Vitis **50** (1), 21–28 (2011)

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

M. Crespan [1)], A. Fabbro[2)], S. Giannetto[1)], S. Meneghetti[1)], C. Petrussi[3)], F. Del Zan[2)] and P. Sivilotti[2)]

[1)] C.R.A. Centro di Ricerca per la Viticoltura, CRA-VIT, Conegliano, Italy
[2)] ERSA Servizio ricerca e sperimentazione, Pordenone, Italy
[3)] Viticulturist, Cividale del Friuli, Udine, Italy

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'.

K e y w o r d s : 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-

Correspondence to: Dr. M. Crespan, C.R.A. Centro di Ricerca per la Viticoltura (CRA-VIT), Via Casoni 13/a, 31058 Susegana (TV), Italy. Fax: +39-438-738489. E-mail: manna.crespan@entecra.it

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 Pantianicco 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 (Crespan 2003) and VMCNG4b9 (Welter *et al.* 2007). The PCR reaction mixture (25 µL final volume) contained 20 ng total DNA, 10 µl Eppendorf HotMaster-Mix (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 µL 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 Crespan 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 explication 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 authoctonous 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 *et al*. 2006		Calò and Costacurta 1991
Aleatico	N	Crespan and Milani 2001	X	
Alicante Bouschet	N	Martin *et al*. 2003		
Auxerrois	B	Bowers *et al*. 1999		
Barbera	N	This *et al.* 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 *et al.* 2004	X	
Chasselas	B	Schneider *et al.* 2008	X	
Cjanorie	N	current paper	X	
Ciavalgian	N	current paper		Calò and Costacurta 1991
Ciliona	N	current paper		
Cilja	B	current paper		Costantini *et al.* 2007
Cividin	B	current paper	X	
Codelunghe	N	current paper		Calò and Costacurta 1991
Corbina	N	Cancellier *et al.* 2006	X	
Cordenossa	N	current paper	X	
Corvinone	N	Cancellier *et al.* 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 *et al.* 2007
Duriese	B	current paper		Costantini *et al.* 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 *et al.* 2007
Humagne B.	B	Vouillamoz *et al.* 2004		
Jacquez	N	This *et al.* 2004		
Kraljevina	B-Rs	Maletic *et al.* 1999		
Lambrusco Maestri	N	DB CRA-VIT	X	
Malvasia bianca lunga	B	Crespan *et al.* 2007	X	
Malvasia istriana	B	DB CRA-VIT	X	
Marzemina bianca	B	Crespan *et al.* 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 *et al.* 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 *et al.* 2009	X	
Pignolo	N	Cancellier *et al.* 2009	X	
Pignoletta	N	current paper	X	
Portugieser	N	This *et al.* 2004	X	
Prosecco lungo	B	Crespan *et al.* 2006	X	
Prosecco tondo	B	Crespan *et al.* 2006	X	
Raboso veronese	N	Crespan *et al.* 2004	X	
Refosco bianco	B	current paper		Costantini *et al.* 2007
Refosco dal peduncolo rosso	N	Costacurta *et al.* 2005	X	
Refosco gentile	N	Costacurta *et al.* 2005		Calò and Costacurta 1991
Refoscone	N	Costacurta *et al.* 2005	X	
Regina	B	DB CRA-VIT	X	
Ribolla gialla	Rs	Costacurta *et al.* 2006	X	
Ruacit	B	current paper		Costantini *et al.* 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 *et al.* 2005	X	
Terrano	N	Costacurta *et al.* 2005	X	
Tintoria Lloyd	N	DB CRA-VIT		
Trebbiano toscano	B	DB CRA-VIT	X	
