Genet Resour Crop Evol (2011) 58:967–975
DOI 10.1007/s10722-011-9731-4

SHORT COMMUNICATION

Intra-varietal genetic diversity of the grapevine (*Vitis vinifera* L.) cultivar 'Nero d'Avola' as revealed by microsatellite markers

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Received: 30 January 2011 / Accepted: 27 June 2011 / Published online: 21 July 2011

Abstract The Sicilian grape cultivar 'Nero d'Avola' is among the oldest and most cultivated in the island, taking part in the production of several red wines exported worldwide, including DOC wines (Etna Rosso and Cerasuolo di Vittoria). Due to the ancient origin and repeated clonally propagation of the cultivar, phenotypic variability has been observed. Clone identification in this important cultivar has so far relied on phenotypic and chemical traits analyses, often affected by environmental conditions. Genetic markers, such as microsatellites, are particularly useful for cultivar identification, parentage testing, pedigree reconstruction and population structure studies. In the present paper, microsatellites were used to analyze the intra-varietal genetic diversity among 118 plants of 'Nero d'Avola', collected in 30 vineyards displaced in different areas of Sicily. Out of 22 microsatellites, 11 showed polymorphism among samples and 15 different phylogenetic groups were identified. Results show that 'Nero d'Avola' actually comprises different genetic profiles, although most of clones share a common origin.

Keywords Clonal identification · Simple sequence repeat · Synonymies and homonyms · *Vitis vinifera* L.

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Introduction

Grape (*Vitis vinifera* L.), one of the earliest domesticated fruit, is a highly valuable crop, especially for the derived products wines and spirits. In Italy, grapevines are extensively cultivated, with about 800,000 ha of vineyards; however, their distribution in the country is unequal, with Sicily representing one of the most significant wine regions. Wine and vine have accompanied the history of Sicily since its beginning; it is believed that wild vines were present in Sicily far earlier than Greeks colonized the island (Costantini 1989). The long history of cultivation has led to a high number of autochthonous grapevine varieties, which represents an important source of genetic diversity. Accordingly, Sicilian varieties are characterized by high variability (Carimi et al. 2010),

having been subjected to mass selection by growers for centuries.

'Nero d'Avola' is currently the most important and diffused red berry cultivar in Sicily, with 15% of the vineyard surface, and this ancient variety is utilized for the production of wines exported worldwide, among which several DOC wines such as Etna Rosso and Cerasuolo di Vittoria. The cultivar, also known as '*Calabrese'* was clearly described by Cupani (1696) in the seventeenth century with the name '*Calaurisi'*; however, its presence in Sicily is known since ancient times. The history and origin of this variety have been mixed with that of '*Nerello'* and '*Calabrese'* groups. The name, as reported by Geremia (1839), suggests a Calabrian origin, but no real evidence supports this theory. An alternative hypothesis, shared by some authors (Di Rosa and Gringeri 1966), derives instead its dialect name '*Calaulisi'* after *Caia-Avola* (grape of Avola). In any case, 'Nero d'Avola' was originally cultivated near Avola (a small town in south-eastern Sicily) several hundred years ago.

Grapevine varieties are asexually propagated in order to maintain their distinctive individual characters. Phenotypic variations among clones belonging to the same cultivar may be due to somatic mutations in the genomes, accumulated during several vegetative propagation cycles. Therefore, in many cases, a cultivar comprises several different populations of clones, sharing a panel of similar phenotypic traits. On the other hand, homogeneity within a variety is often crucial to nursery and wine industries, thus it is rather important to be able to estimate intra-varietal variability and select for different clones. Moreover, the characterization and identifications of clones is an important step for disease control and breeding programs.

The identification of grapevine varieties has traditionally been based on ampelography, the description of morphological plant traits during different stages of development. However, this technique is heavily affected by environmental constraints, and often leads to false attributions such as homonyms and synonymies, especially when used within one cultivar.

