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Cultivar identity control of 'Moscato rosa' grapevines undergoing clonal selection

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Abstract: The variety 'Moscato rosa' is a low yielding red grapevine whose grapes are harvested over-ripened in order to obtain a high quality rose-like flavoured dessert wine. This grape, which is cultivated in northern Italy in small production areas of Trentino, Alto Adige and Friuli, is also known as 'Rosa Muskat', 'Rosenmuskateller' or 'Muskateller rot'. It is very likely that the variety 'Moscato rosa', considered native in Dalmatia, was introduced to South Tyrol towards the end of 19th century following the establishment of important agricultural stations in some countries of the Austria-Hungarian Empire. Two of these schools are now the Institute for Agriculture in Poreč (Croatia) and the Istituto Agrario in San Michele all'Adige (Italy). A clonal selection programme based on evaluation of the sanitary status and performance of about 50 accessions of 'Moscato rosa' collected around five viticultural areas in northern Italy and Croatia was recently started by the Istituto Agrario in San Michele all'Adige. In order to assess the common genetic origin of the grape material investigated and for an initial characterisation of selected vines, which will be subjected to a clonal comparison protocol, the profiles at nine microsatellite loci as well the anthocyanin patterns were determined.

1. Introduction

The 'Moscato rosa' variety is a low-yielding (30-50 q/ha) red grapevine cultivated in small areas of Trentino, Alto Adige and Friuli, in northern Italy. Its grapes are harvested over-ripened in order to obtain a high quality dessert wine, which is characterised by a typical rose-like flavour.

Its origin has been alternatively reported as Italian or Croatian. It is very likely that this variety, considered native in Dalmatia, was introduced to South Tyrol towards the end of 19th century, following the establishment of important agricultural stations in some countries during the Austria-Hungarian Empire. Two of these schools are now the Institute for Agriculture in Poreč (Croatia) and the Istituto Agrario in San Michele all'Adige (Italy).

The first reliable reference to the cultivation of 'Moscato rosa' in Trentino can be attributed to Mach (1888), who reported its presence in the vineyards of the Istituto Agrario in San Michele all'Adige since 1877. Furthermore this variety was included in the list of vines grown in Trentino at the end of 1800s (Mader, 1883).

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Recently, the Istituto Agrario in San Michele all'Adige started a clonal selection programme based on evaluation of the sanitary status and performances of about 50 'Moscato rosa' plants showing different behaviour, spotted around viticultural areas in northern Italy and Croatia.

The present study, which is part of a survey aiming at characterising the wines of coloured muscat grapes (Versini *et al.*, 1993), was planned to verify if those accessions having divergent phenotypic traits can be considered putative clones of plant material originated from the same seedling.

For this purpose, a preliminary characterisation of field-growing 'Moscato rosa' vines before evaluation for clonal selection was performed which regarded the genotype description at nine microsatellite loci and the anthocyanin pattern determination. The high potential of microsatellite markers for grapevine and rootstock cultivar discrimination has been demonstrated in several studies (Thomas and Scott, 1993; Bowers *et al.*, 1996). Due to the stability of polymorphism, microsatellite analysis can be used to determine cultivar identity and to identify grapevine material of unknown varietal origin by comparing the genotype obtained from the sample with reference genotypes of cultivars stored in a database.

In a like manner, the stability of anthocyanin pattern within the same grape variety (Mattivi *et al.*,

1989), has suggested the analysis of these secondary metabolites in germplasm evaluation in order to group land races according to their anthocyanin profile, to make suggestions about the similarity among local varieties found in different locations, differently named but with similar morphological characteristics, or conversely to solve problems of homonym varieties (Valenti *et al.*, 1997).

In the case of 'Moscato rosa', this approach was highly appropriate, since we observed a large variability of the pattern of the anthocyanins among the different accessions of red and pink 'Moscato' varieties present in the ampelographic collection of our Institute. The analysis of the different 'Moscato' vines evidenced both cases of i) varieties having different name and/or origin but with similar anthocyanin profile, as well as ii) varieties with similar name or origin but completely different anthocyanin profile (Fig. 1).

