

Vitis 45 (2), 69–73 (2006)

## Malvasia delle Lipari, Malvasia di Sardegna, Greco di Gerace, Malvasia de Sitges and Malvasia dubrovačka – synonyms of an old and famous grape cultivar

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### Summary

Previously believed to be distinct cultivars Malvasia delle Lipari, Malvasia di Sardegna, Greco di Gerace (Italy), Malvasia de Sitges (Spain) and Malvasia dubrovačka (Croatia) displayed an identical molecular profile when analyzed by 15 SSR markers. Ampelographic comparison supports the genetic analysis indicating that they are all the same variety; they do not differ in any important morphological trait. This genotype is scattered all over the Mediterranean area and as far as the Canaries and Madeira. Historical investigation indicates that the genotype has been imported from Greece. However, so far, the search in Greek databases has produced no useful results to confirm this theory. Allele frequency comparison among cultivars from Greece, Croatia, Italy, Spain and Portugal showed that none of these countries can be pinpointed as the ‘home country’ of this genotype.

**Key words:** grape, Malvasia, synonyms, microsatellite markers.

### Introduction

Malvasias are a large and heterogeneous group of varieties cultivated in Greece, Croatia, Italy, France, Spain and other European countries. Their classification is complex, as they lack common ampelographic descriptors, even if many ampelographers underlined the importance of limiting the use of this name to only those cultivars characterized by aromatic berries with a slight muscat flavor and some bitterness (DI ROVASENDA 1877, DALMASSO 1964).

According to a study on Malvasias by Prof. LOGOTHETIS in 1965 (in: GALET 2000, CALÒ *et al.* 2001), the name Malvasia derives from “Monemvasia”, an old trading seaport in Greece. The first written document of Malvasia wine dates back to 1214, when a citizen of Epheso (Greece), referred to a wine called Monovasia or Monemvasias. The spread of this term is linked to an active wine trade in the Middle Ages, especially by Venetians, who started shipping ‘Vinum de Malvasias’ in 1278. The name was linked to sweet

and aromatic wines from Greece, without any reference to specific cultivars. The increasing demand for this type of wine from northern Europe and the loss of Malvasia wine production after Greece was conquered by the Ottoman Empire, led to the establishment of new centers of production along the shipping routes of the Mediterranean Sea.

Malvasia delle Lipari, Malvasia di Sardegna, Greco di Gerace (Italy), Malvasia de Sitges (Spain) and Malvasia dubrovačka (Croatia) are all believed to be distinct cultivars. Most share the same generic name, together with a geographical name. Nowadays they are not widespread cultivars, but they are grown on delimited sites with a very small wine production.

The earliest written document discovered so far regards Malvasia dubrovačka and dates back to 1385 (Archive of the Republic of Dubrovnik), whereas the oldest citations found for all other synonyms go back no more than to the 18<sup>th</sup> century, excluding Greco di Gerace, *alias* Greco di Bianco (ANTONACCI and PLACCO 1997), for which no historical information is available.

There are numerous ampelographic descriptions of these cultivars, but due to the geographical distance they were not compared. Malvasia de Sitges and Malvasia di Sardegna (under the synonym Malvasia di Bosa) are included in the Genres website (<http://www.genres.de/eccdb/vitis>). Italian accessions have been described by BRUNI (1964), MAZZEI and ZAPPALÀ (1964), PASTENA (1993), ANTONACCI and PLACCO (1997). GALET (2000) classified Malvasias on the basis of berry color and the presence of hairs on the lower side of the leaf. He included the following varieties in the same group (white and hairless): Malvasia bianca di Trani, Malvasia del Lazio, Malvasia di Lipari, Malvasia de Sitges, Malvasia istriana and Malvasia dubrovačka. Malvasia di Sardegna was kept in a different group (white and arachnoid-downy), while, in the same work, this variety is listed among the synonyms of Malvasia de Sitges. Malvasia dubrovačka was described by BULIĆ (1949).

