GENETIC CHARACTERIZATION OF SARDINIA GRAPEVINE CULTIVARS BY SSR MARKERS ANALYSIS

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Abstract

Aim: The objective of the present study is to investigate the molecular characteristics of Sardinian grapevine cultivars to evaluate cases of synonyms and false attributions to protect local agro-biodiversity.

Methods and results: The SSR analysis (13 loci) has been used to define the DNA fingerprint and the relationships with Sardinian grapevine cultivars. Results highlighted a high genetic variability among the accessions, with the Dice coefficients performing from 0 to 0.8. Despite the genetic richness, thirteen groups of redundant genotypes were detected. Molecular analysis refers of cultivars harbouring the same SSR profile but different berry colours such as cultivars Licronaxu Bianco and Nero and Moscatello Bianco and Nero. It could by hypothesized that Licronaxu and Moscatello could derive from a specific retrotransposon-induced mutation event in genes regulating anthocyanin biosynthesis.

Conclusion: Sardinian germplasm has a real problem of cultivar identification probably due to different factors such as the absence of an exhaustive ampelography, problems in the language to name varieties and the existence of cultivars sensitive to biotic and abiotic stresses producing evident morphological modifications leading to mistakes in recognising and identifying properly the affected plants. However, our molecular results suggest that high grape-biodiversity is still preserved in this region.

Significance and impact of study: Results of this work clarified the relationships among grapevine cultivars and provided a solid basis to improve a regional grapevine collection.

Key words: biodiversity, molecular tool, nuclear SSR, Sardinia, *Vitis vinifera*

Abbreviation: SSR = Simple Sequence Repeat

Résumé

Objectif : L'objectif de cette étude est d'analyser les caractéristiques des vignes de la Sardaigne pour évaluer les cas de synonymies et d'identifications erronées pour protéger l'agro-biodiversité locale.

Méthodes et résultats : L'analyse SSR (13 loci) a été utilisée pour caractériser les empreintes génétiques et les relations entre les cépages de Sardaigne. Les résultats mettent en évidence une grande variabilité entre les apports avec des coefficients de Dice compris entre 0 et 0.8. Malgré cette richesse génétique, treize groupes de génotypes redondants ont été identifiés dans les échantillons analysés. Les analyses moléculaires suggèrent que des cépages ont le même profil SSR, mais des baies de couleurs différentes, comme le Licronaxu blanc et noir et le Moscatello blanc et noir. On peut supposer que ces derniers serait le résultat d'une mutation spécifique induite par retrotrasposon dans les gènes de régulation de la biosynthèse des anthocyanines.

Conclusion : Le germoplasme Sarde présente un problème d'identification des cépages, probablement en raison de différents facteurs, comme l'absence d'une ampélographie complète, l'utilisation de langues et de dialectes divers pour dénommer les variétés et l'existence de cultivars sensibles aux stress abiotiques et biotiques qui modifient la morphologie, cause d'identifications erronées. Nos analyses moléculaires permettent de conclure qu'une biodiversité élevée de la vigne est encore préservée dans cette région.

Signification et impact de l'étude : Les résultats du travail clarifient les relations entre les variétés cultivées et fournissent une base solide pour améliorer la collection régionale de la vigne.

Mots clés : biodiversité, moléculaire marqueur, SSR nucléaire, Sardaigne, *Vitis vinifera*

manuscript received: 11th December 2006 - revised manuscript received: 20th September 2007

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J. Int. Sci. Vigne Vin, 2007, **41**, n°4, 175-184

Table 1 - List of the 61 Sardinian traditional cultivars analyzed in this work. Their cultivation area, cultivation state and berry color are listed.

