Vitis **42** (4), 193–197 (2003)

The parentage of Muscat of Hamburg

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Summary

This research demonstrates that Muscat of Hamburg, a fine black table grape variety with muscat flavour, is the progeny of Schiava Grossa x Muscat of Alexandria. Two isozymes (GPI and PGM), 30 nuclear and 5 chloroplastic microsatellite markers were used. Two highly informative microsatellite markers from VMC ('Vitis Microsatellite Consortium') are suggested to enlarge the minimum set of loci selected for grapevine identification in the frame of the European Project GenRes 081.

K e y w o r d s : Muscat of Hamburg parentage, nuclear microsatellites, chloroplast microsatellite markers, identification.

Introduction

For a long while the Muscats attracted and interested ampelographers, although a classification and listing of this great family was difficult due to the abundance of different vines. Recently new technologies, particularly those linked to molecular biology, offered new and valuable tools, flanking classical ampelographic methods of variety characterization and identification. DNA analysis by microsatellite markers revealed to be a very useful approach to clarify synonyms, homonyms and for pedigree analyses (Thomas *et al*. 1994; Bowers and Meredith 1997; Sefc *et al.* 1997; Bowers *et al*. 1999 a).

Muscat of Hamburg is a black table grape grown in many parts of Europe, highly appreciated for its beautiful bunches and for the fair muscat flavour. For these valuable characteristics it has been used by breeders as a parent to obtain new table grape varieties. Information about its origin is very poor: the only fact which seems to be sure is that it appeared first in England in the second half of the 19th century. Viala and Vermorel (1901) assert that it is a very ancient vine variety, sunken into oblivion and rediscovered by a gardener, M. Snow, of West Park (England), who thought to have found a new variety and named it Snow's Muscat Hamburg. The same happened to M. Venn, near Bristol (England), who commercialised it as Venn's Seedling.

In 1948 Pirovano declared that this variety originated from the North of Europe and from the cross of Zibibbo x Frankenthal. Zibibbo is one of the synonyms of Muscat of Alexandria (Molon 1906) and Frankenthal is also known as Trollinger or Schiava Grossa (Branas and Truel 1965). Since Pirovano did not support his opinion by any proof, the French ampelographer Galet (1964) stated that the origin of this variety remains completely unknown. In a previous work (Crespan and Milani 2001) we observed that in fact a parent-offspring link existed for Muscat of Hamburg and Muscat of Alexandria; thus the early indication by Pirovano is object of the present research.

The molecular analysis was performed with 30 nuclear microsatellite markers. Twenty of them, *i.e.* VVS, VVMD and VrZAG loci are well known for their polymorphism and were largely employed in numerous works by many groups of researchers. For the three ISV loci and other 7 derived from the work of different members of VMC, the results of a statistical analysis, performed with the Identity 1.0 software (Wagner and Sefc 1999), are presented. Their informative power was evaluated on a set of heterogeneous table and wine varieties (*Vitis vinifera* L).

The present research includes also the analysis of 5 chloroplastic microsatellite loci, ccmp2, ccmp3, ccmp4, ccmp6 and ccmp10 (Weising and Gardner 1999), with the goal to determine the maternal parent. As in most angiosperms, chloroplasts of grapevine are inherited from the mother: it was shown by Strefeler *et al.* (1992) by analysing progenies of interspecific hybrids and, very recently, by Arroyo-Garcia *et al*. (2002) by testing 20 offsprings derived from a controlled cross between two table grape cultivars.

Material and Methods

P l a n t m a t e r i a l : All varieties were obtained from the collections of the Istituto Sperimentale per la Viticoltura of Conegliano (Italy). For Schiava Grossa, Muscat of Alexandria and Muscat of Hamburg two different accessions for each variety were analysed.

D N A e x t r a c t i o n a n d a n a l y s i s : DNA was extracted from young leaflets according to Dellaporta *et al*. (1983) as modified by Crespan *et al*. (1999).

