

Recognition and genotyping of minor germplasm of Friuli Venezia Giulia revealed high diversity

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Summary

The wealth of vine varieties that used to exist in Friuli Venezia Giulia has been progressively lost. In order to ascertain the current situation regarding vine germplasm in the region, between 2001 and 2008, a wide-ranging study was conducted of recovery, conservation and molecular characterization with microsatellite markers (SSR) of accessions of minor germplasm at risk of erosion or extinction. A total of 178 accessions were analyzed and 93 varieties identified. Of these, 44 are already registered in the Italian Catalogue, 8 have been imported and are well-known foreign varieties even if not registered in the Italian Catalogue, 38 are potentially autochthonous and of these only 15 are described or at least cited in the literature; there are no hypotheses on the remaining three. In order to obtain information on possible genetic similarities, three types of software were used to process the molecular data, but only Structure allowed the existence of two principal groupings to be hypothesized for some of the presumed Friuli autochthons: one that gravitates around ‘Prosecco’ and the other around ‘Refoscone’.

Key words: SSR, microsatellite, minor varieties, endangered cultivars, genetic erosion.

Introduction

Friuli Venezia Giulia (North-East Italy) has a long and consolidated viticultural-enological tradition whose fame extends well beyond the regional and national borders. Nevertheless, in quantitative terms, production is concentrated on very few varieties. From the 2007 data, made available by “Vine card index of Friuli Venezia Giulia region”, we know that on a total land area of slightly less than 20,000 hectares, 7 varieties cover 78 %, with a prevalence of ‘Pinot gris’ (24 %) and ‘Merlot’ (18 %). Areas of more than 1000 ha are cultivated with ‘Tocai friulano’, ‘Chardonnay’ and ‘Sauvignon’ for the whites, ‘Cabernet franc’ and ‘Cabernet Sauvignon’ for the reds. Smaller surfaces are cultivated with ‘Pinot bianco’, ‘Verduzzo friulano’, ‘Prosecco’, ‘Ribolla gialla’, ‘Traminer’, ‘Malvasia istriana’, ‘Riesling italico’, ‘Picolit’, ‘Riesling renano’ and ‘Vitouska’ among the whites, ‘Refosco dal peduncolo rosso’, ‘Pinot nero’, ‘Schioppettino’, ‘Pignolo’, ‘Refosco nostrano’ and ‘Terrano’ among the reds.

We know from the literature that a notable varietal wealth existed in Friuli Venezia Giulia (FVG) in the past, which has gradually been lost. Market globalization has led to production becoming concentrated on a few well-known varieties, with the consequence of strong competition for FVG wine-growers. One possibility for gaining an extra market share could be the cultivation of some traditional local varieties. This product diversification, which is slowly gaining ground, will lead to a strengthening of the bonds between wine and territory through the rediscovery and appreciation of age-old tastes. With adequate marketing strategies, this could also allow the opening of new commercial outlets, and lesser-known wines can be an effective eno-gastronomic tourist attraction. It should also be emphasized that the survival of the minor varieties over time has a high selective value, because they are plants adapted to the specific environments where they are found. This suggests interesting implications regarding the problems of environmental impact linked to plant protection in vineyards, with the possibility of reducing management costs and pesticide use through the selection of genotypes less susceptible at least to some pests and diseases.

Based on these premises, ERSa and CRA-VIT embarked on a wide-ranging work of recovery, conservation and molecular characterization with microsatellite markers (SSR) of accessions of minor germplasm found in the territory of FVG, mainly Centre and East part of the region, and at risk of erosion or extinction.

The aim in the first instance was to identify, preserve and describe the historical, ampelographic, agronomic and enological characteristics of the materials presumed to be native, to understand the extent and characteristics of the varietal heritage currently present in FVG. This paper reports the results relating to the genotyping and the association of at least some of the material with the historically known names. The genetic variability of the material was also evaluated and the existence of any genetic groupings.

Material and Methods

V e g e t a l m a t e r i a l : During eight years of research, from 2001 to 2008, with the aid of technicians and enthusiasts, as well as the existing collections at the University of Udine (Italy) and the “Rinascita” Farm of the Provincial Administration of Pordenone (Italy), a total of 178 accessions were sampled. In addition to the cited collections, the work of retrieval regarded old vineyards, sin-

gle vine rows marking boundaries between holdings, ditches, vegetable gardens and any other places where plants might be identified with characteristics differing from the principal varieties cultivated in the region.

