

'Verdello', 'Verdicchio' and 'Verduschia': an example of integrated multidisciplinary study to clarify grapevine cultivar identity

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Key words: ampelography, 'Duopersico', SSR markers, 'Trebiano di Soave', 'Turbiana', 'Uva Angiola'.

Abstract: 'Verdello', 'Verdicchio' and 'Verduschia' are registered in the official Italian Catalogue as three distinct grapevine varieties. Twenty-five accessions of these cultivars, encompassing known or presumed synonyms, coming from CRA repositories and from vineyards where they are traditionally cultivated, have been genotyped with eleven SSR markers. For morphological comparison, one accession for each variety has been described with 57 characters of OIV 2009 list; phenological and yield traits have also been recorded. In addition, the phenotypic comparison has been extended to the literature descriptions. The same DNA profile has been obtained for all 25 accessions; moreover, present and historical ampelographic data showed a very high similarity. All this information leads to the conclusion that these three varieties are, in fact, the same cultivar.

1. Introduction

The spread of grapevine varieties through different countries over time has produced many of cases of synonymy that now need clarification. Correct identification is very important in order to gain more precise knowledge of the varietal assortment and to better manage grapevine catalogues and germplasm repositories.

The Italian Catalogue of Grapevine Varieties is one of the most copious in the world. Nonetheless, it contains various duplicates or triplicates, such as 'Alicante'/'Cannonao'/'Tocai rosso', 'Vermentino'/'Pigato'/'Favorita', 'Biancame'/'Trebiano toscano', 'Albarola'/'Bianchetta genovese'.

Also 'Verdicchio', one of the most prized white grape varieties of the Marche region (central Italy) and registered in the Italian Catalogue with code no. 254, has an officially recognized duplicate, 'Trebiano di Soave' (code no. 239), the name used in several provinces of the Veneto region (north-eastern Italy). Other synonyms in the same region are 'Trebiano di Lugana' and 'Turbiana'. In the Lazio region (central Italy) it is known as 'Trebiano

verde' (Bruni, 1962). To our knowledge, the first citation of 'Verdicchio' was by the botanist Costanzo Felici as far back as 1569 (in Arbizzoni, 1986).

'Verdello' is considered a minor grapevine variety of the Umbria region (central Italy). It is registered in the Italian Catalogue with code no. 253 and is used for the DOC wines of Orvieto, Colli Amerini, Colli del Trasimeno and Torgiano. Scalabrelli and Grasselli (1988) report that 'Verdello' is also cultivated in the south of Tuscany under the synonym 'Duopersico', mainly in the territory of Piti-gliano DOC, where it has been present for at least two centuries and contributes to the wine of the same name. The two authors refer that in the same territory a variety known as 'Uva Angiola' is also present; they generically link it to the Trebbiano group, but this variety more specifically resembles to those analyzed in the present study.

Furthermore, in the most northern part of Tuscany, a variety morphologically similar to 'Verdicchio' has been grown for a long time. It is called 'Verduschia' (Soderini, 1600; Acerbi, 1825), 'Verdella' or 'Verdarella', depending on the cultivation area. The diffusion of this variety is limited to the small Lunigiana territory (provinces of La Spezia and Massa Carrara) and to a few residual specimens in old vineyards (Scalabrelli and Dodi, 1998). 'Verduschia' falls into the category of minor grapevine varieties on the

Received for publication 16 February 2012.

Accepted for publication 31 May 2012.

verge of extinction; although registered in the Italian Catalogue since 1971 with code no. 297, it does not seem to have been propagated.

DNA genotyping allowed us to hypothesise an unrecognized synonymy among ‘Verdicchio’, ‘Verdello’ and ‘Verduschia’. In fact, based on previous results obtained with molecular analysis of some accessions of ‘Verdello’, interesting for the clonal selection of materials from Lazio, we found that they had the same profile of the ‘Verdicchio’ accession held in the Centro di ricerca per la viticoltura (CRA-VIT) repository. Given the interest aroused by this preliminary information, we extended the molecular comparison to numerous accessions of ‘Verdicchio’, ‘Verdello’ and their synonyms, as well as to ‘Verduschia’, the Tuscan variety morphologically similar to ‘Verdicchio’. The samples for genotypic comparison were singled out both in the CRA-VIT repositories and in the cultivation areas of each variety, for a total of 25 accessions, including a commercial clone of ‘Verdello’ (clone VCR1) and another of ‘Verdicchio bianco’ (clone R2). This study was integrated with morphological comparison: one accession for each variety was characterized with 57 descriptors of the OIV 2009 list; phenological and yield traits were also recorded. In addition, ampelographic comparison was extended to the literature descriptions of these three varieties: most of the traits reported in literature were retrieved and harmonized according to the OIV 2009 descriptors list used for examining the actual materials.