The advent of molecular markers in the '80s, especially those PCR-based such as microsatellites (also called Simple Sequence Repeat, SSR), has revolutionized cultivar identification and parentage analysis of grapevines. In addition, a large number of potential polymorphic sequences are currently available for grapevine molecular analysis. By using microsatellites it is possible to characterize and compare genotypes independently from phenotype. Microsatellite markers are highly polymorphic, which allows the creation of unique genetic profiles for all grapevine cultivars. Moreover, they are inherited in a co-dominant Mendelian fashion, thus allowing the analysis of parentage. In spite of the high number of studies discriminating among varieties, reports for clonal identification within a single

Table 1 List of vineyards/plants analyzed at 22 SSR

Zone of sampling	Vineyard code	Number of plants analyzed	Vineyard age (years)
Syracuse	SR-1	10	>40
Syracuse	SR-2	10	>40
Syracuse	SR-3	10	>40
Syracuse	SR-4	10	>40
Syracuse	SR-5	3	<30
Syracuse	SR-6	3	<30
Syracuse	SR-7	3	<30
Syracuse	SR-8	3	<30
Syracuse	SR-9	3	<30
Syracuse	SR-10	3	<30
Syracuse	SR-11	3	<30
Syracuse	SR-12	3	<30
Syracuse	SR-13	3	<30
Syracuse	SR-14	3	<30
Caltanissetta	CL-1	3	<30
Caltanissetta	CL-2	3	<30
Caltanissetta	CL-3	3	<30
Caltanissetta	CL-4	3	<30
Caltanissetta	CL-5	3	<30
Caltanissetta	CL-6	3	<30
Caltanissetta	CL-7	3	<30
Caltanissetta	CL-8	3	<30
Trapani	TP-1	3	<30
Trapani	TP-2	3	<30
Trapani	TP-3	3	<30
Trapani	TP-4	3	<30
Trapani	TP-5	3	<30
Trapani	TP-6	3	<30
Trapani	TP-7	3	<30
Trapani	TP-8	3	<30
Total	30	118	

Table 2 List of microsatellite loci analyzed

Locus	Forward primer (5′-3′)	Reverse primer (5′-3′)	Ta (°C)*
VVS2	cagcccgtaaatgtatccatc	aaattcaaaattctaattcaactgg	56
VVMD5	ctagagctacgccaatccaa	tataccaaaaatcatattcctaaa	56
VVMD7	agagttgcggagaacaggat	cgaaccttcacacgcttgat	50
VVMD17	tgactcgccaaaatctgacg	cacacatatcatcaccacacgg	56
VVMD21	ggttgtctatggagttgatgttgc	gcttcagtaaaaagggattgcg	56
VVMD24	gtggatgatggagtagtcacgc	gattttaggttcatgttggtgaagg	56
VVMD25	ttccgttaaagcaaaagaaaaagg	ttggatttgaaatttattgagggg	56
VVMD26	gagacgactggtgacattgagc	ccatcaccaccatttctactgc	56
VVMD27	gtaccagatctgaatacatccgtaagt	acgggtatagagcaaacggtgt	56
VVMD28	aacaattcaatgaaaagagagagagaga	tcatcaatttcgtatctctatttgctg	56
VVMD31	cagtggtttttcttaaagtttcaagg	ctctgtgaaagaggaagagacgc	56
VVMD32	tatgatttttttaggggggtgagg	ggaaagatgggatgactcgc	56
VVMD34	ggtacatcagtacttgaaatggttgc	ttctccgtagaagcgtaaacagc	56
VVMD36	taaaataataataggggggacacggg	gcaactgtaaaggtaagacacagtcc	56
VrZAG7	gtggtagtgggtgtgaacggagtgg	aacagcatgacatccacctcaacgg	50
VrZAG12	ctgcaaataaatattaaaaaattcg	aaatcctcggtctctagccaaaagg	50
VrZAG15	ggattttggctgtagttttgtgaag	atctcaagctgggctgtattacaat	50
VrZAG21	tcattcactcactgcattcatcggc	ggggctactccaaagtcagttcttg	50
VrZAG25	ctccacttcacatcacatggcatgc	cggccaacatttactcatctctccc	50
VrZAG62	ggtgaaatgggcaccgaacacacgc	ccatgtctctcctcagcttctcagc	50
VrZAG67	acctggcccgactcctcttgtatgc	tcctgccggcgataaccaagctatg	50
VrZAG79	agattgtggaggagggaacaaaccg	tgcccccattttcaaactcccttcc	50