A comparison of morphological and wine sensory characteristics, as well as of molecular data obtained with microsatellite markers (done separately at Zagreb and at Conegliano, unpubl.) with previous studies (BORREGO *et al.* 2002) led to the hypothesis that Malvasia delle Lipari, Mal-

vasia di Sardegna, Greco di Gerace, Malvasia de Sitges and Malvasia dubrovačka might all be synonyms of the same genotype. The objective of this study was to compare the above-mentioned local cultivars by means of their microsatellite profiles by ampelographic descriptions and by their historical background.

### Material and Methods

**Plant material:** New accessions of the Italian varieties Malvasia delle Lipari, Malvasia di Sardegna and Greco di Gerace were gathered from vineyards in the Lipari (or Eolie) archipelago, Sardinia and Calabria, to confirm preliminary molecular data obtained from the plants cultivated in the Istituto Sperimentale per la Viticoltura (ISV) collection. Malvasia de Sitges was obtained from the Grapevine Germplasm Bank (BGV) at the Finca El Encin, Alcalá de Henares, Madrid (Spain). Malvasia dubrovačka was sampled in the variety collection of the Faculty of Agriculture, University of Zagreb (Croatia) (Tab. 1).

**Microsatellite analysis:** DNA analysis of all 5 varieties was performed with a total of 15 microsatellite loci. The 6 core loci agreed in the format of the European Project Genres 081 (THIS *et al.* 2004), namely VVS2 (THOMAS and SCOTT 1993), VVMD5, VVMD7 (BOWERS *et al.* 1996), VVMD27 (BOWERS *et al.* 1999), VrZAG62 and VrZAG79 (SEFC *et al.* 1999) were independently analyzed in the Istituto Sperimentale per la Viticoltura, Conegliano, Italy and the Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario, Madrid, Spain. Samples were also analyzed at 4 additional loci, ISV2, ISV3, ISV4, VMC NG 4b9, in Italy (CRESPIAN 2003) and at

another 5 loci, VVMD28 (BOWERS *et al.* 1999), VrZAG29, VrZAG67, VrZAG83 and VrZAG112 (SEFC *et al.* 1999) in the Spanish lab.

The procedure adopted in the Spanish lab is reported by IBÁÑEZ *et al.* (2003). In the Italian lab the DNA extraction protocol was similar to that described in CRESPIAN *et al.* (1999). Microsatellite analysis was performed as follows. Groups of 2 or 3 microsatellite loci were amplified as multiplex-PCR using the following combinations: (a) VVMD7 + VrZAG62 + ISV2; (b) VVMD5 + VVMD27 + VVS2; (c) VrZAG79 + VMC NG 4b9; (d) ISV4 + ISV3. The PCR reaction mixture (25 µl final volume) contained 20 ng total DNA, 10 µl Eppendorf HotMasterMix (2.5 x) and 5 pmoles of each primer. The PCR was performed in an AB 9700 thermal cycler with the following steps: 1 min 30 s at 94 °C; 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 65 °C for 30 s; 65 °C for 7 min and a final step of at least 10 min at 8 °C to stop the reaction. Five µl of the PCR product were tested on 2 % agarose gel. On the basis of signal intensity, 2-5 µl of amplified DNA were used for electrophoresis. Samples were denatured at 94 °C for 3 min in a buffer containing formamide and loaded onto a sequencing gel (5 % polyacrylamide, 1x TBE, 7M urea). Electrophoresis was carried out until the xylene cyanol tracking dye had run about 35 cm. Amplification products of cultivars with alleles of known molecular size were used as references for allele sizing. Gel bands were revealed by silver staining, as reported in CRESPIAN and MILANI (2001). Gels were visually scored at least twice.

For a better comparison with other studies, the allele lengths of the 6 core (Genres 081) loci were also expressed in "Genres format" (allele sizes standardized according to alleles of well-known cultivars (THIS *et al.* 2004).