2. Materials and Methods

Plant material

Twenty-five accessions were sampled, coming from CRA-VIT repositories in Spresiano (TV) and Susegana (TV), from Unità di ricerca per la viticoltura (CRA-VIC) repositories in Arezzo, from the Veneto Agricoltura collection and from vineyards in Veneto, Tuscany, Umbria, Marche and Lazio regions (Table 1).

Ampelographic, phenological and yield data comparison

Three accessions were described: one ‘Verdello’ of Umbrian origin (accession no. 4 in Table 1) and one ‘Verduschia’ (no. 15 in Table 1) coming from Lunigiana (province of Massa Carrara), both held in the CRA-VIC germplasm collection in Arezzo, and one ‘Verdicchio’ coming from Marche (no. 25 in Table 1), held at the Azienda Poggio Gagliardo in Montescudaio (Pisa, Italy). Morphological descriptions of the three varieties were performed according to 57 descriptors (OIV, 2009); phyllometric analyses were also carried out on samples of 20 leaves per cultivar using SuperAmpelo software (Soldavini *et al.*, 2009).

The comparison was widened to the ‘Verdello’, ‘Verdicchio’ and ‘Verduschia’ descriptions given in the literature: in particular, the Umbrian ‘Verdello’ was described by Cartechini and Moretti (1989), the ‘Verdicchio’ from Marche by Bruni (1962) and the Tuscan ‘Verduschia’ by Breviglieri and Casini (1965). To facilitate the compari-

Table 1 - List of the analysed accessions

ID	Accession name	Provenance
1	Verdicchio	CRA-VIT repository
2	Verdello	CRA-VIT repository
3	Verdello clone VCR1	Vivai Cooperativi of Rauscedo (Pordenone) - Italy
4	Verdello covio 6	Orvieto (Terni) - Italy
5	Verdello sugano	Orvieto (Terni) - Italy
6	Verdello fausto 1	Porano (Terni) - Italy
7	Verdello 553	Ercolani farm, Capodimonte (Viterbo) - Italy
8	Verdello 550	Ercolani farm, Capodimonte (Viterbo) - Italy
9	Verdello 549	Ercolani farm, Capodimonte (Viterbo) - Italy
10	Verdello 514	Catercia farm, Capodimonte (Viterbo) - Italy
11	Verdello 405	Bianchi farm, Bagnoreggio (Viterbo) - Italy
12	Verdello 146 ar	Pitigliano (Grosseto) - Italy
13	Verdello	Lazio
14	Verdello	CRA-VIT repository
15	Verduschia	Aulla (Massa Carrara) - Italy
16	Verdella	Aulla (Massa Carrara) - Italy
17	Verdicchio carr 10	Terranuova Bracciolini (Arezzo) - Italy
18	Turbiana	Vicenza - Italy
19	Trebbiano verde	Viterbo - Italy
20	Trebbiano di Soave	CRA-VIT repository
21	Trebbiano di Soave	CRA-VIT repository
22	Trebbiano di Lugana	Veneto
23	Duropersico 165 ar	Pitigliano (Grosseto) - Italy
24	Uva angiola	CRA-VIT repository
25	Verdicchio clone R2	Marche

son, all these descriptions were standardized according to the OIV 2009 descriptor list.

DNA extraction

Total genomic DNA was extracted from young leaves of the 25 accessions using NucleoSpin® 8 Plant kit (MACHEREY-NAGEL GmbH, Düren, Germany) automated on the Microlab® STAR liquid handling robot according to the MACHEREY-NAGEL NucleoSpin® 8 Plant protocol. DNA concentration and quality were assessed with a spectrophotometer and by 1% agarose gel electrophoresis. DNA samples were then diluted to 10 ng/μl prior to amplification.