Tsaousi	B	Sefc *et al.* 2000; Aradhya *et al.* 2003		
Ucelut	B	current paper	X	Calò and Costacurta 1991
Veltliner grün	B	Sefc *et al.* 2000	X	
Veltliner rot	B	This *et al.* 2004		
Venere	B	current paper		
Verduzzo trevigiano	B	DB CRA-VIT	X	
Vinoso rosso	N	current paper		
Vitouska	B	Crespan *et al.* 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 (Crespan *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* (Crespan *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 heterozygosis 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 heterozygosis 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 (VMC6F1)		ISV4 (VMC6G1)		VMCNG 4B9	
Aghedene	135	155	226	246	239	247	185	194	247	251	193	195	242	248	141	151	133	133	177	187	150	176
Aleatico	133	135	226	228	239	249	179	194	239	249	185	195	248	254	143	143	131	139	169	169	150	158
Alicante Bouschet	133	145	226	238	239	243	181	194	247	263	187	187	242	256	151	165	135	139	187	187	164	170
Auxerrois	133	151	234	238	239	243	181	189	231	239	187	195	242	244	151	165	139	145	169	197	158	162
Barbera	133	135	226	226	249	253	185	189	237	263	191	199	242	258	143	151	133	133	169	177	158	172
Berzamino	151	155	232	240	257	263	181	185	249	271	193	195	250	258	165	169	139	141	169	177	162	172
Bianchella	151	153	226	228	239	247	181	189	239	251	191	203	242	250	141	143	137	139	177	191	138	164
Blanchias	133	153	226	234	247	249	181	189	239	249	199	203	242	250	141	143	139	139	177	177	138	176
Bontempo	143	145	226	228	249	249	179	194	251	261	189	189	236	250	143	169	139	145	177	187	150	150
Brambana*	135	151	232	236	247	257	185	189	261	271	195	203	242	250	151	161	139	141	169	191	158	158
Cabernet franc	139	147	226	240	239	263	181	189	231	239	193	203	246	258	151	165	139	139	187	191	162	176
Chasselas	133	143	228	236	239	247	185	189	221	271	193	203	250	258	141	165	133	139	169	177	158	162
Ciavalgian	139	151	226	228	247	263	179	194	247	261	193	203	250	250	151	161	137	137	169	169	158	158
Ciliona	133	155	226	232	239	249	181	185	237	249	187	199	242	250	165	169	139	139	169	187	162	164
Cilsa	135	151	226	228	239	263	181	185	239	261	193	193	250	258	151	165	139	141	169	191	158	168
Cividin	141	143	236	238	247	247	181	185	247	261	195	201	242	258	141	143	131	139	169	191	150	162
Cjanorie	133	155	226	228	239	249	185	194	237	251	187	199	242	248	141	151	133	139	169	197	158	162
Codelunghe	133	151	226	228	249	263	179	181	251	261	193	199	250	258	165	165	139	141	169	177	158	178
Corbina	135	155	232	232	249	257	185	189	239	267	191	195	242	250	151	165	139	141	169	169	162	178
Cordenossa	135	155	226	228	239	263	181	189	251	267	187	193	250	258	141	161	139	141	169	187	158	158
Corvinone	135	151	232	240	233	239	179	185	237	261	187	203	250	250	151	165	139	141	169	187	158	178
Cremin	133	143	226	236	239	263	189	191	239	247	193	193	250	250	151	151	139	141	169	177	150	168
Croatina	139	151	236	236	247	249	189	194	237	239	185	195	244	244	141	165	139	145	187	187	166	172
Cuneute/Vercluna	143	155	232	236	247	263	185	185	231	237	193	203	242	250	141	165	133	139	169	197	162	166
Curvin	135	151	232	236	247	257	181	181	239	261	193	195	258	258	151	161	133	141	169	169	158	178
Duriese	133	151	228	228	247	257	185	189	237	237	193	203	248	250	141	165	121	139	169	177	162	166
Durina	143	143	226	234	243	247	185	194	251	263	187	203	236	250	151	165	139	145	169	177	150	172