After DNA analysis varieties that resulted as being original were rescued in a conservation field at Ersagricola's Pantianico Farm. Over three years data were gathered on the phenology, yield, composition characteristics of the berries and sensorial properties of the wines.

DNA analysis: DNA was extracted from young leaves and genotyping was performed with 11 SSR loci routinely employed at CRA-VIT for cultivar identification (VVS2, THOMAS and SCOTT 1993); VVMD5, VVMD7, VVMD27 and VVMD28, BOWERS *et al.* 1996 and 1999; VrZAG62 and VrZAG79, (SEFC *et al.* 1999); ISV2, ISV3, ISV4 (CRESPIAN 2003) and VMCNG4b9 (WELTER *et al.* 2007). The PCR reaction mixture (25 μ L final volume) contained 20 ng total DNA, 10 μ l Eppendorf HotMasterMix (2.5 x) and 5-10 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. On the basis of signal intensity on agarose gel, 0.75-1.5 μ L of amplified DNA were used for electrophoresis onto a sequencing gel (5 % polyacrylamide, 1 x TBE, 7 M urea). Amplification products of cultivars with alleles of known molecular size were used as references for allele sizing. Allele bands were revealed by silver staining and visually scored at least twice, as reported in CRESPIAN and MILANI (2001).

Statistics on SSR data: SSR markers statistics were computed with Identity 1.0 software, *i.e.* number of alleles/locus, allele frequencies, expected and observed heterozygosity, probability of null alleles, and probability of identity (PI). The power of discrimination ($PD = 1 - \sum g_i^2$, where g_i is the frequency of the i^{th} genotype) was calculated for each locus using the genotype frequencies computed with Excel program.

Genotypes correlations: Three different methods were used to determine the genetic relations among the genotypes found: 1) the Analysis of Functional Correspondence (AFC) was performed with GENETIX software (BELKHIR *et al.* 2003), available free at <http://www.univ-montp2.fr/%7Egenetix/genetix/genetix.htm> to compare all the genotypes listed in Tab. 1; 2) to focus on the relationships among all the genotypes found, the dissimilarity index calculated as $-\ln$ (proportion of shared alleles) (DANGL *et al.* 2001) was used to measure the genetic distance between all pairwise combinations. A dendrogram of all these genotypes was elaborated with the UPGMA method and the cophenetic correlation was computed with the NTSYS-pc program; 3) to find indications on population structure. We set the following parameters: length of burn-in period 100,000 generations and 10^6 Markov chain Monte Carlo replications; we used the admixture model, which estimates the fraction of ancestry from each cluster for each individual (PRITCHARD *et al.* 2000), and run the analyses with correlated allele frequencies (FALUSH *et al.* 2003). No information was given on the presumed origin

of the individuals (USEPOPINFO=0). Structure was run for K values ranging from 1 to 10 and the K value where $\ln P(D)$ was minimum was taken in account. Five independent runs were performed for the estimated minimum, in order to valuate the consistency of the datum.

Results and Discussion

The molecular analyses of the 178 accessions sampled demonstrated the presence of 93 different genotypes. The SSR profiles obtained were compared with the CRA-VIT database and data published in the literature. In Tab. 1 the varieties identified are listed in alphabetic order. The results of SSR profile comparison are the following from the 93 different varieties:

