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Molecular characterization of twenty seeded and seedless table grape cultivars (*Vitis vinifera* L.)

by

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Summary: DNA and isoenzyme analyses were used to characterize 20 table grape cultivars including Moscato d'Amburgo, Italia, Sultanina, Bicane and some recently released new varieties. GPI and PGM isoenzyme systems were able to separate the cultivars into 9 groups whereas the 8 microsatellite loci that were analysed revealed a higher discriminating power. In fact, all the cultivars could be distinguished by DNA profiles except Sugraone from its sport Sugrafive. Parentage analysis confirmed that the cultivar Italia was obtained from the crossing Bicane x Moscato d'Amburgo. A difference was observed at one microsatellite locus between Sultanina and the published data for Thompson Seedless, considered to be its synonym. The different microsatellite loci were evaluated for their informativeness.

Key words: microsatellite, SSR, DNA typing, isoenzyme, cultivar.

Introduction

The EU Reg. No. 1592/96, distinguishing between the grapevine cultivars destined for wine making and the varieties for table grape consumption, stated that the latter are to be considered as fruit crops and thus their planting is not ruled by the limitations imposed for winegrapes within the EU. As a consequence, table grape cultivars cannot be used for wine making, except for a few limited areas.

The interest to cultivate new varieties is thus increasing, particularly in Italy, the most important producer of table grapes in the world, with an almost monovarietal cultivation. Despite the good quality of cv. Italia, the request for novelties has favoured, and will further favour, the production of new varieties, in particular seedless ones which had a larger diffusion, so far, in other parts of the world.

All this rises questions about the possibility of distinguishing and identifying table grape genotypes to avoid law infringement, to protect breeders and to safeguard consumers assuring quality products.

Molecular markers represent an objective tool for varietal characterization and give a relevant, sometimes decisive, contribution to identification (Bowers *et al.* 1993; Büscher *et al.* 1994; Tschammer and Zyprian 1994; Bourquin *et al.* 1995; Bowers and Meredith 1997). Among marker types, microsatellites analysed as Sequence Tagged Sites (STMS) have the necessary reliability and informativeness providing data easily exchangeable among the different research institutes (Thomas and Scott 1993; Cipriani *et al.* 1994; Bowers *et al.* 1996; Sefc *et al.* 1997, 1998).

Although DNA markers are usually preferred for genotype identification, the two isoenzymatic systems GPI (Glucose Phosphate Isomerase, E.C. 5.3.1.9) and PGM (Phospho Glucomutase, E.C. 5.4.2.2, *ex* E.C.2.7.5.1) proved to be reliable in comparative experiments (Calò *et al.* 1989) and are requested for the registration of the new cultivars in the National Italian Catalogue of Cultivated Variety (Catalo-go Nazionale delle Varietà di Viti), the official document which lists the varieties authorized for growing in Italy.

By using these two kinds of markers, a group of 20 important seeded and seedless table grapes were analyzed; 12 of them were registered long time ago in the National Italian Catalogue, while 7 are valuable crosses registered more recently (D.M. 01.09.1997).

Material and Methods

Leaf samples were harvested from 20 grapevine cultivars (Tab. 1) grown in the field collection of the Istituto Sperimentale per la Viticoltura in Conegliano. The listed cultivars are grouped according to their origin: the first 4 varieties are ancient, the following 9 are internationally known crosses and the last 7 cultivars are crosses recently obtained by the above mentioned institute (Calò *et al.* 1998).

Isoenzymatic analysis: The isoenzymatic analyses were performed on freshly collected leaves following the method previously described by Crespan *et al.* (1998).

STMS analysis: The procedure used for DNA extraction was the same as in Dellaporta *et al.* (1983) from step 1 to 7, except for the extraction buffer composition, which was integrated by adding polyvinylpyrrolidone (m.w. 10,000) (2 % w/v) and cysteine (60 mM). In addition, the DNA was further purified by two treatments with phenol:chloroform (Sambrook *et al.* 1989) and cleaned from RNA with RNase A.

The samples were analysed at the 8 microsatellite loci VVS1, VVS2, VVS4, VVS29 identified by Thomas and

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Table 1

List of the analysed table grape cultivars and general indications of their origin