In bold, those loci showing polymorphism in this study

*Temperature of annealing

cultivar are limited (Regner et al. 2000; González-Techera et al. 2004; Moncada et al. 2005, 2006; Regner et al. 2006; Moncada and Hinrichsen 2007).

In the present paper, 22 nuclear microsatellites were utilized to assess the intra-varietal genetic diversity in samples of 'Nero d'Avola' collected in different vineyards located in three provinces of Sicily. For the first time, genetic diversity within the 'Nero d'Avola' variety is reported.

Materials and methods

Plant material

Plant material was collected in vineyards located in three provinces of Sicily (Syracuse, Caltanissetta and Trapani). A total of 30 vineyards were sampled, with 14 from Syracuse, 8 from Caltanissetta and 8 from Trapani. The oldest vineyards (more than 40 years) were located in Syracuse (SR-1, SR-2, SR-3 and SR-4), while all the other vineyards were between 10 and 30 years old (Table 1). Ten different plants were sampled for old vineyard whilst, in the other vineyards, 3 plants were chosen. Plants collected in the same vineyard were identified by different capital letters. A total of 118 plants were sampled for DNA extraction and microsatellite analyses (Table 1).

DNA extraction and microsatellite analysis

Total genomic DNA was extract from young leaves or inner wood from young cuts and stored at −80°C until used. The extraction was carried out by the Doyle and Doyle (1990) CTAB method and DNA was quantified in 1% agarose gels.

Microsatellite analysis was based on 22 markers, 13 of VVMD series (#5, 7, 17, 21, 24, 25, 26, 27, 28, 31, 32, 34 and 36), 8 of VrZAG series (#7, 12, 15, 21, 25, 62, 67 and 79) and VVS2 (Table 2) as proposed

Table 3 Genetic profiles of each vineyard (reported in bold)/plant (alphabetic list in bracket) at 11 polymorphic microsatellites analyzed