SSR analysis

SSR analysis was performed in order to verify the varietal identity of the studied accessions. Eleven microsatellite loci were analyzed: the six core loci selected within Genres 081 European Project (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79) (This *et al.*, 2004); VVMD28 (Bowers *et al.*, 1999); ISV2 (VMC6e1), ISV3 (VMC6f1) and ISV4 (VMC6g1) (Crespan, 2003); VMCNG4b9 (Welter *et al.*, 2007).

A multiplex PCR mixture was prepared, amplifying simultaneously all 11 SSR loci. The reaction mixture consisted of: 1 X PCR buffer (Promega; Pharmacia Biotech), 200 μM of each dNTPs, 1 U of Taq DNA polymerase (Promega; Pharmacia Biotech), 2.0 mM MgCl₂ and the primer concentrations ranged between 0.09 μM and 0.40 μM, according to signal intensity; the forward primers were labelled with 6-FAM, VIC, PET or NED fluorescent

dyes; the final volume was 12.5 µl. PCR was carried out in GeneAMP 9700 (Applied Biosystems) with the following thermal profile: 2 min at 94°C, followed by 30 cycles at 94°C for 45 sec, 55°C for 1 min and 30 sec, 72°C for 1 min and a final step at 72°C for 30 min.

PCR products (1 µl) were added to 0.1 µl LIZ 500 size standard and 8.9 µl Hi-Di formamide (Applied Biosystems) and separated by capillary electrophoresis using an ABI Prism 3110xl DNA analyzer (Applied Biosystems) and POP-7 polymer (Applied Biosystems). After data collection, genotyping analysis was performed with ABI Prism® GeneMapper™ software version 3.0.

3. Results

The results of the ampelographic comparison between ‘Verdello’, ‘Verdicchio’ and ‘Verduschia’ are reported in Table 2. The descriptions from the present study show that the three grapevine varieties share 47 out of 57 traits. Ten diverge (in bold in Table 2): in particular, ‘Verdello’ differs from ‘Verdicchio’ and ‘Verduschia’ by having a slight bronze tinge on the young leaf and the mature leaf with less pronounced lower lobes and shorter petiole compared to the length of the middle vein. ‘Verdicchio’ showed a larger average size of the mature leaf, a longer middle vein N1 and a greater density of prostrate hairs on the lower side of

the blade (descriptor no. 84), as well as longer internodes (no. 353). Finally, three morphological traits differ in ‘Verduschia’ with respect to ‘Verdello’ and ‘Verdicchio’: the dorsal side of the shoot is green (OIV codes 007 and 009) and berry size is smaller. Therefore, comparison of our descriptions shows a substantial analogy among these varieties, which share 82% of the expression levels, with very minor discrepancies. Their similarity is also confirmed by the elaboration of the mature leaf measurements with SuperAmpelo software: the cluster analysis performed by the program shows that the degree of similarity is even higher and above 93% (Fig. 1).

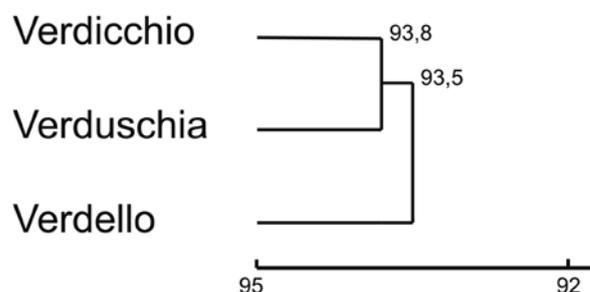


Fig. 1 - Cluster analysis of ‘Verdello’, ‘Verdicchio’ and ‘Verduschia’ phyllometric data elaborated with SuperAmpelo software. Percentage of similarity values are indicated.

Table 2 - List of ampelographic traits of the varieties ‘Verdello’, ‘Verdicchio’ and ‘Verduschia’. There are two types of comparisons: i) among current descriptions: discordant characters observed in the present study are written in bold; ii) among descriptions from other authors: slightly discordant traits inferred from the literature are underlined; clearly discordant characters are underlined in italics. An asterisk indicates the characters defined by the software SuperAmpelo. nr: not recorded character.