Cultivars	Area of cultivation	Cultivation Scale	Berry Colour	Cultivars	Area of cultivation	Cultivation Scale	Berry Colour
Aregu Seulo	Barbagia of Seulo	+ +	W	Moscato – Lodine	Lodine – Barbagia of Ollolai	+ +	W
Aregu Biancu	Barbagia of Seulo	+ +	W	Moscato - Modolo	Modolo - Planargia	+ +	W
Aregu Giallo	Barbagia of Seulo	+ +	W	Moscato – Pattada	Pattada - Logudoro	+ +	W
Argu Mannu	Sardinia	+ +	W	Moscato - su Pinu	Mamoiada - Barbagia of Ollolai	+ +	W
Bianca Tomentosa	Planargia	+ +	W	Moscato 1 - Tempio	Tempio - Gallura	+ +	W
Bovale	Modolo -Planargia	+ + +	B	Moscato 2 - Tempio	Tempio - Gallura	+ +	W
Cannonau	Sardinia	+ + +	B	Moscato 3 - Tempio	Tempio - Gallura	+ +	W
Cannonau Bianco - Oliena	Oliena - Barbagia of Ollolai	+ +	W	Nera Pelosa	Planargia	+ +	B
Cannonau Bianco - Triei	Triei - Ogliastra	+ +	W	Nera Tomentosa	Planargia	+ +	B
Cannonau Nero	Sestu - Campidano	+ +	B	Nero - Bosa	Planargia	+ +	B
Culupuntu	Ogliastra	+ +	W	Nieddu Mannu - Padria	Padria - Logudoro	+ +	B
Falso Gregu	Campidano	+	B	Nieddu Mannu - Pattada	Pattada - Logudoro	+ +	B
Girò - Gonnos	Gonnosfanadiga - Arburese	+ +	B	Nieddu Pedra Serra	Arburese	+ +	B
Granaccia	Oliena - Barbagia of Ollolai	+ +	W	Nieddu Polchinu	Pattada - Logudoro	+ +	B
Granazza	Mamoiada - Barbagia of Ollolai	+ +	W	Nuragus	Sardinia	+ + +	W
Gregu Bianco	Campidano	+ +	W	Nuragus Arrubiu	South Sardinia	+ +	W
Gregu Nieddu - Campidano	Campidano	+ +	B	Nuragus Moscadeddu	Quirra-Ogliastra	+ +	W
Gregu Nieddu - Serramanna	Serramanna - Campidano	+ +	B	Nuragus Moscatello	Nurri - Sarcidano	+ +	W
Licronaxu Bianco	Campidano Oristano	+ +	W	Nuragus Rosso Rompizolla	South Sardinia	+ +	W
Licronaxu Nero	Campidano Oristano	+ +	B	Pascale - Cagliari	Sardinia	+ + +	B
Manzesu	Campidano	+ +	B	Pascale - Oliena	Oliena - Sardinia	+ +	B
Monica	Sardinia	+ + +	B	Pascale - Padria	Padria - Logudoro	+ +	B
Monica - Escalaplano	Escalaplano - Quirra-Ogliastra	+ + +	B	Picciolo Rosso	Sardinia	+ +	B
Monica - Nurri	Nurri - Sarcidano	+ + +	B	Primidivu Nieddu	Padria -Logudoro	+ +	B
Monica - Seulo	Seulo – Barbagia of Seulo	+ + +	B	Sinnidanu	Dorgali - Baronia	+ +	W
Monica - Sorgono	Sorgono - Mandrolisai	+ + +	B	Vernaccia - Escalaplano	Escalaplano - Quirra-Ogliastra	+ +	W
Moscatello - Bianco	Sardinia	+ +	W	Vernaccia	South Sardinia	+	W
Moscatello - Modolo	Modolo - Planargia	+ +	W	Vernaccia – S. Rosalia	Triei - Ogliastra	+ +	W
Moscatello - Murrunzone	Mamoiada – Barbagia of Ollolai	+ +	W	Vernaccia - Solarussa	Campidano of .Oristano	+ +	W
Moscatello Nero	Sulcis	+ +	B	Vertudi	Campidano	+	B
Moscatellone	Sardinia	+ +	W				

+++ largely cultivated; ++ locally cultivated; + cultivated only in CRAS germplasm collection.

INTRODUCTION

Italy is characterized by a rich, complex and diversified viticultural heritage (ALLEWELDT, 1997; CALO *et al.*, 2002), however, in the last years the majority of this rich platform became on the brink of extinction, principally in reason of the selection of few accessions for wine production as an answer to market requirements. As a consequence, cultivars of minor economical interest have been abandoned favouring international varieties such as Cabernet Sauvignon, Chardonnay, Merlot, etc. Despite economics, ancient typical varieties have a longstanding and interesting history related to the places where they were usually grown, becoming well integrated in the landscapes, in the traditional agricultural practices and in the history of the inhabitants (CALO *et al.*, 2002).