Genetic profile	Vineyard/plant number**	Number of plants (%)	VVMD5	VVMD5	VVMD17	VVMD17	VVMD26	VVMD26	VVMD28	VVMD28	VVMD31	VVMD31	VVMD36	VVMD36
I	**SR-1**(A, B, C, D, E, F, G, H, I, L); **SR-5**(A, B, C); **SR-8**(A, B, C); **SR-12**(A, B, C); **SR-14**(A, B, C); **TP-1**(A, B, C); **TP-3**(A, B, C); **TP-6**(A, B, C); **TP-7**(A, B, C); **TP8**(A, B, C); **CL-5**(A, B, C); **CL-6**(A, B, C); **CL-7**(A, B, C); **CL-8**(A, B, C)	41.5	220	234	215	215	245	247	231	239	212	212	244	268
II	**SR-7**(A, B, C); **SR-11**(A, B, C); **SR-13**(A, B, C)	7.6	220	234	215	215	245	247	231	239	212	212	244	268
III	**SR-6**(A, B, C); **SR-9**(A, B, C); **SR-10**(A, B, C)	7.6	220	234	215	215	**245**	**251**	**239**	**251**	212	212	244	268
IV	**TP-2**(A, B, C)	2.6	220	**222**	215	215	245	**245**	**239**	239	212	212	244	268
V	**CL-1**(A, B, C); **CL-2**(A, B, C)	5.1	220	220	215	215	247	251	239	239	212	212	244	268
VI	**CL-3**(A, B, C)	2.6	220	220	215	215	251	251	239	239	212	212	244	268
VII	**CL-4**(A, B, C)	2.6	220	234	215	215	237	251	239	239	212	212	244	268
VIII	**SR-2**(A, C, D, F, G, H, I, L)	6.7	220	234	205	215	237	247	231	231	212	212	244	268
IX	**SR-2**(E)	0.8	220	220	205	215	237	247	231	231	212	212	244	268
X	**SR-3**(A, B, D, E, F, G, H, I, L)	7.6	220	234	205	215	247	251	251	251	212	212	244	268
XI	**SR-3**(C)	0.8	220	234	205	215	247	251	251	251	212	212	244	268
XII	**SR-4**(A, B, C, D, E, F, G, H, I, L)	8.5	220	220	205	215	247	251	251	251	212	212	244	268
XIII	**TP-4**(A, B, C)	2.6	220	234	215	215	247	251	251	251	212	212	244	268
XIV	**TP-5**(A, B, C)	2.6	220	234	215	215	247	251	231	231	212	212	244	268
XV	**SR-2**(B)	0.8	220	234	205	215	247	251	231	231	212	216	264	268

Table 3 continued

Genetic profile	Vineyard/plant number**	Number of plants (%)	VrZAG7	VrZAG7	VrZAG21	VrZAG21	VrZAG25	VrZAG25	VrZAG62	VrZAG62	VrZAG67	VrZAG67
I	**SR-1**(A, B, C, D, E, F, G, H, I, L); **SR-5**(A, B, C); **SR-8**(A, B, C); **SR-12**(A, B, C); **SR-14**(A, B, C); **TP-1**(A, B, C); **TP-3**(A, B, C); **TP-6**(A, B, C); **TP-7**(A, B, C); **TP8**(A, B, C); **CL-5**(A, B, C); **CL-6**(A, B, C); **CL-7**(A, B, C); **CL-8**(A, B, C)	41.5	155	155	194	194	225	225	183	199	152	152
II	**SR-7**(A, B, C); **SR-11**(A, B, C); **SR-13**(A, B, C)	7.6	155	155	194	194	225	225	183	**183**	152	152
III	**SR-6**(A, B, C); **SR-9**(A, B, C); **SR-10**(A, B, C)	7.6	155	155	194	194	225	225	183	199	152	152
IV	**TP-2**(A, B, C)	2.6	155	155	194	194	225	225	183	199	152	152
V	**CL-1**(A, B, C); **CL-2**(A, B, C)	5.1	155	155	194	194	209	225	183	199	152	152
VI	**CL-3**(A, B, C)	2.6	141	155	194	194	225	225	199	199	152	152
VII	**CL-4**(A, B, C)	2.6	141	155	194	194	225	225	183	199	152	152
VIII	**SR-2**(A, C, D, F, G, H, I, L)	6.7	141	155	194	194	209	225	183	199	152	152
IX	**SR-2**(E)	0.8	141	155	194	194	209	225	183	199	152	152
X	**SR-3**(A, B, D, E, F, G, H, I, L)	7.6	141	155	194	194	209	225	199	199	152	152
XI	**SR-3**(C)	0.8	141	155	194	194	209	225	183	199	152	152
XII	**SR-4**(A, B, C, D, E, F, G, H, I, L)	8.5	141	155	194	194	209	225	183	199	152	152
XIII	**TP-4**(A, B, C)	2.6	141	155	194	194	209	225	183	199	152	152
XIV	**TP-5**(A, B, C)	2.6	141	155	194	194	209	225	183	199	152	152
XV	**SR-2**(B)	0.8	141	155	194	206	209	225	183	199	126	152