Organ	OIV Code 2009	Description	Verdello		Verdicchio		Verduschia	
			present study	Cartechini and Moretti, 1989	present study	Bruni, 1962	present study	Breviglieri and Casini, 1965
shoot	002	young shoot: distribution of anthocyanin coloration on prostrate hairs of the shoot tip	absent	<u>absent</u>	absent	<u>absent</u>	absent	<u>piping</u>
	003	young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip	none or very low	<u>none or very low</u>	none or very low	<u>none or very low</u>	none or very low	<u>low</u>
	004	young shoot: density of prostrate hairs on the shoot tip	medium	<u>very high</u>	medium	<u>very high</u>	medium	<u>medium or very high</u>
	006	attitude	semi-erect	semi-erect	semi-erect	semi-erect	semi-erect	semi-erect
	007	colour of the dorsal side of internodes	green and red	<u>green and red</u>	green and red	<u>green and red</u>	green	<u>green</u>
	008	colour of the ventral side of internodes	green and red	green and red	green and red	green and red	green and red	green and red
	009	colour of the dorsal side of nodes	green and red	<u>green and red</u>	green and red	<u>green and red</u>	green	<u>green</u>
	010	colour of the ventral side of nodes	green and red or red	<u>green</u>	green and red or red	<u>green and red</u>	green and red	<u>green and red</u>
	013	density of prostrate hairs on nodes	low	<u>none or very low</u>	low	<u>none or very low</u>	low	<u>low</u>
	015_2	intensity of anthocyanin coloration on the bud scales	weak	weak	weak	nr	weak	nr
016	number of consecutive tendrils	2 or less	2 or less	2 or less	2 or less	2 or less	2 or less	

Organ	OIV Code 2009	Description	Verdello		Verdicchio		Verduschia	
			present study	Cartechini and Moretti, 1989	present study	Bruni, 1962	present study	Breviglieri and Casini, 1965
young leaf	051	colour of upper side of blade (4th leaf)	green / bronze / yellow	<u>green</u>	green / yellow	<u>green / bronze</u>	green / yellow	<u>green</u>
	053	density of prostrate hairs between main veins on lower side of blade (4th leaf)	high	very high	high	very high	high	very high
	055	density of prostrate hairs on main veins on lower side of blade (4th leaf)	low	low	low	nr	low	nr
mature leaf	065	size of blade	medium-small*	<u>medium</u>	medium*	<u>medium</u>	medium-small*	<u>medium-small</u>
	067	shape of blade	wedge-shaped or pentagonal*	<u>circular</u>	wedge-shaped or pentagonal*	<u>circular or pentagonal</u>	wedge-shaped or pentagonal*	<u>pentagonal</u>
	068	number of lobes	five or three	<u>five</u>	five	<u>five or three</u>	five	<u>five or three</u>
	069	colour of the upper side of the blade	between medium green and dark green	<u>medium green</u>	between medium green and dark green	<u>between medium green and dark green</u>	between medium green and dark green	<u>dark green</u>
	070	area of anthocyanin coloration of main veins on upper side of blade	absent	absent	absent	absent	absent	absent
	071	area of anthocyanin coloration of main veins on lower side of blade	absent	absent	absent	absent	absent	absent
	073	undulation of blade between main or lateral veins	present	present	present	present	present	present
	074	profile of blade in cross section	involute, V-shaped or twisted	<u>V-shaped</u>	involute, V-shaped or twisted	<u>flat or twisted</u>	involute, V-shaped or twisted	<u>flat or twisted</u>
	075	blistering of upper side of blade	medium	<u>weak</u>	medium	<u>medium</u>	medium	nr
	076	shape of teeth	mixture between straight and convex sides	<u>one side concave, one side convex</u>	mixture between straight and convex sides	<u>mixture between straight and convex sides</u>	mixture between straight and convex sides	<u>both sides convex</u>
	078	length of teeth compared with their width	short	short	short	short	short	short
	079	degree of opening/overlapping petiole sinus	overlapped	<u>strongly overlapped</u>	overlapped	<u>closed or overlapped</u>	overlapped	<u>overlapped</u>
	084	density of prostrate hairs between main veins on lower side of blade	medium	<u>medium</u>	high	<u>very high</u>	medium	<u>very high</u>
	086	density of prostrate hairs on main veins on lower side of blade	low	<u>low</u>	low	<u>high</u>	low	nr
	090	density of prostrate hairs on petiole	low	none or very low	low	none or very low	low	none or very low
	093	length of petiole compared to length of middle vein (N1)	shorter than N1	longer than N1	equal to N1	nr	equal to N1	nr
	601	length of vein N1	short*	nr	medium*	nr	short*	nr
602	length of vein N2	medium*	nr	medium*	nr	medium*	nr	
603	length of vein N3	medium*	nr	medium*	nr	medium*	nr	
604	length of vein N4	very long*	nr	very long*	nr	very long*	nr	
woody shoot	101	cross section	circular or elliptic	<u>circular</u>	circular or elliptic	<u>circular or elliptic</u>	circular or elliptic	<u>circular or elliptic</u>
	103	main colour	brownish	<u>brownish</u>	brownish	<u>between brownish and grey</u>	brownish	<u>brownish</u>
inflorescence	151	flower: sexual organs	hermaphrodite	hermaphrodite	hermaphrodite	hermaphrodite	hermaphrodite	hermaphrodite
	152	insertion of 1st inflorescence	3rd and 4th node	3rd and 4th node	3rd and 4th node	3rd and 4th node	3rd and 4th node	3rd and 4th node
	153	number of inflorescences per shoot	1.1 to 2	1.1 to 2	1.1 to 2	1.1 to 2	1.1 to 2	1.1 to 2