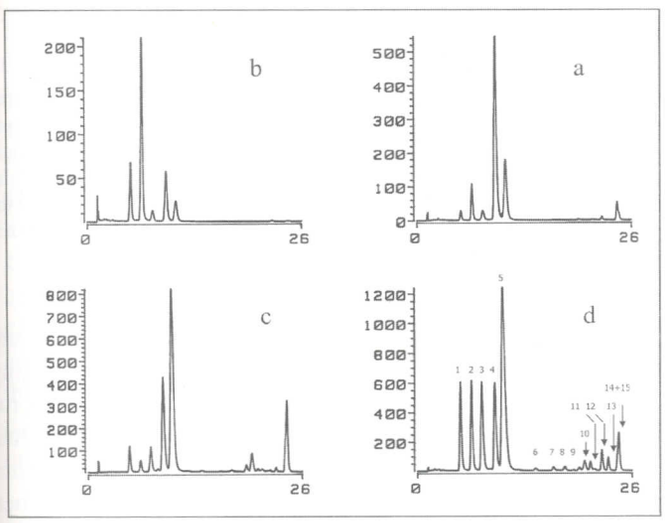

Fig. 1 - HPLC separation of the anthocyanins of a) 'Moscato rosa' (Italy); b) 'Roter Muskateller' (Germany); c) 'Moscato di Scanzo' (Italy); and d) 'Rosen Muskateller' (Germany) showing the wide differences existing in the profile of the anthocyanins of different varieties belonging to the 'Moscato' family.
Legend: x axis, time (min); y axis, absorbance (mau); 1= delphinidin 3-glucoside; 2= cyanidin 3-glucoside; 3= petunidin 3-glucoside; 4= peonidin 3-glucoside; 5= malvidin 3-glucoside; 6-10= acetic esters of 1-5; 11-15= p-coumaric esters of 1-5.

2. Materials and Methods

Microsatellite analysis

Leaf samples were collected from 19 accessions of 'Moscato rosa' field-grown in five viticultural areas in northern Italy and in Croatia (Table 1), among the vines selected for the evaluation of clonal variability. In addition to these accessions, two samples of 'Moscato rosa' grapevines from Umag and Poreč (Croatia), kindly supplied by G. Colugnati (ERSA, Gorizia), were included in the analysis.

Total DNA was extracted following the procedure described by Doyle and Doyle (1990) with the modifications introduced by Steenkamp *et al.* (1994).

To assess the genetic identity at varietal level, the 21 'Moscato rosa' individuals have been compared at the following microsatellite loci: VVS2 (Thomas and Scott, 1993), VVMD5, VVMD7 (Bowers *et al.*, 1996), VVMD25, VVMD27, VVMD28, VVMD31 (Bowers *et al.*, 1999), VrZAG62 and VrZAG79 (Sefc *et al.*, 1999).

PCR was performed in a 25 µl mixture containing 100-120 ng of genomic DNA, 0.5 U Taq DNA polymerase (Bioline), 1X reaction buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl pH 8.8, 0.1% Tween-20), 1.5 mM Mg^{2+}, 0.2 µM of each primer and 100 µM of each dNTP.

One primer of each pair was fluorescently labelled with Dye Phosphoramidities (HEX, 6-FAM and TET, Abi Prism). PCRs were carried out using a Gene Amp PCR System 9600 (Perkin Elmer) under the following conditions: 5 min at 95°C; then 30 cycles of denaturation (45 s at 94°C), annealing (30 s at 50°C), extension (1 min 30 s at 72°C) with the primers VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79 and 35 cycles of denaturation (45 s at 94°C), annealing (45 s at 56°C), extension (1 min at 72°C) with the primers VVMD25, VVMD28, VVMD31; finally, 7 min at 72°C.

To confirm amplifications 6 µl of each reaction mix were checked on 1.5% ethidium bromide stained agarose gels. In order to separate and size SSR alleles, PCR products were analysed on the ABI 310 Genetic Analyzer running GeneScan software (2.1). To this purpose 0.5 µl of the amplified DNA were mixed with 12.5 µl of deionized formamide and 0.3 µl of the inter-

Table 1 - List of the 'Moscato rosa' accessions analysed in this study

Location of the vineyard source of plant material	Code of the mother plant (putative clone)			
Italy - Trentino - Mezzocorona	852	856	857	859
Italy - Trentino - San Michele all'Adige	865	866	868	869
Italy - Alto Adige - Nalles	872	874	876	877
Croatia - Istria - Grisignana	893	897	898	899
Croatia - Istria - Poreč	S1-19	S2-1	S3-8	
Croatia - Istria - Poreč (sample n. 20)				
Croatia - Istria - Umag (sample n. 21)				

nal lane standard (GeneScan 500 TAMRA) and were then denatured for 2 min at 95°C. The run was set at 60°C for 24 min (Grando and Frisinghelli, 1998).