The different alleles between profiles *I*, *II*, *III* and *IV* are reported in bold/underline. The alleles status at the microsatellites not polymorphic in any plant analyzed is reported in footnote (*)

* VVS2 (137:145), VVMD7 (239:249), VVMD21 (241:249), VVMD24 (208:210), VVMD25 (243:253), VVMD27 (176:178), VVMD32 (248:248), VVMD34 (235:243), VrZAG12 (148:148), VrZAG15 (166:166), VrZAG79 (248:248)

** For the vineyards with more than one genotype, representative single profile/plants are reported

Table 4 Genetic parameters at the 11 polymorphic microsatellite loci

Locus	N° alleles	Allele size range (bp)	H_e*	H_o*	r**	PI[#]	PIC[##]	D_j***	Hom[a]	N° Het[b]	S[c]	H_t[d]
VVMD5	3	220–234	0.509	0.909	−0.264	0.586	0.0491	0.2254	9.1	2	0.75	0.50
VVMD17	2	205–215	0.165	0.182	−0.014	0.075	0.0984	0.3068	81.8	1	0.30	0.16
VVMD26	4	237–251	0.674	0.940	−0.158	0.297	0.4204	0.7121	6.1	6	1.20	0.67
VVMD28	3	231–251	0.622	0.606	0.009	0.379	0.3070	0.6951	39.4	3	0.80	0.55
VVMD31	2	212–216	0.029	0.030	−0.001	0.942	0.0265	0.0606	96.9	1	0.08	0.03
VVMD36	3	244–268	0.514	1.000	−0.320	0.588	0.0440	0.0606	–	2	0.76	0.51
VrZAG7	2	141–155	0.257	0.303	−0.036	0.651	0.1244	0.4356	69.7	1	0.42	0.26
VrZAG21	2	194–206	0.029	0.030	−0.001	0.942	0.0265	0.0606	96.9	1	0.08	0.03
VrZAG25	2	209–225	0.257	0.303	−0.036	0.651	0.1244	0.4356	69.7	1	0.42	0.26
VrZAG62	2	183–199	0.499	0.848	−0.232	0.624	0.0009	0.2765	15.6	1	0.96	0.58
VrZAG67	2	126–152	0.029	0.030	−0.001	0.942	0.0265	0.0606	96.9	1	0.08	0.03
All Loci	27	–	–	–	–	6.35×10^{-3}	–	–				
Mean	2.46		0.326	0.471		0.607	0.1134	0.3027	52.9	1.82	0.53	0.32

* 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; [c] Shannon's index; [d] Nei's genetic diversity

by the GENRES 081 Project (European Vitis Database, www.genres.de/vitis/vitis.htm). The forward primer of each marker was labeled with one of the three unique ABI PRISM fluorescent dyes: 6-FAM, JOE, TAMRA. PCR amplification was performed using the Qiagen multiplex PCR kit with the following conditions for all markers: 5 min at 95°C (HotStarTaq DNA Polymerase activation step) followed by 35 cycles consisting of 60 s at 94°C (denaturation), 60 s at 50–56°C (annealing temperatures, Table 2), 2 min at 72°C (extension). In the last cycle, extension time at 72°C was increased to 7 min. Each sample was amplified at least twice to correct for possible mistyping or amplification errors. PCR products were size-separated by capillary electrophoresis performed on a genetic analyzer (ABI Prism 3130, Applied Biosystems, Inc.).

Data analysis

For each microsatellite locus, several parameters were analyzed: the number of alleles per locus (n) and their frequency; the observed (H_o) and expected (H_e) heterozygosities; the screening ability, based on the probability of identity (PI) (Paetkau et al. 1995) and the polymorphic information content (PIC) (Weber 1990) calculated as reported in Carimi et al. (2010). The best microsatellites were those displaying high H_o and PIC and low PI values. Allele frequencies, H_e and H_o, 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). Finally, the discrimination power (D_j) of each microsatellite was 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 analyzed.