Table 1

Alphabetical list of accessions analyzed with molecular markers. If not indicated, only one sample was analyzed

Accession name	Geographic origin
Greco di Gerace <sup>1</sup>	Bianco, Reggio Calabria, Italy
Malvasia de Sitges	Sitges, Barcelona, Spain
Malvasia delle Lipari <sup>2</sup>	Salina, Lipari's archipelagos, Messina, Italy
Malvasia di Sardegna <sup>3</sup>	Sardinia, Italy
Malvasia dubrovačka	Croatia

1 - Two samples were analyzed, coming from two farms: Az. Pasquale Sergio and Az. Stefano Sergio, contrada Palmetta Saporita in Agro di Bianco, Reggio Calabria, Italy.

2 - Samples analyzed:

- Germplasm collection, ISV Susegana (Treviso), Italy;
- Salina (Messina) Sicily - Italy (Azienda Virgona);
- Salina (Messina) Sicily - Italy (Azienda Matarazzo);
- Salina (Messina) Sicily - Italy (Azienda of Prof. C. Nicolosi-Asmundo)

3 - Samples analyzed:

- Germplasm collection, ISV Susegana (Treviso), Italy;
- Mogoro (Cagliari), Italy;
- Maracalagonis (Cagliari), Italy
- Sassari, Italy (II 17);
- Dolianova (Sassari), Sardinia, Italy;
- Sassari, Sardinia, Italy (20/34).

**Statistical analysis:** Probability of identity was computed with the freeware program “Identity 1.0” of WAGNER and SEFC (1999) (<http://www.boku.ac.at/zag/identity.htm>), using microsatellite data on 10 loci of 400 grapevine varieties (ISV molecular database). In order to check whether alleles of the studied genotype in this paper are most frequent in the group of varieties considered to be native in a particular country, the allele frequencies at 7 SSR loci (the 6 core Genres081 loci, plus VrZAG83) were compared with those of cultivars from Greece (n=32), Italy (n=30), Croatia (n=30), Spain (n=20) and Portugal (n=27). SSR profiles of the above-mentioned cultivars were taken from Sefc *et al.* (2000) (freely available at <http://www.boku.ac.at/zag/forsch/grapeSSR2.htm>). Part of the data was generated by the Croatian partner (Croatian cultivars not reported in SEFC *et al.* (2000)).

**Ampelographic and ampelometric description:** Seven accessions were described according to the OIV descriptor list (OIV 1983) as modified by the EU project GENRES 081 (2001): Malvasia delle Lipari, Malvasia di Sardegna and Greco di Gerace - for Italy<sup>1)</sup>; Malvasia de Sitges, Malvasia (from Tenerife, Canaries) and Malvasia de Banyalbufar (from Mallorca, Balearics) - for Spain; Malvasija dubrovačka - for Croatia. Thirty-five ampelographic and 25 ampelometric descriptors were used, including leaf, shoot, inflorescence, cluster and berry morphology, fruit composition and phenology.

For the Croatian and Italian accessions, phyllometric measurements were conducted on a computer graphical board.

## Results and Discussion

**DNA analysis:** A unique SSR profile was obtained for all analyzed varieties (Tab. 2). All Italian samples, analyzed at the first 10 microsatellite loci listed in ‘Material and Methods’, had the same molecular profile. Probability of identity (uniqueness) was  $1.93 \times 10^{-13}$ , which means that the probability of finding another distinct cultivar with the same molecular profile is extremely low. Thus, with high probability our samples are genetically uniform, are derived from the same seedling and are the same variety (BOURSIQUOT and THIS 1999), and do not differ at any important morphological trait (see below).

Our results do not agree with conclusions of FANIZZA *et al.* (2003), who, using AFLP markers, considered Malvasia delle Lipari and Malvasia di Sardegna to be different cultivars. Instead, our results agree well with the classification of Galet, where three of the Malvasia synonym accessions identified in the present study are grouped together. Comparing our SSR profile with others recorded in our databases, we conclude that this Malvasia is different from Malvasia istriana and Malvasia del Lazio; the percentage of common alleles is low, 33.3 and 26.7 %, respectively. Computation was performed on 15 loci, namely the 6 core loci plus VVS1, VVMD28, VVMD32, VVMD36, VrZAG21, VrZAG64, ISV2, ISV3 and ISV4. No data are available for Malvasia bianca di Trani.