Anthocyanin analysis

Berry samples of the 12 'Moscato rosa' vines selected in the vineyards from Trentino Alto Adige were collected at the ripeness, extracted with methanol, separated and analysed by high-performance liquid-chromatography (HPLC) and quantified at 520 nm, according to the method reported by Iacono *et al.* (1994) and extensively described in Mattivi (1997). Identification of the compounds was based on the relative retention times and on UV-VIS spectra, according to Castia *et al.*, (1992). Results were expressed as equivalent of malvidin 3-glucoside chloride, mg/kg of grapes. The characterisation of each plant as respect to its grape production (one year) is reported in Table 2.

3. Results

Microsatellite analysis

When compared at microsatellite loci, all the 19 'Moscato rosa' accessions in Table 1, as well as the two Croatian vines included in the clonal selection programme, produced the same profile of molecular markers (Table 3). If the base pair offset at each locus due to the different electrophoretic conditions used for allele sizing in the two laboratories is considered, the microsatellite pattern of 'Moscato rosa' resulted consistent with the genotype obtained by Maletič *et al.* (1999) for the cultivar 'Muškat Ruža Porečki'.

Anthocyanin analysis

The anthocyanin profiles of the 12 putative clones of 'Moscato rosa' from Trentino Alto Adige is shown in figure 2. In this figure, below the pattern reporting the absolute amounts, a graph describing the percentage of each class of anthocyanin out of the total amount is also reported. This figure facilitates understanding of the similarities, since the use of percentage as proposed by Mattivi *et al.* (1989), instead of the absolute amounts, allows for easier verification of similarities between vines belonging to the same variety when grown under different viticultural conditions.

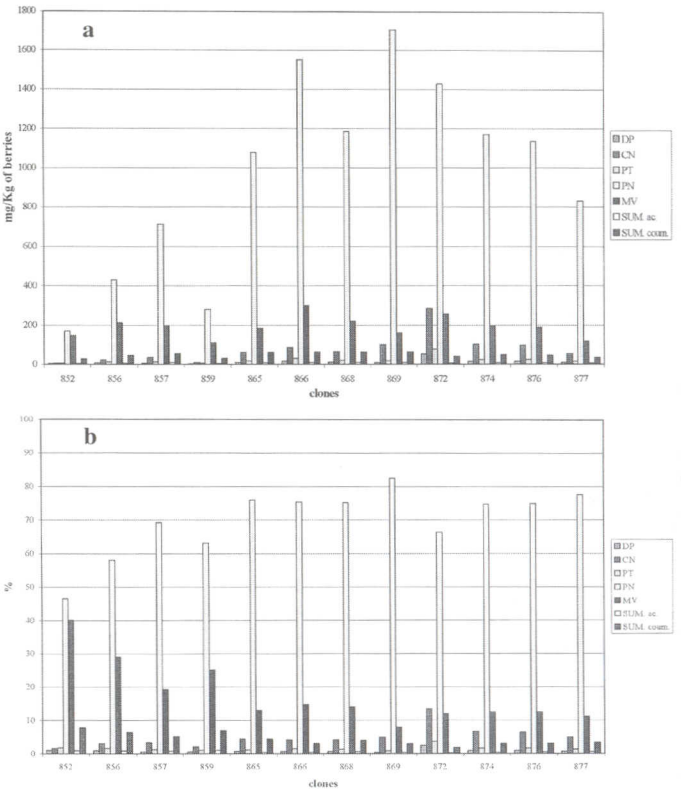

Fig. 2 - Anthocyanin content of the individual 'Moscato rosa' grapes expressed as absolute quantities (mg/kg of berries) (a) and percentage (b). DP= delphinidin 3-glucoside; CN= cyanidin 3-glucoside; PT= petunidin 3-glucoside; PN= peonidin 3-glucoside; MV= malvidin 3-glucoside; SUM. ac.=sum of the acetic acid esters; SUM. coum.=sum of the p-coumaric esters).

Table 2 - Agronomic characteristics of the 'Moscato rosa' individual plants from Trentino A. Adige

Agronomic characteristics	Code of the mother plant (putative clone)											
	852	856	857	859	865	866	868	869	872	874	876	877
Cluster/vine (N)	50	77	106	120	22	35	20	17	14	18	33	28
Yield/vine (Kg)	9.80	12.05	12.80	12.20	3.40	4.20	2.25	2.00	3.30	4.50	8.20	8.40
Cluster's weight (g)	196	156	121	102	154	120	112	117	236	250	248	300
Sugar of must (°Babo)	17.4	19.4	24.8	18.6	24.2	25.8	25.8	36.0	17.9	18.5	16.5	15.0
Total acidity (g/l)	9.50	8.70	10.60	10.80	11.00	11.00	11.20	10.40	8.14	12.66	12.32	11.37
pH	3.22	3.25	3.18	3.31	3.28	3.26	3.27	3.39	3.39	3.19	3.05	3.22