In order to protect grapevine biodiversity and to improve Italian viticulture and oenology an extensive investigation on Italian local varieties is required. In this frame, Sardinia (Italy), the second largest islands in the Mediterranean sea, represents an interesting Italian region where to perform a similar investigation. The spatial isolation of the island harboured and protected the genetic richness of grapevine accessions limiting the opportunities of contamination from out-coming material. Sardinia is characterized by a huge number of cultivars, performing different morphological and chemical characteristics (CASTIA *et al.*, 1992; CALO *et al.*, 2002), traditionally considered as local varieties. This variability could be the result of an heterogeneous origin characterizing the Sardinian grapevine platform. Some of these cultivars are considered to be the product of different breeding events, such as direct domestications from local wild grapes (GRASSI *et al.*, 2003) and others could have been imported from elsewhere (REALE *et al.*, 2006). The Sardinian grapevine germplasm could also have been enriched through breeding events among local varieties and wild plants present in the island spontaneous flora (BAKELS, 2002; GRASSI *et al.*, 2003). This is the results of the complex history of Sardinian island, colonized from different populations each with their specific grapevine varieties and viticultural techniques. During the Iron Age the island was interested from Phoenician commercial networks (DAVISON, 1990; NEGBI, 1992). Around 500 B.C. Sardinia was a Carthage colony, thereby entering in the Punic period (BAKELS, 2002) and starting from 238 B.C. the Island was a part of the Roman Empire. Subsequently the island was invaded by Mediterranean civilizations belonging to nations such as Spain (BLÁZQUEZ, 2000). We can suppose that invaders influenced the island viticulture by new introductions (CRESPAN *et al.*, 2006), improving the constitution of the local germplasm, there where inhabitants may have, in the mean time, already started the domestication and cultivation and of local wild grapes.

Despite the large and complex grapevine platform, a lot of Sardinian cultivars were unknown and only few varieties were commonly used for wine production. To evaluate Sardinian grapevine biodiversity and to protect the minor varieties a suitable characterization of Sardinian grapevine germplasm is necessary. The availability of molecular tools to screen biodiversity among plant genomes (KARP *et al.*, 1998) provides an answer to these needs. Specifically in grapevines, SSR (Simple Sequence Repeat) analysis has been successfully used in genotyping (BOWERS *et al.*, 1996, 1999; MERDINOGLU *et al.*, 2005) and in the evaluation of genetic relationships among cultivars (SEFC *et al.*, 2001; LABRA *et al.*, 2003; COSTANTINI *et al.*, 2005; LADOUKAKIS *et al.*, 2005).

The objective of the present study is to investigate the molecular characteristics of local cultivars growing in the Sardinia Island to evaluate cases of synonyms and false attributions aiming to provide a solid basis to develop a regional germplasm collection to protect local agro-biodiversity.

MATERIALS AND METHODS

Plant material and morphological characterization

The 61 grapevine cultivars (*Vitis vinifera* subsp. *vinifera* L.) listed in table 1 were obtained from CRAS (Centro Regionale Agrario Sperimentale), Sardinia, Italy. They include minor and major local cultivated varieties, as well as ancient varieties from CRAS germplasm collections. Table 1 lists names, berry colours, areas of cultivation and a scale of cultivation for each variety.

DNA extraction and SSR analysis

Young leaflets were collected from rooted cuttings, frozen in liquid nitrogen and ground to fine powder. Genomic DNA was extracted from this powder as described by LABRA (2001). Samples were genotyped at 13 microsatellite loci: VVS2 (THOMAS and SCOTT, 1993), VVMD5, VVMD7 (BOWERS *et al.*, 1996), VVMD21, VVMD24, VVMD25, VVMD27 (BOWERS *et al.*, 1999), VrZAG21, VrZAG47, VrZAG62, VrZAG64, VrZAG67 and VrZAG79 (SEFC *et al.*, 1999). PCR was performed in a volume of 20 µl containing 10-20 ng of genomic DNA, 1X reaction buffer (200 mM Tris-HCl pH 8.0, 500 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µMol of each primer. PCRs were carried out in a PTC 100 thermal cycler (MJ Research Inc.), under the following cycling conditions: 2 min at 94 °C, followed by 35 cycles of 30 sec at 94 °C, 30 sec at 50 °C and 60 sec at 72 °C; and a final hold of 30 min at 72 °C. The forward primer, in each pair, was labeled with a fluorescent dye (6-FAM, VIC, NED, and PET). The fragment analysis was performed on an ABI 310 Genomic Analyser (Applera) using the POP-4 polymer

and a 47 cm-long capillary; allele sizing was performed with the GeneMapper software 3.7 (Applera).

Statistical analysis

Each microsatellite allele was scored as a binary character for its absence (0) or presence (1) for all the 61 samples. Obtained data were analysed using the software NTSYSpc. A matrix of similarities, based on the Dice index (SNEATH and SOKAL, 1973), was computed and used to draw a UPGMA tree.

The software IDENTITY (WAGNER and SEFC, 1999) was used to calculate the number of alleles (n), the expected (He) and observed (Ho) heterozygosity, the estimated frequency of null alleles (r) and the probability of identity (P.I.).

RESULTS

SSR markers analysis

A total of 13 SSR markers have been used to characterize Sardinian grapevine germplasm. Table 2 shows the allele profiles obtained for each accession at the analyzed SSR loci. Number of alleles (N), allele size range, expected (He) and observed (Ho) heterozygosity, frequencies of null alleles (r) and probability of identity (P.I.) of all genotypes are listed for each locus in table 3.