Code No.*	Cultivar Old varieties	Colour**	Geographic or genetic origin	Reference
not registered	Bicane	w	unknown	VIALA and VERMOREL 1991
517	Moscato d'Amburgo	b	Great Britain	Ministero 1975
527	Regina	w	Anatolia	Ministero 1975
531	Sultanina bianca	w	Anatolia	Ministero 1975
	Well known crosses			
555	Conegliano precoce	b	Italia x Volta	Istituto 1989
558	Conegliano 218	b	Italia x Volta	Istituto 1989
514	Italia	w	Bicane x Moscato d'Amburgo	Istituto 1989
522	Perlette	w	Regina dei vigneti x Sultanina Marble	Istituto 1989
568	Perlon	b	Emperador x Perlette	Istituto 1989
551	Red flame	b	Cardinal x Sultanina x Red Malaga x Tifafhi Ahmer x (Zibibbo x Sultanina)	FREGONI 1998
552	Ruby Seedless	b	Emperor x Sultana moscata	
554	Sugraone	w	not declared, patent covered	Istituto 1989
553	Sugrafive	w	sport of Sugraone, patent covered	Istituto 1989
	More recent crosses			
578	Damina	w	not declared, patent pending	CALÒ *et al.* 1998
579	Fiorenza	w	not declared, patent pending	CALÒ *et al.* 1998
580	Helena	w	not declared, patent pending	CALÒ *et al.* 1998
581	Lara	w	not declared, patent pending	CALÒ *et al.* 1998
582	Maxia	w	not declared, patent pending	CALÒ *et al.* 1998
583	Paula	w	not declared, patent pending	CALÒ *et al.* 1998
584	Rubinia	b	not declared, patent pending	CALÒ *et al.* 1998

* Number which identifies each variety registered in the Italian Catalogue.
** Berry colour: w = white, b = black.

SCOTT (1993) and by THOMAS *et al.* (1994), and VVDM5, VVMD6, VVDM7, VVDM8 isolated and described by BOWERS *et al.* (1996). Amplification products were electrophoresed on a sequencing gel, then transferred onto a nylon membrane by Southern blotting and hybridized with the appropriate probe. The PCR reaction mixture (25 µl) contained: 50 ng total DNA, 0.5 U *Taq* DNA polymerase (HT Biotechnology, Cambridge, UK), 10 mM Tris HCl pH 9, 50 mM KCl, 0.01 % (w/v) gelatin, 0.1 % Triton X-100, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 10 pmoles of each primer for the VVS loci and 20 pmoles for the VVMD loci. The PCR was performed in a PTC-100 thermocycler (MJR) at the following conditions: 5 min at 95 °C, 6 min at 80 °C, then 25 cycles of denaturation (1 min at 94 °C), annealing (50 s at 55 °C), extension (1 min at 72 °C) with a final elongation step of 7 min at 72 °C.

For the electrophoresis 2.0 µl of each sample were denatured at 94 °C for 3 min in 2.0 µl loading buffer containing formamide and loaded on a sequencing gel (6 % polyacrylamide, TBE 1 x, urea 8 M).

Amplification products of cultivars having alleles of known molecular weight (THOMAS and SCOTT 1993; BOTTA *et al.* 1995) were used as reference molecular weight markers. Southern blotting was performed by capillary transfer using a Hybond N+ (Amersham) nylon membrane on the gel for 3 h. Bands were visualized by hybridization with either a $(GA)_{13}$ or a $(GT)_{13}$ probe, labelled with biotin at the 5' end, depending on the locus. In the case of loci VVS1 and VVMD6, amplifications were done using biotinilated primers and hybridization was unnecessary. The chemiluminescence detection was performed with the Southern-Light Chemiluminescent Detection System by Tropix (Perkin Elmer, USA), according to the manufacturer's instructions. The membrane was exposed on a X-Omat Kodak film for 1-4 h.

Data analysis: The informativeness of each microsatellite locus in the studied group of cultivars was evaluated by applying two indexes: n_e, effective number of alleles and δ_T, that estimates the proportion of the population that would carry different alleles at a locus (MORGANTE *et al.* 1994); they were computed by considering the homozygosity in the presence of single alleles at one locus.

Results and Discussion

Isoenzyme analysis: GPI and PGM patterns found for each of the 20 table grape cultivars were listed

Table 2

GPI and PGM codes of 20 table grape cultivars, according to CALÒ *et al.* 1989

Variety	GPI	PGM
Bicane	2	0
Moscato d'Amburgo	1	4
Regina	2	1
Sultanina bianca	2	4
Conegliano precoce	1	5
Conegliano 218	4	5
Italia	2	3
Perlette	3	4
Perlon	2	3
Red flame	3	4
Ruby Seedless	2	3
Sugrafive	1	5
Sugraone	1	5
Damina	2	3
Fiorenza	1	3
Helena	3	3
Lara	1	5
Maxia	1	3
Paula	3	4
Rubinia	2	3