Some authors correlated Malvasia delle Lipari (PAS-TENA 1993) and Malvasia de Sitges (BORREGO *et al.* 2002)

Table 2

SSR profile of the 5 accessions studied. Allele lengths are expressed in bp.  
For the first 6 loci, alleles are codified in „Genres format“

Microsatellite loci	allele lengths		analytical laboratory/ies
VVS2	143 (CH2)	145 (SU1)	IMIDRA and ISV
VVMD5	226 (CF1)	226 (CF1)	IMIDRA and ISV
VVMD7	243 (GE1)	249 (MU2)	IMIDRA and ISV
VVMD27	179 (MU1)	183 (FE1)	IMIDRA and ISV
VrZAG 62	187 (CH1)	201 (SCH2)	IMIDRA and ISV
VrZAG 79	242 (CH1)	246 (CF1)	IMIDRA and ISV
VVMD28	234	256	IMIDRA
VrZAG29	109	109	IMIDRA
VrZAG67	149	149	IMIDRA
VrZAG83	195	195	IMIDRA
VrZAG112	227	240	IMIDRA
ISV2	143	161	ISV
ISV3	133	139	ISV
ISV4	187	187	ISV
VMC NG 4b9	168	172	ISV

IMIDRA: Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario, Madrid, Spain; ISV: Istituto Sperimentale per la Viticoltura, Conegliano, Italy.

<sup>1)</sup> Malvasia delle Lipari and Greco di Gerace plants were grown at the farm of Saverio Ladogana in Agro of Orta Nova (Foggia, Italy); Malvasia di Sardegna plants from the ISV collection at Susegana (Treviso, Italy).

to Malvasia fina cultivated on the island of Madeira. Nevertheless, the molecular profile of this later variety differs from our Malvasia genotype (LOPES *et al.* 1999 and ISV database). Another variety, locally named Malvasia candida, is the most widely cultivated Malvasia on the island and is considered to be the authentic and oldest Malvasia from Madeira (PEREIRA 1956). The Spanish partner found that Malvasia de Sitges and Malvasia candida present the same profile for 9 microsatellites (VVS2, VVMD5, VVMD27, VVMD28, VrZAG29, VrZAG67, VrZAG83 and VrZAG-112) and so should be considered synonyms.

Cv. Malvasia presented in this paper produces aromatic berries, reminding some oenologists of a certain similarity to Muscat varieties: Moscato bianco (Muscat à petits grains) and Muscat of Alexandria (Zibibbo), two cultivars presumed to be the oldest of the entire Muscat family. A comparison of SSR profiles excludes a parent-progeny relationship among these varieties, as with the 18 other Muscats identified previously (CRESPIAN and MILANI 2001).

**Ampelographic description:** Ampelographic description of different accessions (not shown) supports the genetic analysis indicating that they are all the same cultivar. For most characteristics the data of the majority of accessions are very similar or overlapping. According to the ampelographic and ampelometric data, this Malvasia genotype can be described as follows: Shoot tip opened with sparse prostrate hairs; young leaves bronze to copper-reddish; mature leaf pentagonal or circular with 5-7 lobes; deep lateral sinuses; petiole sinus slightly-open to open and density of prostrate hairs between the main vein on the lower side very-weak to weak; bunch long, cylindrical or narrow-conical, usually with wings; berry small, roundish to slightly elliptic, berry skin yellow, pronounced particular flavor; yield very-low to low, quality high; must sugar content high, acidity medium.