Thirty nuclear microsatellite loci were analysed: VVS1 and VVS2 (Thomas and Scott 1993), VVS29 (Thomas, CSIRO Plant Industry, Adelaide, Australia, pers. comm.), VVMD5, VVMD7, VVMD8 (Bowers *et al*. 1996), VVMD17, VVMD21, VVMD24, VVMD25, VVMD26, VVMD27, VVMD28, VVMD31, VVMD32, VVMD36 (Bowers *et al.* 1999), VrZAG21, VrZAG62, VrZAG64, VrZAG79 (Sefc *et al*. 1999), ISV2 (VMC 6e1), ISV3 (VMC 6f1), ISV4 (VMC 6g1) isolated by the Istituto Sperimentale per la Viticoltura as a member of the 'Vitis Microsatellite Consortium' (primers are given in Tab. 1) and 7 additional microsatellite loci from other members: VMC 2g2 (for primer sequences see Di Gaspero *et al*. 2000), VMC 1e12, VMC NG 4b9, VMC 4g6, VMC 6e10, VMC 3d7 and VMC 2h9. Five chloroplast microsatellite loci were analysed by using the consensus primer pairs designed by

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Table 1

Primer sequences for the ISV microsatellite loci

Locus	forward primer (5' → 3')	reverse primer (5' → 3')
ISV2 (VMC 6e1)	CAC TGG CCT GTT GGG AGA TAA T	CCT TCA ACT GGA AAA GCC TGT C
ISV3 (VMC 6f1)	AAG GAG GAG TTG AGA TGT AGT A	GAG TAA GAG AGA AGC AAG AAA A
ISV4 (VMC 6g1)	TGC ATA GTG CTG TAG GCC ATT G	TCT GTC ATT GCT GTC CCT TTC A

WEISING and GARDNER (1999) for ccmp2, ccmp3, ccmp4, ccmp6 and ccmp10.

The PCR reaction mixture (25 µl final volume) for nuclear microsatellites contained 20 ng total DNA, 0.5 U Taq DNA polymerase with relative reaction buffer (Polymed, Florence, Italy), 1.5 mM $MgCl_2$, 200 µM of each dNTP and 20 pmoles of each primer. In the reaction mixture for chloroplast microsatellite markers the amount of $MgCl_2$ was increased to 2.5 mM.

The PCR was performed in an AB 9700 thermal cycler with the following steps: 5 min at 95 °C; 40 cycles at 95 °C for 1 min, 55 °C for 30 s, 72 °C for 30 s; 72 °C for 10 min and a final step of 8 °C for at least 10 min to stop the reaction.

Five µl of the PCR product were tested on 2 % agarose gel. On the basis of signal intensity, 1-2 µl of amplified DNA were used for electrophoresis. Samples were denatured at 94 °C for 3 min in a buffer containing formamide and loaded on to a sequencing gel (5 % polyacrylamide, TBE 1 x, urea 7 M). Amplification products of cultivars with alleles of known molecular size were used as references for allele sizing.

Bands on the gel were revealed by silver staining, as indicated in CRESPAN and MILANI (2001). The gels were scored by eye at least twice.

Data analysis: The freeware program "Identity 1.0" by WAGNER and SEFC (1999) was used for data processing (http://www.boku.ac.at/zag/forsch/MANUAL.rtf).

Results

Characterisation of ISV and 7 other VMC microsatellite markers: From 23 to 110 different grape varieties were analysed to evaluate the characteristics of 7 additional microsatellite markers, together with 3 ISV loci. Statistical elaboration of data was performed with the identity 1.0 program and results are exposed in Tab. 2. All ten loci showed very clear patterns and revealed to be useful for parentage analysis. The number of detected alleles ranged from 5 to 13; the observed heterozygosity from 0.695 to 0.918, with an average of 0.820; the probability of null alleles was very low or absent. Therefore the use of these additional markers increased the solidity of the whole data acquired to test the initial hypothesis about the parentage of Muscat of Hamburg. Moreover two of them, ISV2 and VMC NG 4b9, revealed to be particularly informative for identification purposes, due to the great number of alleles found, the absence of null alleles and the low probability of identity (see Tab. 2).