- 44 are registered in the Italian National Catalogue of Vine Varieties (RNVV) (marked with an X in Tab. 1). Fifteen out of these are considered as pure FVG: 'Cividin', 'Cjanorie', 'Cordenossa', 'Forgiarin', 'Piccola nera', 'Picolit', 'Piculit neri', 'Refosco dal peduncolo rosso', 'Refoscone', 'Ribolla gialla', 'Schioppettino', 'Sciaglin', 'Tazzelenghe', 'Ucelut' and 'Verduzzo friulano'. 'Cividin' and 'Cjanorie' were registered in 2006 and 'Cordenossa' even more recently in 2008. The registration of these three minor varieties is due to the passion of some winegrowers in the region aiming the cultivation of mainly endangered local varieties.
- 7 are known foreign varieties and not registered in the RNVV, *i.e.* 'Alicante Bouschet', 'Auxerrois', 'Humagne', 'Jacquez', 'Kraljevina', 'Tsaousi' and 'Veltliner rot'. They are heterogeneous varieties of highly disparate provenance (VIVC, Vitis International Variety Catalogue, <http://www.vivc.de>). Apart from 'Kraljevina' and 'Veltliner rot', which are grown in the neighboring regions of Croatia and Austria, there is no explanation for the presence of the other varieties in FVG.
- 38 are presumed original Friuli varieties (in bold in Tab. 1) not registered in the RNVV. Of these, 21 (55 %) have black grapes and 17 (45 %) white. Only 15 have already been described or even just mentioned in the literature (CALÒ and COSTACURTA 1991; COSTANTINI *et al.* 2007), while the other 22 have new molecular profiles and are not referable to varieties cited in the literature (at least with that name), so they represent a heritage that we have just begun to explore.
- A difficult case is that of 'Pienel', which corresponds to the 'Bela glera' found in Slovenia by ŠTAJNER *et al.* (2008) but, contrary to what is reported in the VIVC, it is not a synonym of 'Chasselas'. Nothing is known about the geographical origin of this genotype.
- One is 'Siciliana', whereas two other no named genotypes could not be identified. They are indicated as unknown G1 and unknown G2.

On the basis of our analyses, but unlike what was previously stated (CALÒ and COSTACURTA 1991), 'Cuneute' and 'Vercluna' are synonymous varieties. In the majority of cases the identified genotypes grouped between one and four accessions together, but some varieties were better represented. In particular, 11 samples were identified as

Table 1

List of the 93 varieties found after analysis of 178 accessions, ordered by identity. In bold those presumed autochthonous of Friuli Venezia Giulia. DB CRA-VIT: SSR database of Centro di Ricerca per la Viticoltura. N: black; B: white, and Rs: pink