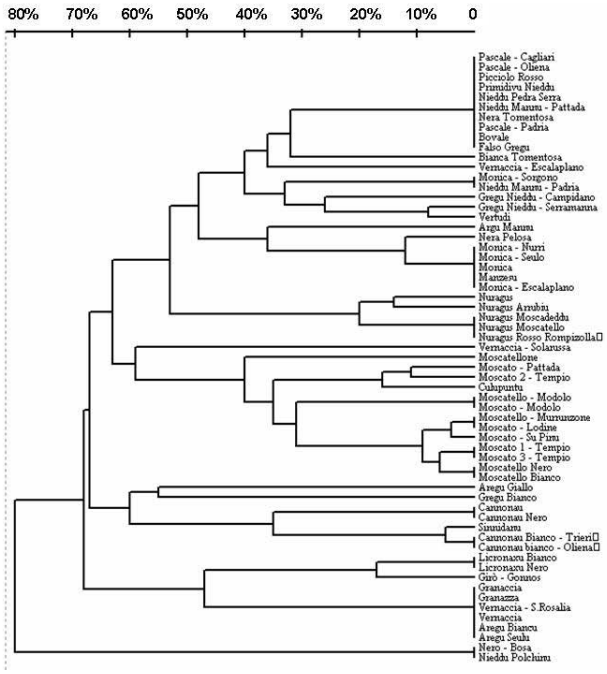

Figure 1 - Dendrogram based on the Dice similarity index, showing the genetic relationship among 61 Sardinian cultivars as determined by SSR analyses.

The number of alleles detected ranged from 4 (VVMD24) to 11 (VrZAG67), with a total of 98 alleles overall loci with an average value of 7.5 alleles per locus. These SSR markers have already been used in other sets of grapevine cultivars (SEFC *et al.*, 2001; MARTIN *et al.*, 2003) however this is the first application for the characterization of Sardinian germplasm. In general the level of polymorphism detected at the 13 analyzed loci was high and confirmed data detected in previous investigations (MARTIN *et al.*, 2003; ROSSONI *et al.*, 2003; THIS *et al.*, 2004).

The expected and observed heterozygosity values were very high, ranging from 0.70 to 0.90, with the exception of VVMD24 where only four alleles were detected and the expected and observed heterozygosity values were lower. The estimated frequencies of null alleles (r) was very low, mostly negative, with the only exceptions of VVMD7 and VVMD24 loci. The positive value detected for those two SSRs has not to be considered necessarily as the existence of null alleles but, only an indication of this possibility (SEFC *et al.*, 1998).

Values of P.I. ranged between 0.072 and 0.362, almost near and higher than the value of 0.05 at which a grapevine microsatellite is considered hyper polymorphic (SEFC *et al.*, 2001).

The high number of deteceted alleles for each SSR locus and the low P.I (P.I.=5.063 ·10-11) supports, once again, the high descriptive power of the chosen markers for the investigation of grapevines.

Analysis of synonyms and false attributions

The visual inspection of table 2 and the IDENTITY software were used to define synonyms and false attributions. A total of 13 groups of redundant genotypes were detected in the analysed germpasm at the 13 investigated SSR loci.

The first group of synonyms (group 1) is composed by Picciolo Rosso, Primidivu Nieddu, Pascale - Cagliari, Nera Tomentosa, Pascale - Oliena, Pascale - Padria, Falso Gregu, Nieddu Pedra Serra and Nieddu Mannu - Pattada. All these varieties proved to have the same SSR profiles and their identities were also confirmed by the sharing of common morphological traits such as: large bunch (more or less loose), great pentalobate tomentose leaves and the berry skin color, ranging from red-blue to dark blue. Basing on these results we can consider these accessions as genetic synonyms of the cultivar named Pascale.

The second group is composed from the Monica samples (group 2) with the exclusion of Monica - Sorgono. Monica varieties are all characterized by being black varieties, with a large bunch and pentalobate leaves,

performing characteristics suitable in the production of different kind of red wines. Monica is a cultivar largely diffused in Sardinia, usually the in the variety name the term Monica s followed by a name indicating the cultivation area, or the place of supposed origin. Our molecular investigation suggests a common genotype for all accessions thus a common place of origin. Referring to our data, cultivar known as Monica - Sorgono has to be considered as an incorrect identification (wrong name) because this accession performs common SSR profile and morphological traits with Nieddu Mannu - Padria (group 3 of table 2).

In the case of Nuragus (group 4) only 3 accessions, Nuragus Moscadeddu, Nuragus Rosso Rompizzolla and Nuragus Moscatello, proved to have identical SSR profiles while Nuragus Arrubiu and Nuragus showed few different alleles.