Organ	OIV Code 2009	Description	Verdello		Verdicchio		Verduschia	
			present study	Cartechini and Moretti, 1989	present study	Bruni, 1962	present study	Breviglieri and Casini, 1965
bunch	202	length	medium-long	<u>medium</u>	medium-long	<u>medium-long</u>	medium-long	<u>long</u>
	204	density	dense	<i>very dense</i>	dense	<i>dense or medium</i>	dense	<i>medium</i>
	206	length of peduncle	short	<i>very short</i>	short	<i>medium</i>	short	<i>short</i>
	208	shape	conical / funnel shaped / cylindrical	<u>conical</u>	conical / funnel shaped / cylindrical	<u>conical or cylindrical-conical</u>	conical / funnel shaped / cylindrical	<u>conical</u>
	209	number of wings of the primary bunch	1-2/3-4	with wings	1-2/3-4 wings	with wings	1-2/3-4	with wings
berry	220	length	medium	<u>medium</u>	medium	<u>medium</u>	between medium and short	<u>short</u>
	222	uniformity of size	uniform	uniform	uniform	uniform	uniform	uniform
	223	shape	globose	globose	globose	globose	globose	globose
	225	colour of skin	yellow	yellow	yellow	yellow	yellow	yellow
	227	bloom	medium	medium	medium	medium	medium	medium
	228	thickness of skin	thin	<i>thick</i>	thin	<i>thin</i>	thin	<i>thick</i>
	229	hilum	visible	visible	visible	visible	visible	visible
	241	formation of seeds	complete	complete	complete	complete	complete	complete
vegetation	306	autumn coloration of leaves	yellow	yellow	yellow	yellow	yellow	yellow
	351	vigour of shoot growth	medium-strong	<u>medium</u>	medium-strong	<u>medium-strong</u>	medium-strong	<u>medium-strong</u>
	352	growth of lateral shoots	weak	<u>weak</u>	weak	<u>medium</u>	weak	<u>weak</u>
	353	length of internodes	medium	<u>long</u>	long	<u>medium-long</u>	medium	<u>medium</u>
	354	diameter of internodes	medium	<u>small</u>	medium	<u>medium</u>	medium	<u>medium</u>

With regard to the descriptions from the literature, most of the 57 OIV descriptors we used were retrieved. Only the measurements from code 601 to 604 relative to the mature leaf, three traits of ‘Verdicchio’ and five of ‘Verduschia’ were excluded (indicated as not recorded characters in Table 2) and so a total of 50 descriptors for ‘Verdello’ and ‘Verdicchio’ and 48 for ‘Verduschia’ were taken into consideration. As expected, the literature data comparison highlighted greater differences, given that the three descriptions were made by different authors. Twenty-eight descriptors, underlined in Table 2, showed slightly discordant expression levels, as they are limited to a single interval or with different coexisting or intermediate expression levels. These are mainly characters whose expression may be influenced by environmental factors like the exposure to the light or by cropping factors and vegetative vigour, in addition to the subjectivity of the observer. Other descriptors (7 out of 52), underlined in italics in Table 2, showed markedly different expression levels. Four of them regard the mature leaf and in particular the density of prostrate hairs on the lower side of the blade (no. 84 and 86) and the profile and shape of the teeth (no. 74 and 76); the other three regard the bunch and the berry (no. 204, bunch density; no. 206, peduncle length; no. 228, thickness of skin).