Variety	Color	SSR profile of reference used	Registered in the Italian Catalogue	where ampelographic description of FVG cultivars may be found
Aghedene	B	CRESpan <i>et al.</i> 2006		CALÒ and COSTACURTA 1991
Aleatico	N	CRESpan and MILANI 2001	X	
Alicante Bouschet	N	MARTIN <i>et al.</i> 2003		
Auxerrois	B	BOWERS <i>et al.</i> 1999		
Barbera	N	THIS <i>et al.</i> 2004	X	
Berzamino	N	current paper		CALÒ and COSTACURTA 1991
Bianchella	B	current paper		
Blanchias	B	current paper		
Bontempo	N	current paper		
Brambana	N	current paper		CALÒ and COSTACURTA 1991
Cabernet franc	N	THIS <i>et al.</i> 2004	X	
Chasselas	B	SCHNEIDER <i>et al.</i> 2008	X	
Cjanorie	N	current paper	X	
Ciavalgian	N	current paper		CALÒ and COSTACURTA 1991
Ciliona	N	current paper		
Cilja	B	current paper		COSTANTINI <i>et al.</i> 2007
Cividin	B	current paper	X	
Codelunghe	N	current paper		CALÒ and COSTACURTA 1991
Corbina	N	CANCELLIER <i>et al.</i> 2006	X	
Cordenossa	N	current paper	X	
Corvinone	N	CANCELLIER <i>et al.</i> 2006	X	
Cremin	N	current paper		
Croatina	N	DB CRA-VIT	X	
Cuneute/Vercluna	N	current paper		CALÒ and COSTACURTA 1991
Curvin	N	current paper		COSTANTINI <i>et al.</i> 2007
Duriese	B	current paper		COSTANTINI <i>et al.</i> 2007
Durina	B	current paper		
Forgiarin	N	current paper	X	
Freisa	N	DB CRA-VIT	X	
Friularo	B	current paper		
Fumat	N	current paper		CALÒ and COSTACURTA 1991
Gragnelut	N	current paper		
Gran Rap	N	current paper		COSTANTINI <i>et al.</i> 2007
Humagne B.	B	VOUILLAMOZ <i>et al.</i> 2004		
Jacquez	N	THIS <i>et al.</i> 2004		
Kraljevina	B-Rs	MALETIC <i>et al.</i> 1999		
Lambrusco Maestri	N	DB CRA-VIT	X	
Malvasia bianca lunga	B	CRESpan <i>et al.</i> 2007	X	
Malvasia istriana	B	DB CRA-VIT	X	
Marzemina bianca	B	CRESpan <i>et al.</i> 2004	X	
Mocula	B	current paper		
Mostosa	B	DB CRA-VIT	X	
Mueller Thurgau	B	DB CRA-VIT	X	
Negrat	N	DB CRA-VIT		COSTANTINI <i>et al.</i> 2007
Negretto	N	DB CRA-VIT	X	
Nerata	N	current paper		
Nigrut	N	current paper		
Palomba nera	N	current paper		
Pelena	B	current paper		
Peverina	N	current paper		
Piccola nera	N	current paper	X	
Piciule	N	current paper		
Picolit	B	DB CRA-VIT	X	
Piculit neri	N	DB CRA-VIT	X	
Pienel	B	current paper		
Pignola della Valtellina	N	CANCELLIER <i>et al.</i> 2009	X	
Pignolo	N	CANCELLIER <i>et al.</i> 2009	X	
Pignoletta	N	current paper	X	
Portugieser	N	THIS <i>et al.</i> 2004	X	
Prosecco lungo	B	CRESpan <i>et al.</i> 2006	X	
Prosecco tondo	B	CRESpan <i>et al.</i> 2006	X	
Raboso veronese	N	CRESpan <i>et al.</i> 2004	X	
Refosco bianco	B	current paper		COSTANTINI <i>et al.</i> 2007
Refosco dal peduncolo rosso	N	COSTACURTA <i>et al.</i> 2005	X	
Refosco gentile	N	COSTACURTA <i>et al.</i> 2005		CALÒ and COSTACURTA 1991
Refoscone	N	COSTACURTA <i>et al.</i> 2005	X	
Regina	B	DB CRA-VIT	X	
Ribolla gialla	Rs	COSTACURTA <i>et al.</i> 2006	X	
Ruacit	B	current paper		COSTANTINI <i>et al.</i> 2007
Sagrestana	B	current paper		
Sangiovese	N	CRESpan and MILANI 2001	X	
Sbulcisa	B	current paper		
Sbulzina	N	current paper		
Schiava gentile	N	DB CRA-VIT	X	
Sciaglin	B	current paper	X	

Tab. 1, continued

Variety	Color	SSR profile of reference used	Registered in the Italian Catalogue	where ampelographic description of FVG cultivars may be found
Siora	B	current paper		
Siciliana	N	current paper		
Tazzelenghe	N	COSTACURTA <i>et al.</i> 2005	X	
Terrano	N	COSTACURTA <i>et al.</i> 2005	X	
Tintoria Lloyd	N	DB CRA-VIT		
Trebbiano toscano	B	DB CRA-VIT	X	
Tsaousi	B	SEFC <i>et al.</i> 2000; ARADHYA <i>et al.</i> 2003		
Ucelut	B	current paper	X	CALÒ and COSTACURTA 1991
Veltliner grün	B	SEFC <i>et al.</i> 2000	X	
Veltliner rot	B	THIS <i>et al.</i> 2004		
Venere	B	current paper		
Verduzzo trevigiano	B	DB CRA-VIT	X	
Vinoso rosso	N	current paper		
Vitouska	B	CRESPIAN <i>et al.</i> 2007	X	
Vubola	B	current paper		
Turca	N	DB CRA-VIT	X	
unknown G1	N			
unknown G2	B			

‘Piculit neri’, 9 as ‘Berzamino’, 5 as ‘Prosecco lungo’ and 5 as ‘Gragnelut’, indicating a wider diffusion of these varieties in the studied territory.

The term “Glera” also gives cause for reflection as 4 accessions with this name refer to 4 different varieties: ‘Aghedene’, ‘Mocula’, ‘Prosecco lungo’ and ‘Vitouska’. A previous study (CRESPIAN *et al.* 2006 a) revealed that the name “Glera”, considered just a synonym of ‘Prosecco’, was more often associated to ‘Prosecco lungo’ and, marginally, also to other minor white grape varieties.