Verifying the hypothesised parentage of Muscat of Hamburg: The GPI and PGM data were obtained from the archive of Istituto Sperimentale per la Viticoltura. Molecular data are shown in Tab. 3 and are compatible with the preliminary hypothesis, since at each locus Muscat of Hamburg has one allele derived from Schiava Grossa and the other from Muscat of Alexandria. In

Table 2

Characteristics of the three ISV and of seven additional microsatellite markers from VMC

Locus*	Number of detected alleles	Allele size range (bp)	Observed heterozygosity	Expected heterozygosity	Estimated frequency of null alleles	Probability of identity	Number of analysed cultivars
ISV2 (VMC 6e1)	**13**	**133 - 171**	**0.918**	**0.834**	**-0.045**	**0.089**	**110**
ISV3 (VMC 6f1)	6	131 - 145	0.855	0.635	-0.134	0.334	83
ISV4 (VMC 6g1)	8	169 - 197	0.795	0.772	-0.012	0.155	93
VMC 1e12	9	240 - 260	0.775	0.791	0.009	0.135	40
VMC NG 4b9	**11**	**140 - 178**	**0.857**	**0.811**	**-0.025**	**0.090**	**35**
VMC 2g2	7	125 - 143	0.885	0.784	-0.056	0.147	35
VMC 4g6	5	117 - 125	0.695	0.717	0.012	0.229	23
VMC 6e10	6	159 - 175	0.826	0.707	-0.069	0.226	23
VMC 3d7	7	119 - 141	0.756	0.756	0	0.172	37
VMC 2h9	12	93 - 129	0.843	0.880	0.019	0.048	32

*The most interesting loci are in bold.

Tab. 4 the cumulative likelihood ratios are reported of Muscat of Hamburg being the cross between Schiava Grossa and Muscat of Alexandria *vs.* alternative parents, including close relatives. The ratios were computed by using the observed allele frequencies and the 95% upper confidence limits. These data demonstrate clearly that the putative parents of Muscat of Hamburg are Schiava Grossa and Muscat of Alexandria, since other possible cross combinations are highly less probable. The two parents of Muscat of Hamburg exhibited a polymorphism at ccmp3 locus (Tab. 3) and so it was possible to determine the direction of the cross: as Muscat of Hamburg showed the haplotype of Schiava Grossa, this last is the female parent and the male is Muscat of Alexandria.

Discussion

Additional microsatellite data here provided confirm that Muscat of Hamburg derived from Muscat of Alexandria, as previously reported (Crespan and Milani 2001). The other

Table 3

Isozyme (GPI and PGM) patterns and microsatellite data at 30 nuclear and 5 chloroplastic loci. Allele lengths are in base pairs

Varieties	Schiava Grossa		Muscat of Hamburg		Muscat of Alexandria	
Berry color*	N		N		B	
GPI**	ab (2)		aa (1)		aa (1)	
PGM	aa (1)		ab (4)		ab (4)	
Nuclear microsatellite markers						
VVS1	181	190	181	190	181	180
VVS2	135	155	135	149	133	149
VVS29	171	179	171	179	171	179
VVMD5	236	238	232	238	228	232
VVMD7	247	247	247	249	249	251
VVMD8	157	167	141	157	141	141
VVMD17	222	222	220	222	220	220
VVMD21	249	249	249	256	256	266
VVMD24	210	214	214	214	214	214
VVMD25	245	259	253	259	253	253
VVMD26	249	251	249	251	249	251
VVMD27	181	185	179	185	179	194
VVMD28	239	247	239	247	247	271
VVMD31	212	212	212	216	216	224
VVMD32	253	273	273	273	265	273
VVMD36	264	298	254	298	254	264
VRZAG21	202	206	190	206	190	206
VRZAG62	191	193	185	191	185	203
VRZAG64	159	191	139	191	139	141
VRZAG79	238	258	238	254	246	254
ISV2 (VMC 6e1)	161	165	141	161	141	143
ISV3 (VMC 6f1)	139	141	139	139	133	139
ISV4 (VMC 6g1)	177	187	169	187	169	195
VMC 1e12	246	246	246	248	248	260
VMC NG 4b9	158	178	158	158	158	158
VMC 2g2	121	123	123	125	125	125
VMC 4g6	143	143	129	143	127	129
VMC 6e10	97	113	99	113	99	113
VMC 3d7	169	171	161	169	161	161
VMC 2h9	119	121	121	123	123	123
Chloroplastic microsatellite markers						
ccmp2	208		208		208	
ccmp3	107		107		106	
ccmp4	128		128		128	
ccmp6	107		107		107	
ccmp10	115		115		115	