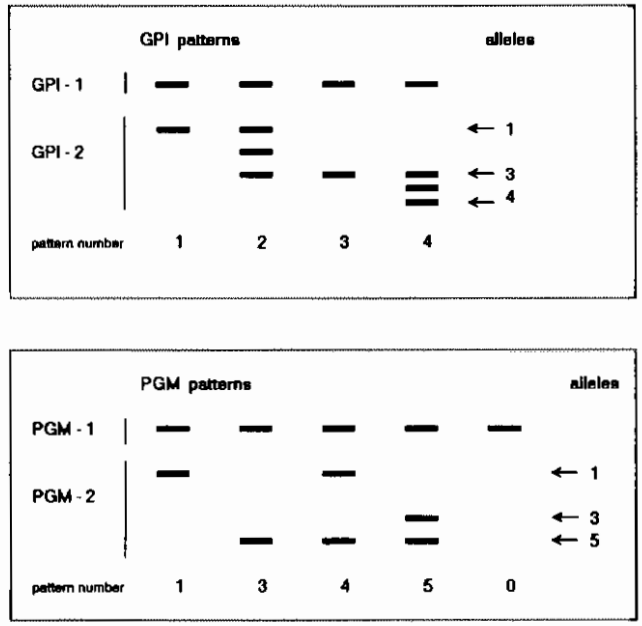

Fig. 1: GPI and PGM patterns found by analysing 20 table grape cultivars. The alleles are indicated by arrows.

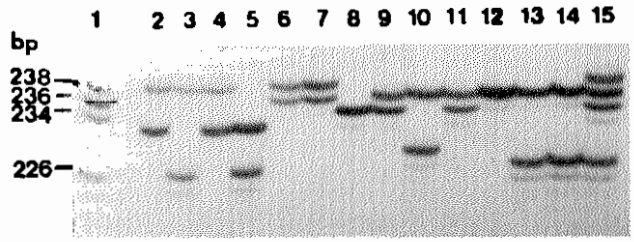

Fig. 2: Microsatellite STMS genotypes at the VVMD5 locus. Lane 1: Size standard; 2: Italia; 3: Bicane; 4: Moscato d'Amburgo; 5: Regina; 6: Conegliano precoce; 7: Conegliano 218; 8: Sultanina; 9: Red flame; 10: Ruby Seedless; 11: Perlette; 12: Perlon; 13: Sugrafive; 14: Sugraone; 15: Size standard.

(Tab. 2) using the numbers of the coded profiles according to CALÒ *et al.* (1989) and were drawn in Fig. 1 where the alleles, useful for comparing parents and crosses, were marked with arrows. The PGM profile attributed to cv. Bicane was indicated with "0" since, in spite of the repeated attempts, the analysis revealed only the PGM-1 plastidial band and no bands were detected for PGM-2.

The discriminating power of isoenzymes appeared immediately very interesting, although not high enough for a full resolution of the studied cultivars. They were in fact divided into 9 different groups, 5 of which included a single cultivar while the most numerous group (pattern GPI n. 2, PGM n. 3) comprised 5 varieties.

The varietal characterization performed in the collections at the Istituto Sperimentale per la Viticoltura in Conegliano allowed to identify 6 GPI-2 alleles and 6 PGM-2 alleles; in the studied table grape cultivars only three alleles were found for each isoenzymatic system: data related to allele frequencies computed on about 500 cultivars of table and wine grapes (CALÒ *et al.* 1991) showed that they are in fact the most common.

STMS analysis: The results of the DNA analyses (Fig. 2, Tabs. 3 and 4) performed on the 20 table grape cultivars, allowed to identify all varieties, except Sugrafive and Sugraone, which showed identical profiles for all the analyzed loci. Sugrafive is known to be a sport of Sugraone (FANIGLIULO 1998) with smaller berry size and earlier ripening. The distinction of Sugrafive as a separate cultivar may be an object of discussion since the two isoenzyme systems and the 8 SSR markers were not able to detect differences between Sugraone and its sport. Yet, this does not contradict the designation of Sugrafive as a different cultivar, since it shows stable and distinctive traits of commercial and agronomical interest, thus fitting the definition of cultivar, as stated in the International Code of Nomenclature for Cultivated Plants (FANIGLIULO 1995).

By comparing the DNA profiles of grapevines of ancient origin with those of crosses, it may be observed that there is higher variability in the first group (33 different alleles for 4 cultivars) in comparison with the second group (31 different alleles for 16 cultivars). This result can easily be explained, since these crosses often share some parents (*e.g.* Italia, Sultanina).