Some differences among accessions have been observed as well, e.g. in intensity of anthocyanin coloration of the shoot tip, density of erect hairs on the main veins of mature leaves, or bunch length. Despite a standardized description method (according to the OIV descriptors) we cannot exclude some subjectivity on the part of different experts from Italy, Spain and Croatia, who made descriptions in the field. Supposing that the differences are objective, it remains difficult to decide, for the moment, if they are due to flexibility in the interaction genotype x environment or if they have a genetic basis, *i.e.* mutations accumu-

lated over centuries. GALET (2000) classified Malvasia di Sardegna in a different group just due to hairs on the lower side of the leaf. PASTENA (1993) also underlines the great variability of some Malvasia delle Lipari characters, in particular bunch length (18-40 cm) and berry dimensions, suggesting that this vine could be very ancient.

**Geographic origin assignment:** The results of this study suggest that this group of synonyms is very old. The time and origin of this cultivar and its dissemination path remain unclear. Our knowledge about the SSR genotypes of the present Greek cultivars does not support a Greek origin. Information from the Greek Vitis database (<http://www.biology.uoc.gr/gvd>, LEFORT and ROUBELAKIS-ANGELAKIS 2000) gave molecular profiles for 4 Malvasia cultivars: Malvasia del Chianti, Malvasia di Candia, Malvasia aromatica and Malvasia istriana. None of these is consistent with our genotype. A more general search in the Greek database, independently of cultivar name, produced no useful results and the same is true when our data were compared with those shown in LEFORT and ROUBELAKIS-ANGELAKIS (2002).

A comparison of allele frequencies among groups of cultivars from different Mediterranean countries (Greece, Italy, Croatia, Spain and Portugal) and the studied Malvasias showed that the highest allele frequencies at different loci were randomly scattered across countries (Tab. 3). Thus, none of them can be pinpointed as the 'home country' of this variety.

Our conclusions based on nuclear microsatellite marker comparisons do not support the results of ARROYO-GARCÍA *et al.* (2002) based on chloroplast microsatellite markers haplotypes: Malvasia de Sitges showed a haplotype with a very high frequency in the Spanish cultivars and very low frequency in the Greek varieties analyzed in that work.

Finally it is interesting to note that the original name of Malvasia was preserved in almost all cases, except for Greco di Gerace. An explanation for this may be the genuine quality and the long-lasting commercial importance of this cultivar.

### Acknowledgements

The authors thank all those who contributed to the present work, supplying plant material and bibliographic information: O. GRAVIANO (Consorzio per la Frutticoltura di Cagliari), C. NICO-

Table 3

Malvasia alleles frequencies (the highest in bold) in groups of varieties considered native in the selected European countries

SSR loci alleles	VVS 2		VVMD 5		VVMD 7		VVMD27		VrZAG 62		VrZAG 79		VrZAG 83	
	143	145	226	226	243	249	179	183	187	201	242	246	195	195
Country														
SP (n=20)	0.200	<b>0.325</b>	0.175		<b>0.225</b>	0.125	0.050	0.050	0.100	0.075	0.150	0.300	0.325	
PT (n=27)	0.148	0.185	0.278		0.093	0.037	0.111	0.037	<b>0.111</b>	0.037	0.037	<b>0.444</b>	0.315	
HR (n=30)	0.183	0.067	0.300		0.017	0.217	<b>0.450</b>	0.033	0.067	0.083	0.033	0.017	<b>0.517</b>	
GR (n=32)	<b>0.391</b>	0.109	0.219		0.172	<b>0.328</b>	0.250	<b>0.078</b>	0.047	0.156	0.094	0.234	0.500	
IT (n=30)	0.150	0.017	<b>0.333</b>		0.033	0.250	0.317	0.067	0.033	<b>0.217</b>	<b>0.167</b>	0.033	0.467	

SP: Spain. PT: Portugal. HR: Croatia. GR: Greece. IT: Italy.

LOSI-ASMUNDO (University of Catania), G. NIEDDU (University of Sassari), K. ROUBELAKIS-ANGELAKIS (University of Crete, Greece), N. KARAMAN and I. OREŠKOVIĆ (State Archive Dubrovnik), D. OLIVA, F. CAPRARO and A. PICCIOLO (Istituto Regionale delle Vite e del Vino of Palermo). Thanks also to M. D. VELEZ (IMIDRIA, Spain) for her technical assistance.

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Received July 28, 2005