Another interesting example of synonyms (group 5) is represented from the Vernaccia group: Vernaccia S. Rosalia, Vernaccia, Aregu Biancu, Aregu Seulo, Granazza, and Granaccia. These varieties share common morphological traits such as the white berry colour, no hairy leaves (nor erect or prostrate), yellow colour woody shoots. In addition the names Vernaccia - Granaccia - Granazza are very similar and in Sardinia Island are commonly used to define the same varieties. However Vernaccia - Escalaplano and Vernaccia - Solarussa showed different SSR profiles suggesting that the name Vernaccia is related to a group of varieties showing similar morphological traits such as berry colour but not always with a common genetic constitution.

Two different cases of identity were observed between the two black Cannonau accessions (Cannonau Nero and Cannonau) and between the two white Cannonau cultivars (Cannonau Bianco - Triei and Cannonau Bianco - Oliena) of group 6 and 7, respectively. The comparison between white and black Cannonau cultivars showed that these two groups share 50 % of alleles, suggesting a direct relationship. Other synonyms were described in table 2.

Group 12 and 13, composed respectively from Moscatello Nero - Moscatello Bianco and Licronaxu Nero - Licronaxu Bianco are examples of varieties showing different berry color but common SSR profiles.

Genetic relationships among cultivars

Basing on SSR markers an UPGMA analysis was performed. The resulting dendrogram (figure 1) defines the genomic relationships among analyzed cultivars. The Dice's coefficient among cultivars varies from 0 (full genomic similarity) to 0,8 (high genomic dissimilarity) thus demonstrating the high polymorphism of the analyzed genotypes. The synonym groups are evidenced in the dendrogram (figure 1) as all the synonym varieties share the same branch of the tree. Homonyms (cultivars showing the same name but a different genetic constitution), such as some Moscato accessions, are also clearly scorable in the dendrogram.

DISCUSSION

SSR markers have already been used to solve cases of homonyms and synonyms (FOSSATI *et al.*, 2001), to fingerprint varieties, for germplasm exploitation, in the definition of conservation strategies (ROSSONI *et al.*, 2003; MORAVCOVA *et al.*, 2006), and in the identification or confirmation of pedigrees for grapevine varieties (SEFC *et al.*, 2001). In this specific case, the analysis identified a high genetic variability among the Sardinian accessions, however also a lot of synonyms or close related varieties were observed. The 61 accessions analyzed produced only 33 distinct SSR profiles. This suggest that Sardinian germplasm has a real problem of cultivar identification due to different factors. Primarily, the absence of an extensive ampelographic investigation. This lack does not allow to identify synonyms and false attributions at the morphological level in the proper way. This situation is also complicated by the existences of different languages, commonly known as dialects and used by populations living in different places in the Island. Giving rise, during the centuries, to a consistent number of different words describing the same thing (MORAL *et al.*, 1994; CAVALLI-SFORZA *et al.*, 1994). Dialectal differences lead to the production of distinct names used to describe the same grapevine cultivar. An example is the case of Granazza-Granaccia-Vernaccia, indicating a white fine wine and the grape from which it is produced. Our SSR analysis showed a common SSR profile for Granazza, Granaccia, Vernaccia S. Rosalia and Vernaccia - Oristano, confirming that, in Sardinia, these names are used for the same cultivar. On the other hand the comparison of morphological characteristic and of SSR profiles among the Sardinian Vernaccia - Garnaccia (Group 5) with the Spanish accessions of Garnacha (MARTIN *et al.*, 2003) highlights clearly the existence of differences. This indicates that Spanish Garnacha has not to be confused with the Sardinian Vernaccia - Garnaccia despite the similarities in their names. In the mean time we must stress that also in Sardinia some of the Vernaccia accessions (Vernaccia - Escalaplano and Vernaccia - Solarussa) harbour differences in their genetic constitution.

Previous considerations and results enable us to conclude that similar names for Sardinian cultivars are not always good indicators for having identical genotypes. An extensive DNA analysis should by used to clarify synonyms and homonyms among semantically closely related accessions.

Table 2 - Genetic profile of 61 Sardinian varieties analysed at 13 SSR loci. Allele size are given in base pairs. First colum indicate the group of accession considered synonyms.