Comparing this last group of seven morphological features with the set of results from our field measurements, it appears that our descriptions match better with those of ‘Verdicchio’ described by Bruni (1962); instead, fewer similarities are noted with the descriptions in the literature for ‘Verduschia’ and even fewer for ‘Verdello’.

Lastly, the comparison of the phenological and yield data collected in the two environments [Arezzo and Montescudaio (Pisa)] are reported in Table 3. The data obtained from the same references for ampelographic descriptions are also shown for comparison and ‘Trebbiano toscano’ is proposed as common reference variety to enhance data significance. ‘Verduschia’ differs by the longer vegetative cycle and the lower berry weight. Instead, there are no relevant differences between the other two varieties.

Molecular analysis of the 25 accessions listed in Table 1 produced the same DNA profile, reported in Table 4 together with ‘Sangiovese’, ‘Pinot noir’ and ‘Muscat blanc à petits grains’ profiles, proposed as reference varieties for easier data comparison. To our knowledge this is the first time that the synonymy with ‘Trebbiano verde’ from Lazio, ‘Duropersico’, ‘Verduschia’ or ‘Verdella’ and ‘Uva Angiola’ from Tuscany and ‘Turbiana’ from Veneto, is confirmed by molecular data.

Table 3 - Phenology and yield data for ‘Verdello’, ‘Verdicchio’ and ‘Verduschia’: average of 2007-2010 for the vineyard in Arezzo, average of 2004-2008 for the vineyard in Montescudaio (Pisa). The data from some reference ampelographic descriptions are also shown for comparison. ‘Trebiano toscano’ is proposed as common reference variety to enhance data significance

	‘Verdello’		‘Verdicchio’		‘Verduschia’		‘Trebiano toscano’	
Place of data collection	Arezzo ¹	Orvieto (Terni) ²	Montescudaio (Pisa) ³	Iesi (Ancona) ⁴	Arezzo ¹	Firenze ⁵	Arezzo ¹	Montescudaio (Pisa) ³
Bud burst	13 April (medium)	medium	7 April (medium)	medium-late	12 April (medium)	10 - 20 April (medium)	16 April (medium)	7 April (medium)
Flowering	3 June (medium - early)	medium	2 June (medium -early)	early	7 June (medium)	1 - 10 June (medium)	8 June (medium)	3 June (medium)
Veraison	11 August (medium)	medium	5 August (medium)	medium	13 August (medium late)	21 - 31 August (late)	17 August (medium late)	6 August (medium)
Ripening	25 September (medium)	late	20 September (medium)	medium-late	27 September (late)	1 - 10 October (late)	4 October (late)	28 September (late)
Average weight of the bunch (g ± SD)	320±95	269	455±43	280	352±66	245	357±51	402±60
Average weight of the berry (g ± SD)	1.87±0.18	1.80	1.94	2.05	1.60±0.64	0.80	1.71±0.21	1.82±0.19
° Brix (± SD)	22.0±1.2	22.3	21.4±0.6	20.0	20.6±0.7	21.0	18.9±1.2	21.1±0.8
Titrateable acidity (g/l ± SD)	6.71±1.36	8.90	6.80±0.40	8.25	6.42± 0.75	6.97	7.32±0.48	5.29±0.62

¹ CRA-VIC repository, Lon: 11°49’29” E, Lat: 43°28’30” N.

² Data from Cartechini and Moretti (1989). Lon: 12° 12’ E, Lat: 42° 42’ N.

³ Poggio Gagliardo Farm, Lon: 10°32’53” E, Lat: 43°18’52” N.

⁴ Data from Bruni (1962), Lon: 13° 11’ E, Lat: 43° 38’ N.

⁵ Data from Breviglieri and Casini (1965), Lon: 11°19’21” E, Lat: 43°45’11” N.