We also found some peculiarities: the two accessions of ‘Brambana’ analyzed have three alleles at locus VVS2 (135, 151 and 153), whereas the accession of ‘Brambana’ held in the CRA-VIT collection had alleles 135 and 151. The two accessions identified as ‘Sbulzina’ have a small difference at locus VVMD7, accession n. 122 having 239 and 255 alleles and n. 88 having 239 and 257. Lastly, of the four accessions identified as ‘Pignolo’, two have three alleles at VVMD7 (247, 257 and 259).

Some genotypes, *i.e.* ‘Jacquez’, ‘Pignoletta’, ‘Siciliana’ and unknown G1, display alleles typical of rootstocks or other *Vitis* species and absent in *V. vinifera* (CRESPIAN *et al.* 2009), which are highlighted in bold in Tab. 2. ‘Jacquez’ is a known interspecific hybrid (VIVC), while we would hypothesize that the others are hybrids. Some ampelographic characteristics (colour of the bud and internodes, colour, hairiness and shape of the leaf) reveal signs of “hybrid blood”, while no phylloxera attacks were found on the leaves, probably because the vineyard is still very young.

Results of the elaborations with the Identity software: To evaluate the genetic variability present within the presumed FVG varieties, the statistics of the molecular data of the 58 genotypes in bold in Tab. 1 were elaborated with the Identity software, as well as those of ‘Malvasia istriana’, ‘Marzemina bianca’ and ‘Corbina’, which are also found outside the region. The results are summarized in Tab. 3. A total of 96 alleles were found, with an average of 8.7 alleles per locus. The observed heterozygosity was higher than expected at all the loci, excluding ISV4 and VMCNG4b9. There is a very big

difference between the two values in the case of locus VrZAG79, where the observed heterozygosity is much higher than that expected on the basis of the allelic frequencies. The probability of null alleles is less than or very close to zero at all loci, excluding ISV4 and VMCNG4b9. The three most informative loci, on the basis of both PI and PD, are VVMD28, VMCNG4b9 and VVS2, in that order, while those least informative are ISV3, ISV4 and VrZAG79. The probability of total identity is 5.72×10^{-11} , therefore a high value, notwithstanding the small number of genotypes being compared. The average PD is also high at 0.9073.

The high heterogeneity of the varieties of presumed FVG origin thus emerges, but the molecular data are too limited to make any suppositions on possible parent/offspring relationships.

Genotypes relationships: Genetic relations among the 89 unique genotypes (‘Jacquez’, ‘Pignoletta’, ‘Siciliana’ and unknown G1 were not considered) were analysed by Genetix software. It turned out that the varieties presumed to be native to FVG intermixed with the others, with exception of ‘Malvasia istriana’, ‘Fumat’ and ‘Alicante Bouschet’ (data not shown).

The dendrogram of genetic dissimilarity, elaborated using all 93 identified genotypes, showed a similar result, in which the two groups (presumed Friuli vs. non Friuli cv.) are uniformly mixed and ‘Malvasia istriana’ is confirmed as being one of most divergent samples (data not shown).

The Structure software was also applied to the same 89 genotypes, because it gives an estimate on the possibility to find realistic groups according to allele frequencies. The $\ln P(D)$ showed a slight minimum for $k = 3$ and 4, therefore none distinct population was inferred. This result is in agreement with that obtained by CIPRIANI *et al.* (2010) analyzing 795 genotypes of *Vitis vinifera*: Structure failed to divide them in any sub-population. Nevertheless, the 5 repetitions fluctuated very closely around the same values; moreover, comparing the results of the two bar plots, we observed that a more clear separation was obtained with $k = 4$. Also with this elaboration the presumed FVG varieties were disseminated in all groups individuated by the software. In an attempt to interpret the group-

Table 2
SSR profiles. In bold are the alleles found in rootstocks and not in *V. vinifera*