	Campione	VVS2		VVMD5		VVMD7		VVMD27		ZAG62		ZAG79		VVMD21		VVMD24		VVMD25		ZAG21		ZAG47		ZAG64		ZAG67	
G1	Picciolo Rosso	145	151	226	234	244	250	179	181	188	204	248	262	245	245	205	205	238	260	202	204	155	157	136	159	127	134
	Primidivu Nieddu	145	151	226	234	244	250	179	181	188	204	248	262	245	245	205	205	238	260	202	204	155	157	136	159	127	134
	Pascale - Cagliari	145	151	226	234	244	250	179	181	188	204	248	262	245	245	205	205	238	260	202	202	155	157	136	159	127	134
	Nera Tomentosa	145	151	226	234	244	250	179	181	188	204	248	262	245	245	205	205	238	260	202	204	155	157	136	159	127	134
	Pascale - Oliena	145	151	226	234	244	250	179	181	188	204	248	262	245	245	205	205	238	260	202	204	155	157	136	159	127	134
	Pascale - Padria	145	151	226	234	244	250	179	181	188	204	248	262	245	245	205	205	238	260	202	204	155	157	136	159	127	134
	Bovale	145	151	226	234	244	250	179	181	188	204	248	262	245	245	205	205	238	260	202	204	155	157	136	159	127	134
	Falso Gregu	145	151	226	234	244	250	179	181	188	204	248	262	245	245	205	205	238	260	202	202	155	157	136	159	127	134
	Nieddu Pedra Serra	145	151	226	234	244	250	179	181	188	204	248	262	245	245	205	205	238	260	202	204	155	157	136	159	127	134
	Nieddu Mannu -Pattada	145	151	226	234	244	250	179	181	188	204	248	262	245	245	205	205	238	260	202	204	155	157	136	159	127	134
G2	Manzesu	143	151	222	234	240	250	179	193	188	204	258	262	239	245	205	213	252	260	198	204	155	170	136	140	121	127
	Monica - Escalaplano	143	151	222	234	240	250	179	193	188	204	258	262	239	245	205	213	252	260	198	204	155	170	136	140	121	127
	Monica - Nurri	143	151	222	234	240	250	179	193	188	204	258	262	239	245	205	213	252	260	198	204	155	170	136	140	121	127
	Monica - Seulo	143	151	222	234	240	250	179	193	188	204	258	262	239	245	205	213	252	260	198	204	155	170	136	140	121	127
	Monica	143	151	222	234	240	240	179	193	188	204	258	262	239	245	205	213	252	260	198	204	155	170	136	140	121	127
G3	Monica - Sorgono	133	145	226	234	240	250	179	193	188	188	258	262	239	245	205	205	238	238	198	204	155	170	140	142	134	146
	Nieddu Mannu - Padria	133	145	226	234	240	250	179	193	188	188	258	262	239	245	205	205	238	238	198	204	155	170	140	142	134	146
G4	Nuragus Moscatello	133	145	234	242	248	248	179	185	190	204	242	262	245	251	205	205	238	260	188	204	155	161	140	159	121	134
	Nuragus Rosso Rompizolla	133	145	234	242	248	248	179	185	190	204	242	262	245	251	205	205	238	260	188	204	155	161	140	159	121	134
	Nuragus Moscadeddu	133	145	234	242	248	248	179	185	190	204	242	262	245	251	205	205	238	260	188	204	155	161	140	159	121	134
G5	Vernaccia - S. Rosalia	143	151	226	240	248	248	179	179	194	204	252	260	239	251	205	205	252	252	200	202	155	155	140	194	121	150
	Vernaccia	143	151	226	240	248	248	179	179	194	204	252	260	239	251	205	205	252	252	200	202	155	155	140	194	121	150
	Aregu Biancu	143	151	226	240	248	248	179	179	194	204	252	260	239	251	205	205	252	252	200	202	155	155	140	194	121	150
	Aregu Seulu	143	151	226	240	248	248	179	179	194	204	252	260	239	251	205	205	252	252	200	202	155	155	140	194	121	150
	Granazza	143	151	226	240	248	248	179	179	194	204	252	260	239	251	205	205	252	252	200	202	155	155	140	194	121	150
	Granaccia	143	151	226	240	248	248	179	179	194	204	252	260	239	251	205	205	252	252	200	202	155	155	140	194	121	150
G6	Cannonau Nero	137	145	226	240	240	244	193	193	188	188	258	258	239	245	207	213	238	252	200	202	170	170	136	142	127	144
	Cannonau	137	145	226	240	240	244	193	193	188	188	258	258	239	245	207	213	238	252	200	202	170	170	136	142	127	144
G7	Cannonau Bianco - Trieri	135	137	240	240	244	244	189	193	188	188	244	258	245	245	205	213	238	252	198	200	165	170	136	136	127	127
	Cannonau Bianco - Oliena	135	137	240	240	244	244	189	193	188	188	244	258	245	245	205	213	238	252	198	200	165	170	136	136	127	127
G8	Nero - Bosa	133	139	236	236	240	264	183	189	186	196	248	254	245	249	205	213	252	268	200	202	159	165	142	148	146	159
	Nieddu Polchinu	133	139	236	236	240	264	183	189	186	196	248	254	245	249	205	213	252	268	200	202	159	165	142	148	146	159
G9	Moscato 1 - Tempio	133	133	228	238	234	250	179	193	186	196	252	252	245	261	209	213	238	246	204	204	155	170	140	159	121	134
	Moscato 3 - Tempio	133	133	228	238	234	250	179	193	186	196	252	252	245	261	209	213	238	246	204	204	155	170	140	159	121	134