Based on microsatellite data, Simple Matching (SM) coefficients were estimated with the SIMQUAL program from NTSYS-pc ver. 2.02j package (Exeter Software, Setauket, NY). 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 the TREE program of the NTSYS-pc ver. 2.02j package (Rohlf 1998). Total genetic diversity of Nei (Ht) (Siegfried 2000) and Shannon's index (S) (Gryta et al. 2005) were used to summarize

the data and their standard deviations. All calculations and analyses were conducted using POPGENE program version 1.31 (Yeh et al. 1999). The genetic relationship among 'Nero d'Avola' clones was further investigated by principal coordinate analysis (PCoA) by using the GenAlEx 6 program (Peakall and Smouse 2006).

Results and discussion

The molecular analysis on 118 plants of 'Nero d'Avola' (coming from 30 different vineyards) showed that 11 out of 22 loci (50%) were polymorphic and revealed 15 different genetic profiles. Polymorphic and common genetic profiles are reported in Table 3. Polymorphisms at different loci were observed among vineyards and in two cases (SR-2 and SR-3) genetic differences were detected within the same vineyard (Table 3). In particular, the vineyard SR-2 showed three different genetic profiles (*VIII*, *IX*, *XV*), with one (profile *VIII*) characterizing eight out of ten plants; in the same way, the genetic profile *X* was prevalent in vineyard SR-3 (nine out of ten plants). The most frequent genetic profile (*I*) was carried by samples coming from all provinces and seems to be the main genetic profile describing the 'Nero d'Avola' cultivar group.

The 11 polymorphic loci led to the detection of a total 27 polymorphic alleles (Table 4). 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), Shannon's index, Nei's genetic diversity are reported (Table 4).

The number of observed alleles per locus was not high, as expected for clones of the same variety. In particular, the highest information content was provided by locus VVMD26 with four different alleles, while only three were observed in VVMD5, VVMD28 and VVMD36; only two alleles were observed for seven microsatellites (VVMD17, VVMD31, VrZAG7 VrZAG21 VrZAG25, VrZAG62 and VrZAG67) (Table 4). The observed heterozygosity (H_o) ranged from 0.03 for VVMD31, VrZAG21 and VrZAG67 to 1 for VVMD36. The mean of H_o was 0.471, slightly higher than expected by random sorting of gametes (0.326). This result agrees with what reported by Sefc et al. (2000), where a certain level of heterozygosity is explained as a consequence of both natural and human selection against homozygosity in grapevine plants. Estimated frequency of null alleles (r) was negative for ten out of 11 loci. However, r parameter developed by Brookfield (1996) estimates null allele frequency in the case of panmixya rather than breeding selection (Costantini et al. 2005). The probability of identity (PI) ranged from 0.075 to 0.942; the highest value was provided by loci VVMD31, VrZAG21 and VrZAG67. However, the probability to find different plants with the same profile at whole loci was low ($PI = 6.35 \times 10^3$).

The major discrimination power (D_j) was found for locus VVMD26 (71%) and the lowest (6%) for

