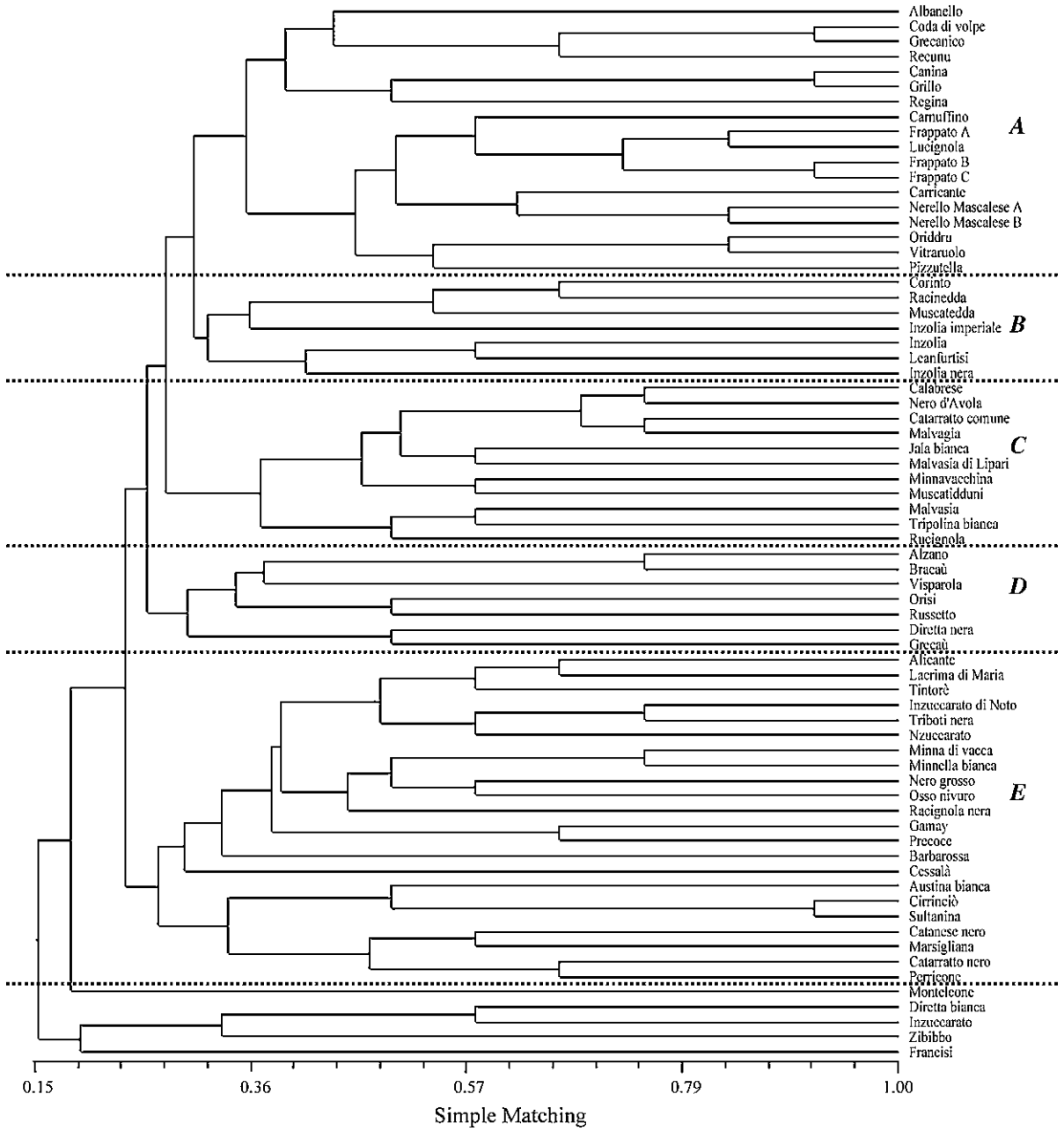

Fig. 3 Dendrogram of genetic relationships among the 70 grapevine cultivars generated with simple matching coefficient and UPGMA cluster analysis using the SAHN-clustering and TREE program of the NTSYS-pc ver. 2.02j package (Rohlf 1998)

that is a possible parent (the possible parent offsprings combinations was calculated by software IDENTITY) for Frappato A, B, C and Vitraruolo, another cultivar of minor interest from the province of Messina. In several cases, Lucignola is also indicated as possible offspring between Frappato (A, B and C), Carricante, Catarratto, Nerello Mascalese and Nero d'Avola. The tight relationships among the Nerello Mascalese and Carricante clones clearly indicates a common origin from the Contea of Mascali, a small city at the slope of Etna (Sestini 1812b). In cluster A, the important cultivar Grillo of relatively recent introduction in Sicily is also present, which seems to be related to the cultivar of minor

interest Canina cultivar sampled in the province of Messina.