Table 2 - suite

	Campione	VVS2		VVMD5		VVMD7		VVMD27		ZAG62		ZAG79		VVMD21		VVMD24		VVMD25		ZAG21		ZAG47		ZAG64		ZAG67	
G10	Moscato - Modolo	133	135	226	228	250	250	179	193	186	200	252	256	239	261	209	213	236	238	188	204	155	170	136	159	127	134
	Moscatello - Modolo	133	135	226	228	250	250	179	193	186	200	252	256	239	261	209	213	236	238	188	204	155	170	136	159	127	134
G11	Moscatello - Murrunzone	133	139	228	238	238	250	179	193	186	196	252	256	245	261	209	213	238	246	204	204	155	170	140	159	121	134
	Moscato - Lodine	133	139	228	238	238	250	179	193	186	196	252	256	245	261	209	213	238	246	204	204	155	170	140	159	121	134
G12	Moscatello Nero	133	133	228	236	234	250	179	193	186	196	252	256	245	261	209	213	238	246	204	204	155	170	140	159	121	134
	Moscatello Bianco	133	133	228	236	234	250	179	193	186	196	252	256	245	261	209	213	238	246	204	204	155	170	140	159	121	134
G113	Licronaxu Nero	143	143	228	240	240	260	179	181	188	194	252	260	239	251	205	205	238	252	188	202	155	157	136	142	144	144
	Licronaxu Bianco	143	143	228	240	240	260	179	181	188	194	252	260	239	251	205	205	238	252	188	202	155	157	136	142	144	144
	Bianca Tomentosa	133	145	226	234	244	250	181	181	188	200	262	262	245	251	205	205	236	238	198	204	155	170	136	159	127	164
	Culupuntu	133	143	228	240	240	250	179	179	188	204	248	248	251	261	209	213	238	252	188	204	155	161	140	159	121	134
	Vertudi	145	151	226	234	244	250	179	193	188	204	258	262	239	245	205	213	238	260	202	202	155	170	142	159	134	146
	Gregu Nieddu - Campidano	145	151	226	232	244	250	179	193	188	204	258	262	243	249	205	215	242	264	202	202	155	170	142	159	134	146
	Gregu Nieddu - Serramanna	145	151	226	236	244	250	179	193	188	204	258	262	239	245	205	213	238	260	202	202	155	170	142	159	134	148
	Gregu Bianco	133	155	226	246	240	248	191	193	188	204	248	250	239	245	205	205	236	252	200	204	165	167	136	138	134	150
	Nera Pelosa	143	151	224	238	240	250	179	195	188	204	258	262	239	245	205	213	252	260	198	204	155	170	136	140	121	127
	Argu Mannu	137	151	236	238	240	250	189	193	188	204	244	258	245	251	205	205	252	252	198	204	165	170	136	138	121	127
	Aregu Giallo	133	143	238	240	244	250	181	189	188	204	244	248	245	251	205	207	252	252	198	204	157	165	136	159	134	134
	Nuragus Arrubiu	133	145	234	242	250	250	179	185	190	200	240	258	245	251	205	205	238	260	188	204	155	161	140	159	121	134
	Nuragus	133	145	236	246	248	250	179	185	190	202	240	258	245	251	205	205	238	260	188	204	155	161	140	159	121	134
	Vernaccia - Escalaplano	143	151	226	240	248	248	179	179	194	204	252	260	245	245	205	205	238	260	202	204	155	157	136	159	134	134
	Vernaccia - Solarussa	133	155	232	246	250	264	181	193	194	200	252	260	239	245	205	213	238	246	188	188	157	170	148	159	134	159
	Girò - Gonnos	143	143	226	238	240	256	179	181	188	194	252	260	239	251	205	205	238	252	188	204	155	157	136	142	144	144
	Sinnidanu	133	135	240	240	244	244	189	193	188	188	244	258	245	245	205	213	238	252	198	200	165	170	136	136	127	127
	Moscato 2 - Tempio	133	143	228	240	240	250	179	179	186	186	252	252	251	261	209	213	238	246	188	204	155	155	140	159	121	134
	Moscato - Pattada	133	143	228	240	240	250	179	189	186	188	252	256	251	261	209	213	238	252	188	204	155	155	140	159	121	134
	Moscato - Su Pinu	133	135	228	238	238	250	179	193	186	196	252	256	245	261	209	213	238	246	204	204	155	170	140	159	121	134
	Moscatellone	133	149	228	232	250	252	179	193	186	204	248	256	251	261	209	209	246	246	188	204	155	170	138	140	121	121