The present data on Sultanina bianca showed the presence of two alleles at the locus VVMD8 instead of one, as found by BOWERS *et al.* (1996) in Thompson Seedless. This result, which was confirmed on further three clones, may be due to the detection of clonal polymorphism at the locus or may support the hypothesis that Thompson Seedless is a cross obtained by Thompson between a *V. vinifera* cultivar and Sultanina bianca, as reported in early papers (LONGO 1948). Further investigations should run Thompson Seedless and Sultanina samples on the same gel, to directly com-

Table 3

DNA profiles of 13 table grape cultivars analysed at 8 microsatellite loci; allele length in base pairs

Locus	Bicane	Moscato d'Amburgo	Regina	Sultanina	Conegliano precoce	Conegliano 218	Italia	Perlette	Perlon	Red flame	Ruby Seedless	Sugraone	Sugrafive
VVS1	190-162	190-181	188-181	181-188	181-162	181-162	190-162	181-188	181	181	181	181	181
VVS2	137-133	149-135	135-133	151-145	135-133	151-133	149-133	145-133	135-133	151-133	151-133	135	135
VVS4	175-168	175-168	175-169	175	175-168	168	175-168	175-168	175-168	175	175	175-168	175-168
VVS29	181-171	179-171	171	179-171	179-171	179-171	181-171	179-171	179-171	179-171	179-171	179-171	179-171
VVMD5	238-226	238-232	232-226	234	238-236	238-236	238-232	236-234	236	236-234	236-228	236-226	236-226
VVMD6	214-212	214-212	212-210	214-212	214-212	214-212	214-212	214-212	212-210	214-212	214-212	214-212	214-212
VVMD7	249-243	249-247	249-239	253-239	249-243	249-247	247-243	253-247	247-243	253-239	249-239	249-239	249-239
VVMD8	143	157-141	147-143	157-145	141	141	141	157-143	143	157	0-0	157-143	157-143

Table 4

DNA profiles of 7 recently released table grape cultivars analysed at 8 microsatellite loci; allele length in base pairs

Locus	Damina	Fiorenza	Helena	Lara	Maxia	Paula	Rubinia
VVS1	162	190-188	181-162	181-190	181-162	181-162	181-162
VVS2	135-133	133	151-149	135-133	135-133	151-133	135-133
VVS4	175-168	175	175-168	175-168	168	175	175
VVS29	181-171	179-171	171	179-171	181-171	179-171	171
VVMD5	238	238-236	238-228	238-236	238-226	236-232	238-236
VVMD6	214-212	214-212	214-212	214-212	214-212	214-212	214-212
VVMD7	243	253-243	243-239	243-239	243-239	253-243	243-239
VVMD8	141	143	141	157	141-157	157	141

Table 5

Informativeness of 7 grapevine microsatellite loci analysed on 20 table grape cultivars

Locus	n	n_e	δ_T
VVS1	4	2.762	0.671
VVS2	6	3.584	0.758
VVS4	3	2.000	0.526
VVS29	3	2.247	0.583
VVMD5	6	4.310	0.807
VVMD6	3	2.202	0.574
VVMD7	5	4.504	0.818

n: number of alleles per locus;
n_e: effective number of alleles per locus;
δ_T: within-population differentiation.

pare allele sizes, and analyse other loci to look for polymorphisms.

The values of n_e and δ_T indexes (Tab. 5) were computed by considering the condition of homozygosity when a single allele was detected at one locus; these indexes were not calculated for VVMD8 locus for the probable presence of null alleles. The highest values of the indexes were found for the loci VVS2, VVMD5 and VVMD7 which thus resulted the most informative while the locus VVS4 was the least. As the number of individuals was low and as they were not chosen at random, these evaluations are valid strictly for the group of varieties analyzed here and may be generalized only with caution.

Parentage analysis: In this study it was possible to verify the parentage of the cultivar Italia (synonyms Incrocio Pirovano 65 or Ideal in France), obtained by Prof. Pirovano in 1911 by crossing Bicane x Moscato d'Amburgo. The isoenzyme patterns of Italia for both systems were consistent with the expected combinations inheritable from the parents and its DNA profile had at least one allele in common with each of the parents with the exception of locus VVMD8. In this case, in fact, Italia shared the allele 141 with Moscato d'Amburgo but showed probably a null allele which could derive from Bicane. Although it cannot be demonstrated with the present analyses only, this hypothesis is highly probable since null alleles at the locus VVMD8 were found in Ruby Seedless and are reported in the database by Bowers *et al.* (1996) for Greek cultivars.

Conclusions

Isoenzymes are useful markers for screening cultivars which need to be identified; they are interesting due to the simplicity and speed of their analysis and for an appreciable level of polymorphism and can be a precious, although not conclusive, help to ampelography.

Microsatellites, by having a higher discriminating power, confirmed to be very good markers for varietal characterization and identification, and also for verifying the origin of crosses. In this case some caution must be taken in the choice of the loci, to avoid null-allely cases; the definition of a common set of loci to be used in cultivar characterization and the construction of a reference molecular weight marker will allow proper and comparable evaluation of the allele sizes in all the analyses. This is a prerequisite to the building of a common international database.

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