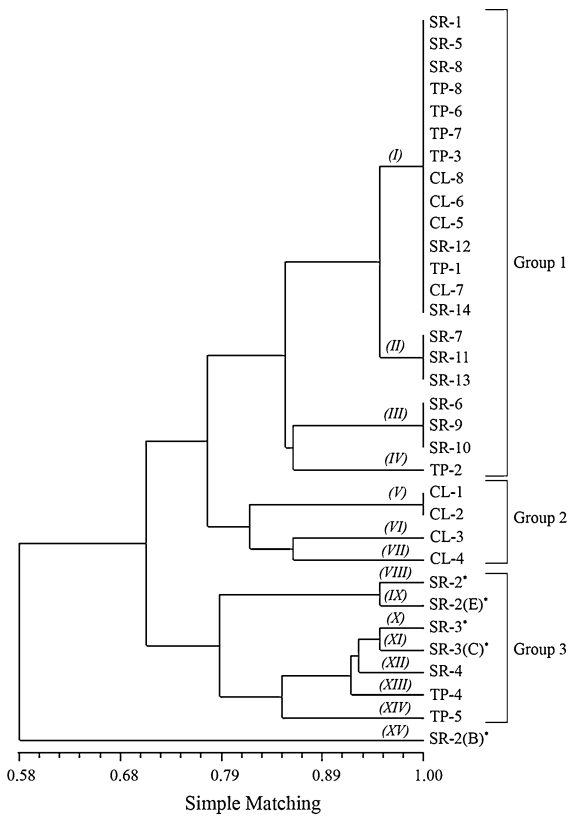

Fig. 1 Dendrogram of genetic relationships among 118 plants, sampled in 30 different vineyards, based on 11 genomic microsatellites. Genetic distances were estimated by simple matching (*SM*) coefficient and UPGMA cluster analyses were adopted using the SAHN-clustering and TREE program of the NTSYS-pc ver. 2.02j package (Rohlf 1998). The three main groups and the genetic profiles (from *I* to *XV*) are shown (plants with different genetic profiles but coming from the same vineyards are indicated with *asterisks*)

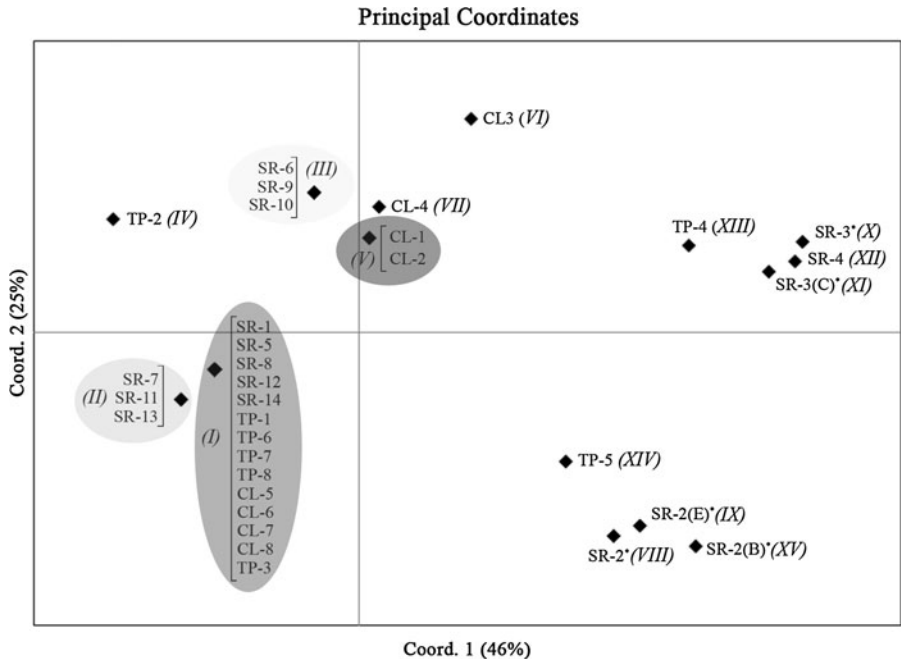

Fig. 2 Principal Coordinate Analysis (*PCoA*) based on genetic profiles from microsatellite analysis on 118 grapevine plants, coming from 30 vineyards. The *first axis* accounts 46% of the genetic variation while the *second axis* 25%. Genetic profiles are shown in *brackets* (plants with different genetic profiles coming from the same vineyards are indicated with *asterisks*)

loci VVMD31, VVMD36, VrZAG21 and VrZAG67, with a mean of 30% for all loci, indicating a low efficient discrimination of the genotypes, as expected for a single cultivar. Finally, an additional measure of genetic variation was obtained using Shannon's index (S) and Nei's genetic diversity (Ht) with means of 0.53 and 0.32, respectively.