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The high number of synonyms, detected among the Sardinian grapevine germplasm, could also be the result of a biological response, of the cultivars, to environmental changes. Some cultivars could be more sensitive than others to biotic and abiotic stresses leading to evident morphological modifications. In this frame we could explain some of the mistakes made in the identification of cultivars such as the ones resulted as synonyms of Pascale. This variety is grown in different places characterized from dissimilar soil and climatic conditions and is also know with different names (Nera Tomentosa, Picciolo Rosso, Primidivu Nieddu, etc.) used according with the area of cultivation or morphological characteristics. The environmental characteristics could be the reason of small morphological differences (leaf tomentosity, berries size, etc) observed in the Pascale accessions.

Our analysis also showed cases of cultivars harboring the same SSR profile but different berry colours: Licronaxu (Bianco and Nero) and Moscatello (Bianco and Nero). The berry colour is determined by the accumulation of anthocyanins. A recent research (KOBAYASHI *et al.*, 2004) showed that Myb-related genes regulate anthocyanin biosynthesis and a retrotransposon-induced mutation in these genes is associated with the loss of pigmentation in cultivars of *Vitis vinifera* L. In his work, Kobayashi showed that some red cultivars are derived from white-skinned progenitors by a reversion in the Myb-genes mutation. Perhaps, it could by hypothesized that the changes in berry colour in Licronaxu and Moscatello could derive from a retrotransposon-induced mutation event.

In conclusion we would stress that, considering the origin of Sardinian viticulture, an extensive investigation on the genetic relationships among Sardinia varieties and other countries (especially Greece and Spain) could be of help to verify the existence of international synonyms and to clarify the real history of Sardinian varieties and of the migration of grapevine varieties in the Mediterranean Basin during the ancient times. One of the most amazing cases capturing the attention of international investigators is related to the Moscato cultivars, defined from CRESPAN and MILANI (2001), COSTACURTA and co-workers (2003) as a complex family of varieties. In Sardinia a lot of Moscato - Moscatellone varieties were detected and analysed. Some of these resulted synonyms while others showed clear genetic differences. This complexity could be traced back to three centres of origin referring to Moscato bianco (Greece), Moscato d'Alessandria (Egypt) and Moscato giallo (Middle East) from which all other varieties have derived, either directly or indirectly. Although our analysis does not consider Moscato varieties from other regions, we can state that the Moscato cultivars of Sardinia represent an heterogeneous group of varieties related one to the others only basing on common oenological characteristics: e.g. alcohol, sweetness and specific aromatics, and often leading to wines produced from overripe grapes with different genetic profiles.

Finally, we would like stress that a clear and correct ampelographic characterisation is essential this work to be considered as a contribution in the comprehension of the intricate relationships linking grapevine accessions and in the verification of cultivar identities. Our efforts should lead to the protection of local grape biodiversity by defining a characterisation system combining molecular and morphological investigations. All this could have a sense especially if framed in future efforts leading to the constitution of a standardize international SSR database grouping varieties from different countries; this should be made to identify once and forever synonyms, homonyms and false attributions.

Table 3 - Genetic parameters of the 13 SSR loci analyzed within the 61 grapevine varieties.

Locus	N	Allele (bp)	H_e	H_o	r	P.I.
VVS2	8	133-155	0.810	0.887	-0.043	0.118
VVMD5	10	222-246	0.840	0.920	-0.043	0.082
VVMD7	10	234-264	0.782	0.726	0.031	0.137
VVMD21	5	239-261	0.681	0774	-0.055	0.232
VVMD24	4	205-213	0.548	0.500	0.031	0.362
VVMD25	7	236-268	0.737	0.822	-0.049	0.198
VVMD27	6	179-195	0.693	0.790	-0.057	0.221
VrZAG21	6	188-212	0.747	0.790	-0.024	0.177
VrZAG47	7	155-170	0.685	0.839	-0.090	0.227
VrZAG62	8	186-204	0.771	0.870	-0.056	0.147
VrZAG64	7	136-194	0.772	0.951	-0.101	0.161
VrZAG67	9	121-164	0.780	0.854	-0.042	0.145
VrZAG79	11	240-262	0.853	0.887	-0.018	0.072

N: number of alleles detected; Allele (bp): allele size range; He/o: expected and observed heterozygosity; r: probability of null alleles; P.I.: probability of identity.

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