Variety	VVS2	VVMD5	VVMD7	VVMD27	VVMD28	VRZAG 62	VRZAG 79	ISV2 (VMC 6E1)	ISV3 (VMCGF1)	ISV4 (VMCG6G1)	VMCNG 4B9
Aghedene	135	226	239	185	194	247	251	141	133	177	187
Aleatico	133	226	239	179	194	249	249	143	131	169	169
Alicante Bouschet	133	238	239	181	194	243	263	151	135	187	187
Auxerrois	133	238	239	181	189	243	239	165	139	169	197
Barbera	133	226	249	185	189	253	263	143	133	169	177
Berzaminio	151	232	257	181	185	263	271	165	139	141	169
Bianchella	151	226	239	181	189	247	251	143	137	177	191
Blanchias	133	226	247	181	189	249	249	143	139	177	177
Bontempo	143	226	249	179	194	251	261	169	139	177	187
Brambanat*	135	232	247	185	189	261	271	161	139	141	169
Cabernet franc	139	226	239	181	189	263	239	165	139	187	191
Chasselas	133	228	239	185	189	247	271	165	133	169	177
Ciavalgian	139	226	247	179	194	263	261	161	137	169	169
Cillona	133	226	239	181	185	249	249	165	139	169	187
Cilsa	135	226	239	181	185	263	261	151	139	141	169
Cividin	141	236	247	181	185	247	261	143	131	169	191
Cjanorie	133	226	239	185	194	249	251	151	133	169	197
Codelunghe	133	226	249	179	181	263	261	165	139	141	169
Corbina	135	232	249	185	189	257	267	151	139	141	169
Corbina	135	226	239	181	189	263	267	161	139	141	169
Corvossola	135	232	240	179	185	239	261	165	139	141	169
Corvinone	135	232	240	185	189	239	261	151	139	141	169
Cremin	133	226	236	189	191	239	247	151	139	141	169
Croatina	139	236	247	189	194	249	239	141	139	141	169
Cuneute/Vercluna	143	236	247	185	185	243	237	165	133	169	187
Curvin	135	232	247	181	181	257	261	161	133	169	169
Durtese	133	228	247	185	189	247	237	141	139	141	169
Durina	143	234	243	185	194	247	263	165	139	141	169
Forgiarini	133	240	247	181	189	257	267	151	133	141	187
Freisa	133	226	247	185	189	249	271	161	135	187	197
Fritularo	135	226	232	179	189	247	271	151	131	169	169
Fumât	133	228	249	185	191	261	271	165	133	191	191
Gragnelut	139	238	239	185	189	249	251	151	133	169	158
Gran Rap	155	226	247	185	189	249	267	165	133	169	176
Humagne B.	133	236	243	185	189	253	261	151	133	169	187
Jacquez	139	244	237	179	189	239	239	141	139	175	187
Kraljevina	135	234	239	179	183	247	237	169	133	169	177
Lambrusco Maestri	135	228	253	191	194	257	267	143	133	169	177
Malvasia bianca lunga	145	226	239	179	179	253	251	165	133	177	158
Malvasia istriana	143	222	239	179	179	239	281	165	133	191	197
Marzemina bianca	133	226	239	185	194	253	239	141	133	169	166
Mocula	153	226	239	179	194	247	251	165	133	177	177
Mostosa	133	246	243	189	191	237	261	169	133	177	188
Mueller Thurgau	143	226	247	181	181	237	247	161	133	169	197
Negrat	133	232	247	185	189	231	239	151	139	169	158
Negretto	133	226	247	183	189	237	239	165	133	177	177
Nerata	135	236	239	179	181	237	249	169	133	141	169
Nigrut	155	226	239	185	189	247	239	151	139	141	169
Palomba nera	135	226	247	189	194	239	251	165	133	169	162
Pelena	133	240	239	179	179	247	257	151	133	177	197
Peverina	143	226	239	179	189	261	267	143	139	141	169