The dendrogram of genetic relationships among the clones, based on simple matching (SM) coefficient and UPGMA algorithm, showed 15 different genetic profiles clustered in three groups and one outgroup, as reported in Fig. 1. The most abundant and diffuse genetic profile (*I*) was present in 14 vineyards and suggests a genetic relationships among plants of 'Nero d'Avola' sampled in different areas of Sicily. A larger group including about 60% of plants sampled, coming from 21 vineyards, showed >85% of similarity (profiles *I–IV*). The genetic profile *II* differed from *I* only for one allele at locus VrZAG62 (183:183 instead of 183:199), while the genetic profile *III* differed from *I* for one allele at locus VVMD26 (245:251 instead of 245:247) and one at locus VVMD28 (239:251 instead of 231:239). Profiles *I* and *IV* differed at the three loci VVMD5, VVMD26 and VVMD28. Moreover, four vineyards of Caltanissetta (showing profile *V*, *VI* and *VII*) were sorted in a second small group rather close to the main one. This observation suggests a similar origin of the samples harbouring profiles *I–VII* with the presence of limited events of mutation.

Plants collected in the oldest vineyards (SR-2, SR-3 and SR-4, more than 40 years old) together with two vineyards of Trapani (TP-4 and TP-5) differed from the major group (profiles *I*, *II, III* and *IV)* described above, with a similarity level of 0.79 (Fig. 1). Only one plant (SR-2B), sampled in the oldest vineyard of Syracuse (SR-2) seemed to be an outgroup, clearly distinguished from the other clusters, displaying a heterozygous status at three loci (VVMD31, VrZAG21 and VrZAG67) in contrast with any other plants (Table 3) and a diallelic status at the locus VVMD36 (alleles 264:268), different from the genotype (244:268) observed in all other plants. This sample (SR-2B), collected in the most ancient vineyard (48 years old), might even not be a real 'Nero d'Avola' plant, taking into account that farmers in the past used to cultivate 'Nero d'Avola' mixed with plants belonging to the 'Nerello' group of red berry cultivars.

Genetic relationships among genotypes are well highlighted also by PCoA analysis (Fig. 2), where the main group including the genetic profiles *I*, *II*, *III* and *IV* (coming from 21 out of 30 vineyards analyzed) was maintained; in particular, the coordinate 1 (46%) was able to distinguish these profiles from the others.

Conclusion

This is the first report on genetic characterization of 'Nero d'Avola', the most important red berry grape cultivar in Sicily. The molecular analysis of 118 plants, proceeding from 30 vineyards, revealed 15 different genetic profiles. Among them, profile *I* was prevalent (41.5%) and present in samples coming from all the studied provinces. Eleven out of 22 microsatellite markers utilized in the present study were highly informative among the analyzed clones of 'Nero d'Avola' cultivar group. According to these results, most of the studied plants/vineyards are characterized by close kinship; only one plant seemed to be genetically distant from the others, and this observation suggests either a different origin or its belonging to a different group of red berry. No correlation was found between genetic clustering and sampling areas. Ampelographic studies and phenotypic descriptions might be used to complement the genetic data of this famous cultivar.

Acknowledgments This research was partly supported by a grant from "Assessorato Agricoltura e Foreste, Regione Sicilia" in the frame of the project "Caratterizzazione tecnologica e genetica di microrganismi autoctoni ed interazione con i migliori cloni dei vitigni 'Nero d'Avola' ed Inzolia per migliorare alcune produzioni vitivinicole tipiche della Regione Sicilia" (Project Leader Prof. Patrizia Romano—Università degli Studi della Basilicata) and by the grant from the Italian Ministry of the Research in the frame of the project 'Tracciabilità della filiera vitivinicola' (OR 2.1.2—CISIA DGLS 191/2009).

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