* B = blanc; N = noir.

** GPI and PGM patterns are expressed as combined alleles in letters and as code numbers in brackets. The codes refer to Calò *et al.* 1989.

Table 4

Cumulative likelihood ratios of Muscat of Hamburg being the cross between Schiava Grossa (1) and Muscat of Alexandria (2) versus alternative parents, including close relatives, calculated over 30 nuclear microsatellite loci. The reference database for allele frequency computation ranged from 23 to 295 different varieties, depending from the locus*

Parents combinations	X x Y	(1) x X	rel (2) x (1)	(2) x X	rel (1) x (2)
with observed allele frequencies	3.48×10^{24}	1.45×10^{17}	1.18×10^{5}	1.08×10^{14}	3.69×10^{4}
with 95 % upper confidence limits for allele frequencies	5.93×10^{16}	9.98×10^{12}	1.34×10^{4}	2.17×10^{10}	4.41×10^{3}

* VVS1 = 144, VVS2 = 295, VVS29 = 65, VVMD5 = 295, VVMD7 = 295, VVMD8 = 65, VVMD17 = 27, VVMD21 = 35, VVMD24 = 36, VVMD25 = 37, VVMD26 = 35, VVMD27 = 280, VVMD28 = 134, VVMD31 = 41, VVMD32 = 66, VVMD36 = 92, VRZAG21 = 223, VRZAG62 = 292, VRZAG64 = 217, VRZAG79 = 287, ISV2 = 110, ISV3 = 83, ISV4 = 93, VMC1e12 = 40, VMC NG 4b9 = 35, VMC 2g2 = 35, VMC 4g6 = 23, VMC 6e10 =23, VMC 3d7 = 37, VMC 2h9 = 32.

parent was demonstrated to be Schiava Grossa. Chloroplast microsatellite markers, when polymorphic, may give a further contribution for a more precise determination of pedigree relationships among varieties, by clarifying the cross direction, otherwise impossible with nuclear markers. In this case, the only polymorphism found at locus ccmp3 was useful to establish the maternal parent.

In a previous work (CRESPAN and MILANI 2001) Muscat of Hamburg showed the same microsatellite profile as Muscat of Madresfield Court (only one accession available), which is a known cross between Muscat of Alexandria and Black Morocco (VIALA and VERMOREL 1991). The question of this presumed identity remains open even in the light of the results here presented, since the two cultivars share one parent, Muscat of Alexandria, and the identity of Black Morocco is highly uncertain, because this name is used as synonym of many different varieties, such as Alphonse Lavallée, Augster Blau, Coarna Rosie, Geisdutte Blau, Damaszener Blau, Maroc Gros, Marocain Noir (E. MAUL, pers. comm.).

Two microsatellite markers from VMC, ISV2 and VMC NG 4b9, revealed to be very interesting for their clear patterns and highly informative power. Therefore we suggest their employment in addition to the six most suitable (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79) selected for grapevine by the research groups working in the European Project Genres 081 (THIS and DETTWEILER 2002), as in some cases closely related varieties may not be distinguished with only those six markers (MARTÍN *et al*. 2003).

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Received July 21, 2003