Tab. 2, continued

Variety	VVS2	VVMD5	VVMD7	VVMD27	VVMD28	VRZAG 62	VRZAG 79	ISV2 (VMC 6E1)	ISV3 (VMC6F1)	ISV4 (VMC6G1)	VMCNG 4B9
Piccola nera	133	228	239	181	239	251	248	141	133	187	158
Piccole	133	228	239	181	239	251	250	141	139	169	176
Picolit	135	232	247	181	231	237	238	141	131	169	164
Picolit neri	135	232	246	179	239	267	248	141	133	169	168
Pienel	143	226	239	179	239	257	250	143	139	169	176
Pignola della Valtellina	155	232	253	181	237	239	250	159	135	169	177
Pignoletta	125	226	239	185	255	263	244	133	139	185	158
Pignolo**	133	226	247	179	239	247	250	141	133	177	150
Portugeser	143	226	243	181	231	263	248	141	135	169	177
Prosecco lungo	133	226	246	179	239	247	248	141	133	169	150
Prosecco tondo	133	226	246	179	239	247	258	141	133	169	176
Raboso veronese	133	232	238	185	237	239	242	151	133	169	158
Refosco bianco	135	228	249	185	247	271	250	165	133	169	166
Refosco dal peduncolo rosso	133	226	239	181	237	239	250	141	133	169	158
Refoscone	133	226	247	179	237	247	258	143	133	169	162
Regina	151	226	249	185	237	261	242	165	133	169	168
Ribolla gialla	143	232	234	179	231	237	250	165	133	169	150
Ruait	143	226	247	181	237	249	248	141	139	191	172
Sagrestiana	139	226	239	181	231	249	250	169	131	177	164
Sangiovese	133	226	239	179	237	247	258	143	139	177	158
Sbuleisa	135	234	239	181	239	251	242	165	139	187	176
Sbulzima***	143	226	239	181	249	263	250	141	133	177	150
Schiava gentile	139	236	247	181	239	257	238	151	141	169	158
Sciaglin	133	228	242	179	231	237	258	141	131	169	162
Siciliana	133	234	243	181	201	245	242	165	139	177	151
Stora	133	228	239	181	237	239	248	141	133	169	178
Tazzelenghe	151	228	249	185	237	261	242	151	133	169	162
Terrano	135	226	247	189	221	237	238	141	139	169	150
Tintoria Lloyd	133	236	239	189	231	237	244	151	131	169	158
Trebbiano toscano	133	226	249	179	247	251	244	161	133	177	162
Tsaousi	135	228	247	183	261	261	246	143	131	177	138
Turca	151	228	249	189	231	247	250	141	133	169	176
Ucelut	133	228	249	179	237	237	250	151	133	169	162
unknown G1	133	236	247	181	186	251	242	151	137	177	158
unknown G2	133	226	249	179	237	251	246	143	133	177	178
Veltliner grün	133	232	247	189	237	251	248	141	133	169	158
Veltliner rot	131	240	246	183	249	271	250	143	133	187	138
Venerè	135	226	247	181	239	239	238	141	133	169	164
Venerè	135	226	247	181	239	239	238	141	133	169	164
Verduzzo trevigiano	143	226	247	179	231	237	250	159	133	169	150
Vinoso rosso	155	226	247	185	237	267	242	151	133	169	162
Vitouska	133	240	249	179	239	257	242	143	133	177	176
Vubola	135	226	239	185	247	251	242	143	133	177	162

*The two accessions analyzed have three alleles at VVS2 locus: 135, 151 and 153.

**Two accessions out of the four analyzed have three alleles at VVMD7: 247, 257 and 259.

***The two accessions analyzed showed a difference at VVMD7, one having 239-255 and the other 88 239-257.

Table 3

Statistics on the 11 SSR markers applied on the 58 genotypes presumed to be autochthonous of Friuli Venezia Giulia region. He: expected heterozygosity, Ho: observed heterozygosity, Δ = Ho-He, r: probability of null alleles, PI: probability of identity; PD: power of discrimination

Locus	no of alleles	He	Ho	Δ	r	PI	PD
VVMD28	13	0,881	0,931	0,050	-0,026	0,047	0,9625
VMCNG4b9	11	0,869	0,844	-0,025	0,013	0,055	0,9548
VVS2	9	0,842	0,844	0,002	-0,001	0,084	0,9435
VVMD27	6	0,809	0,810	0,001	0,000	0,120	0,9239
VrZAG62	8	0,816	0,896	0,080	-0,044	0,108	0,9227
ISV2	9	0,807	0,879	0,072	-0,039	0,117	0,9197
VVMD7	8	0,780	0,810	0,030	-0,016	0,147	0,9174
VVMD5	9	0,791	0,844	0,053	-0,029	0,124	0,9132
VrZAG79	10	0,754	0,913	0,159	-0,091	0,168	0,8596
ISV4	5	0,682	0,603	-0,079	0,047	0,228	0,8489
ISV3	8	0,689	0,810	0,121	-0,071	0,249	0,8145
mean	8,7	0,793	0,835			5,72 E-11*	0,9073