Table 4 - SSR profiles of ‘Verdicchio’/‘Verdello’/‘Verduschia’ and three cultivars proposed as reference to favour comparison with other databases

SSR loci	Verdicchio, Verdello and Verduschia	Sangiovese	Pinot noir	Muscat blanc à petits grains
VVS2	133	133	137	133
	155	133	151	133
VVMD5	228	226	228	228
	240	236	238	236
VVMD7	239	239	239	233
	247	263	243	249
VVMD27	179	179	185	179
	185	185	189	194
VVMD28	239	237	221	249
	261	247	239	271
VrZAG62	195	193	187	185
	195	195	193	195
VrZAG79	248	242	238	250
	256	258	244	254
ISV2 (VMC6e1)	165	143	151	141
	165	165	165	143
ISV3 (VMC6f1)	135	139	133	133
	139	139	145	139
ISV4 (VMC6g1)	169	177	169	169
	197	197	177	187
VMCNG4b9	164	158	158	158
	166	168	162	166

4. Discussion and Conclusions

The large number of accessions analyzed, most of which come from traditional cultivation areas, together with the results of the ampelographic comparisons, allow us to affirm that 'Verdicchio', 'Verdello' and 'Verduschia' represent a new group of synonyms. This discovery is important because the three varieties are registered as distinct in the Italian Catalogue. This is further supported by the results of the molecular analysis on the commercial clone of 'Verdello' (clone VCR1), which showed to be identical to the other 'Verdello' accessions analyzed in this study.

It emerges from present work that the extent of the cultivation area in Italy and the interest in 'Verdicchio'/'Verdello'/'Verduschia' are greater than previously supposed from the information available on the already known synonyms. According to the Italian census of Agriculture Data (ISTAT, 2004) the total area under 'Verdicchio', 'Verdello', 'Verduschia' and 'Trebiano di Soave' cultivation in Italy is about 6000 ha, mainly in Marche (53%) and Veneto (30%) and, to a much lesser extent, in Umbria (8.3%) and Lazio (1.6%). Cultivation is also authorized in Emilia Romagna, Tuscany, Abruzzo, Molise and even in Sardinia. The success with which this variety has been cultivated for centuries in central and north-eastern Italy is obvious; the broad and historic diversification of the denominations, even in very close areas, supports the old age of the variety and also the interest in the locally produced wines, with recognised quality.

The synonymy 'Verdicchio'/'Verdello'/'Verduschia' is added to a growing number of redundancies found in the Italian Catalogue and highlights the value of molecular analysis to facilitate rapid comparison among varieties. This tool has been very useful in revealing other, never previously suspected cases of synonymy, such as 'Greco di Tufo' and 'Asprinio' (Costantini *et al.*, 2005), 'Malvasia delle Lipari' and 'Malvasia di Sardegna' (Crespan *et al.*, 2006), or to definitively clarify long disputed synonymies, such as 'Malvasia nera di Brindisi' and 'Malvasia nera di Lecce' (Crespan *et al.*, 2008; Gasparro *et al.*, 2008).

It is extremely difficult to hypothesize the centre of diffusion of 'Verdicchio'/'Verdello'/'Verduschia', also because the pedigree of this variety is unknown. One not scientifically supported hypothesis infers that this cultivar arrived in Marche from northern Italy in the second half of the 15th century, with colonies of farmers from Veneto and Lombardy, and from there it moved to Lazio, to finally turn up in Tuscany (Pollini, 2006).

The discovery of this new group of synonyms has been greatly facilitated by the use of SSR markers, which suggest comparisons among varieties independently from preliminary ampelographic indications. Given the wealth of varieties registered in the Italian Catalogue, molecular analysis, associated with the building of a reliable and complete molecular database of reference, has been shown to be very useful to highlight redundancies. Ampelographic comparison is indispensable to confirm the preliminary data acquired via molecular fingerprinting. Indeed, the

definition of a cultivated variety is tied to the DUS criteria (Distinctness, Uniformity and Stability), since the somatic mutants for agronomically important characters are legally registered as separate cultivars, as are mutants for berry colour or earliness of ripening. Lastly, genotyping represents a strategic tool in order to avoid the recording of duplicates in the future.

Acknowledgements

This research was financed by the Ministry of Agricultural, Food and Forestry Policies as part of the RGV-FAO and ASER-IDENTIVIT projects, and with funds from the regional agency 'Veneto Agricoltura'. The authors thank Francesco Anaclerio (VCR) for providing the clone of 'Verdello' VCR1 and Marina Niero for the information on the clonal selection of 'Verdello' in the Lazio region.

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