Cluster B is characterized by the presence of Inzolia clones (Inzolia, Inzolia nera and Inzolia imperiale) together with some cultivars frequently used to make sweet and dessert wines, confirming the frequent sweet feature of Inzolia berries. In the same cluster, it is possible to distinguish two small clusters; Inzolia imperiale is in a cluster with Muscatedda and Corinto that are utilized for sweet wine production, while Inzolia and Inzolia nera are in a cluster with the cultivar of minor interest Leanfurtisi from the province of Syracuse.

Cluster C is characterized by the presence of several important Sicilian cultivars of ancient origin (Nero d'Avola, Catarratto comune, Malvasia, Malvasia di Lipari, Malvagia and Calabrese) together with some rare white berry cultivars from the provinces of Palermo and Messina, of which only Minnavacchina and Jala bianca are reported in the literature as cultivated in Sicily since ancient times (Minà Palumbo 1853). The first branch of this cluster indicates that Calabrese and Nero d'Avola, considering our samples, do not appear to be synonyms as reported by Costantini et al. (2005). The second branch related to the first clearly indicates a genetic relationship between Nero d'Avola and another important Sicilian cultivar such as Catarratto comune that is tightly linked to Malvagia. This cultivar, confirming the genetic similarity to Nero d'Avola, is found to be a possible parent of Nero d'Avola (together with Albanello) or as offspring from Calabrese, Nero d'Avola or Frappato.

Cluster D is very small and includes 7 rare cultivars of minor interest from the Etna area, of which only Visparola and Grecaù are reported in the ancient literature as cultivated in Sicily (Cupani 1696; Sestini 1812a).

Cluster E is the largest, with 22 cultivars included; the most important cultivars are Alicante and Perricone, which are noir berry cultivars from the provinces of Catania and Trapani, respectively. Perricone, in the lower part of the cluster, is tightly linked with Catarratto nero (sampled in the province of Palermo), a noir berry cultivar probably derived from the most famous white berry cultivar, namely Catarratto comune. In this cluster, Lacrima di Maria (Acerbi 1825), Triboti nera (Minà Palumbo 1853), Nero grosso (Acerbi 1825), Barbarossa (Cupani 1696), Marsigliana (Cupani 1696) and Catanese nero (Bollettino Ampelografico 1878) are cited as having been cultivated in Sicily since ancient times. In the analysis of possible parent offsprings combinations (calculated by software IDENTITY), in several cases Lacrima di Maria is reported as a possible parent of Alicante (important noir berry cultivar from the province of Catania), while Lacrima di Maria and Catanese nero are proposed as possible parents of Marsigliana; all three cultivars included in cluster E. Finally, above cluster E, the cultivars Monteleone, Diretta Bianca, Inzuccarato, Zibibbo and Francisi (this cultivar was sampled in the province of Palermo but considering its name it is clear that it was introduced in that area from France, not in ancient times) appear to form another small cluster. The cultivar Francisi, supporting its supposed origin, can be considered as an outgroup genotype.

Conclusion

The 82 cultivars analysed in this study are distributed mainly in the eastern part of the region (46 cultivars) in the provinces of Syracuse (23), Catania (11) and Messina (12). In the western part of the island, the province of Palermo is the richest in terms of biodiversity (13 cultivars), followed by Trapani (7) and Agrigento (6). Finally, there are 7 cultivars from small islands (Eolie Islands and Pantelleria) that are most important in terms of biodiversity. The distribution of the cultivars does not seem to reflect the evolution and the increase of the area cultivated with grapevine. Although Syracuse is still an important province in terms of Sicilian viticulture, Trapani and Agrigento are probably the provinces with the greatest increase in vineyard areas during the last decades. The intensive grapevine cultivation could have caused a considerable loss of biodiversity. In conclusion, the Sicilian grape germplasm has been introduced in different periods from several geographic areas. Considering the popularity of wines obtained from local Sicilian cultivars (Nero d'Avola, Catarratto, Inzolia, Nerelli and Frappato) in the international market, the results of the present work shed light either on the genetic relationships among cultivars of regional interest and further demonstrate that the large genetic diversity of grapevines is still unexploited in Sicily.

Finally, it is important to observe that biotypes of the important cultivars analysed, such as Nero d'Avola and Catarratto, displayed identical SSR profiles. Given the importance and ancient origin of these cultivars, however, further investigation, especially in the presumed province of origin, should be carried out to isolate their different biotypes.

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