*PI over all 11 SSR loci was computed as the product of PI at each locus.

ing proposed, we set an arbitrary limit on the recognition of origin and imposed a threshold equal to 85 % or above for a genotype to be included in one of the four groups. With this limit, two groups encompassing respectively 21 and 17 genotypes resulted “anonymous”, because none variety could be picked out. A third group included 13 varieties, 10 of them being above the threshold limit, mainly white grapes; they were, in decreasing order: ‘Pelena’, ‘Vitouska’, ‘Pienel’, ‘Prosecco’, ‘Piccola nera’, ‘Malvasia bianca lunga’, ‘Sbulcisa’, ‘Mocula’, ‘Prosecco lungo’ and ‘Aghedene’. The presence of ‘Malvasia bianca lunga’ in this group is no surprise, it being the parent, with ‘Prosecco’, of ‘Vitouska’ (Crespan *et al.* 2007); almost all the others are presumed to be autochthonous (Tab. 1). Lastly, in the fourth group of 38 varieties, 12 overcame the threshold limit: ‘Corbina’, ‘Refoscone’, ‘Nigrut’, ‘Vinoso rosso’, ‘Refosco bianco’, ‘Negrat’, ‘Curvin’, ‘Tazzelenghe’, ‘Cilja’, ‘Raboso veronese’, ‘Gran rap’ and ‘Brambana’. In this case they are mainly red grape and are all minor varieties of FVG, except ‘Raboso veronese’. The presence of ‘Raboso veronese’ suggests the existence of a link between Veneto and FVG as it is a spontaneous hybrid between ‘Raboso Piave’ and ‘Marzemina bianca’ (CRESPIAN *et al.* 2006): the cultivation area of the former is confined to Veneto, mainly in the province of Treviso, while the latter has a more extensive distribution area that also includes FVG, as proved by the 4 accessions identified as ‘Marzemina bianca’ in this work. Nevertheless, ‘Marzemina bianca’ is related to ‘Garganega’, another centuries-old variety of Veneto, with which it has a parent-offspring relationship (CRESPIAN *et al.* 2008); it was attributed by Structure to the previous group with a percentage of 65 %, much lower than the threshold. We therefore re-processed the data with Structure, adding ‘Raboso Piave’ and imposing $k = 4$: the variety was classified in the fourth group, with a presumed recognition of origin of 91 %, demonstrating a close similarity to those varieties, previously unsuspected, and which explains the position of ‘Raboso veronese’ in the same group. ‘Pignola’ is instead typical of the province of Sondrio (North Lombardy), but it has also been found in Veneto, under name of ‘Groppello di Breganze’ (CANCELLIER *et al.* 2009), and now also in FVG. With the current information it is difficult to

hypothesize on its spread from east to west or vice versa, nevertheless its similarity to the other varieties of FVG would suggest the former possibility. Based on the Structure results, therefore, at least two principal groups can be identified among the presumed varieties from FVG, one referring to ‘Prosecco’ and the other to ‘Refoscone’. The elaboration of further comparisons between these materials, when their ampelographic, phenological and chemical characterization has been completed, will allow the validity of these preliminary findings to be verified.

Conclusions

The meticulous work in the field, flanked by literature searches and molecular analyses of the materials sampled over six years of research on the FVG territory, has allowed much of the autochthonous material of the region to be rescued, to characterize it genetically, identify the original materials and evidence the synonymies. The elaboration of the molecular data has also highlighted the presence of two groups of varieties that might have a common origin.

The results of this research are a prerequisite for moving on to the successive stages of the project of requalification of the ampelographic heritage of FVG, which are underway and involve the ampelographic, ampelometric, agronomic and oenological characterization of all the “potentially original” accessions. A third phase will consist in a more detailed evaluation in different environments of the varieties considered potentially more interesting, aimed at reintroducing cultivation of the best ones, with registration in the National Catalogue of Italian Vine Varieties.

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