Table 3 - Genotype at microsatellite loci of the 'Moscato rosa' individuals analysed

Variety	Locus								
	VVS2	VVMD5	VVMD7	VVMD27	VrZAG62	VrZAG79	VVMD25	VVMD28	VVMD31
Moscato rosa (all 21 accessions listed in Table 1)	130 132	233 237	237 247	177 191	186 188	247 253	239 253	245 265	209 213
Mŭskat Ruža Porečki (Maletič *et al.*, 1999)	132 134	234 238	236 246	157 172 VrZAG47	185 187	248 254			

Table 4 - Anthocyanin content (mg/kg of berries) of the 'Moscato rosa' individual plants from Trentino Alto Adige

	Code of the mother plant (putative clone)											
Antocyanin	852	856	857	859	865	866	868	869	872	874	876	877
Delphinidin 3-glucoside	3.87	6.97	6.64	2.60	9.53	15.76	11.17	8.66	53.48	14.76	14.46	8.24
Cyanidin 3-glucoside	5.97	22.75	35.08	9.36	62.28	86.11	65.33	100.55	287.15	103.05	97.35	54.34
Petunidin 3-glucoside	6.70	12.35	12.81	5.06	16.79	31.42	20.77	18.10	78.12	24.67	24.14	14.43
Peonidin 3-glucoside	172.13	428.93	712.04	279.02	1079.26	1553.23	1185.69	1707.22	1427.89	1170.89	1137.31	832.40
Malvidin 3-glucoside	148.37	213.50	197.66	110.89	183.65	301.19	220.67	162.59	255.68	195.12	189.19	119.35
Sum of the monoglucosides	337.04	684.50	964.23	406.93	1351.51	1987.71	1503.63	1997.12	2102.32	1508.49	1462.45	1028.76
Sum of the acetic acid esters	3.72	6.58	8.34	4.64	6.85	9.88	8.83	8.64	5.95	7.79	7.90	6.58
Sum of the p-coumaric esters	29.08	47.06	54.17	30.59	62.83	64.48	63.77	63.22	40.33	49.43	46.77	36.75
Total anthocyanins	369.84	738.14	1026.74	442.16	1421.19	2062.07	1576.23	2068.98	2148.60	1565.71	1517.12	1072.09

The anthocyanin content of the grapes is shown in Table 4 and in figure 1. Under normal viticultural conditions for the vineyards and vintage considered, this character ranges typically between 1400 and 2100 mg/kg of grapes. All the samples analysed present the same peculiar pattern, with the main anthocyanin, the peonidin 3-glucoside (PN) being 4 to 10 times higher than the malvidin 3-glucoside (MV), the cyanidin 3-glucoside (CN) being the third compound, always higher than the sum of delphinidin- and petunidin 3-glucoside (DP + PT) and, finally, the sum of the acetic acid esters (SUM. ac.) being 6 to 10 times lower than the sum of the p-coumaric esters (SUM. coum.). Such data are in very good agreement with previous studies on the same variety (Mattivi *et al.*, 1989).

A few samples which were collected from vines having excessive production (clones 852, 856, 857, 859 in Tables 1 and 2) present both lower content of total anthocyanins in the grape skin and a pattern characterised by lower values of the ratios PN/MV, and CN/(DP+PT) thus indicating that stress conditions could increase the variability of the profile of the anthocyanins and, in particular, the relative abundance of di- and tri-substituted pigments.

4. Conclusions

Due to the high discriminative power of co-dominant DNA markers, the finding of identical genotypes in different plants is a strong evidence that these materials in fact belong to the same cultivar. In the present case, all the 'Moscato rosa' accessions collected in northern Italy and Croatia shared the same microsatellite genotype at the nine loci investigated. This finding certainly supports a common genetic origin of the individual vines to be further evaluated for clonal variation and selection.

Putative clones of 'Moscato rosa' also present a relatively similar pattern of the anthocyanins having the typical features of the known 'Moscato rosa' profile (Fig. 1 a). Some deviations observed from this profile are reasonably due to crop overload in the year of the study and deserve further confirmation. Indeed, the reduced anthocyanin contents under excessive crop load suggest that the cultural practices and environment can strongly affect the performance of these plants, which was consistent with the classification of 'Moscato rosa' into the "low yielding" grapevine varieties.

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