Forgiarin	133	141	232	240	247	257	181	189	247	267	193	203	250	258	151	165	133	141	187	187	164	184
Freisa	133	155	226	232	247	249	185	189	247	271	193	199	250	250	151	161	135	139	187	197	150	178
Friularo	135	135	226	232	247	247	179	189	239	271	193	195	250	258	141	151	131	139	169	169	150	166
Fumat	133	151	228	236	249	261	185	191	231	271	193	199	242	260	161	165	133	133	191	191	168	172
Gragnelut	139	151	226	238	239	249	185	189	231	251	187	199	244	250	151	151	133	139	169	169	158	158
Gran Rap	155	155	226	232	247	249	185	189	247	267	191	193	242	250	141	165	133	141	169	177	176	178
Humagne B.	133	151	226	236	243	253	185	189	247	261	187	199	238	246	151	165	133	139	169	187	150	168
Jacquez	139	143	228	244	237	239	179	189	233	239	185	197	248	250	141	151	139	143	**175**	187	154	172
Kraljevina	135	143	234	240	239	247	179	183	231	237	193	195	250	250	143	169	133	139	169	177	150	150
Lambrusco Maestri	135	155	228	232	253	257	191	194	237	267	191	201	244	250	151	169	133	145	169	177	158	184
Malvasia bianca lunga	145	145	226	240	239	253	179	179	251	257	195	199	242	250	143	165	133	139	177	177	150	176
Malvasia istriana	143	143	222	240	239	239	179	179	257	281	187	187	236	254	161	165	133	139	191	197	138	168
Marzemina bianca	133	133	226	238	239	253	185	194	239	239	193	199	248	250	141	165	133	139	169	177	166	176
Mocula	153	155	226	234	239	247	179	194	247	251	195	203	248	258	151	165	133	133	177	177	138	150
Mostosa	133	145	226	246	243	247	189	191	237	261	187	195	250	256	141	169	133	139	177	187	158	166
Mueller Thurgau	143	151	226	228	247	257	181	181	237	247	193	193	242	244	151	161	133	139	169	197	138	158
Negrat	133	135	232	232	247	257	185	189	231	239	195	203	250	250	141	151	139	139	169	169	158	158
Negretto	133	155	226	232	247	257	183	189	237	239	193	203	244	248	151	165	133	139	177	177	158	158
Nerata	135	151	236	236	239	249	179	181	237	249	187	199	258	258	169	169	133	141	169	177	166	172
Nigrut	155	155	226	228	239	247	185	189	239	239	193	195	242	250	151	165	139	141	169	169	162	166
Palomba nera	135	143	226	232	247	247	189	194	239	251	193	195	250	256	151	151	133	139	169	169	150	158
Pelena	133	145	240	246	239	239	179	179	247	257	187	195	242	258	151	165	133	133	177	197	150	166
Peverina	143	151	226	226	239	261	179	189	221	267	193	199	250	258	143	165	139	141	169	169	150	158

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	135	228	246	239	239	181	194	239	251	187	195	248	258	141	141	133	139	187	197	158	176
Piciule	133	155	228	232	239	257	181	189	251	267	195	195	250	258	141	151	139	141	169	197	166	176
Picolit	135	139	232	238	247	247	181	185	231	237	191	203	238	258	141	161	131	133	169	177	164	168
Piculit neri	135	151	232	246	239	263	179	194	239	267	193	195	248	250	141	145	133	141	169	187	158	162
Pienel	143	145	226	246	239	239	179	179	239	257	187	195	250	258	143	151	139	139	169	177	176	176
Pignola della Valtellina	155	155	232	236	253	263	181	189	237	239	193	193	250	258	159	165	135	139	169	177	162	178
Pignoletta	**125**	133	226	244	239	243	185	194	**255**	263	187	187	244	258	**133**	151	139	139	185	197	158	158
Pignolo**	133	155	226	232	247	257	179	191	239	247	193	195	250	258	141	167	131	133	177	177	150	158
Portugieser	143	151	226	232	243	255	181	194	231	263	187	203	248	258	141	151	135	139	169	177	138	158
Prosecco lungo	133	143	226	246	239	247	179	194	239	247	187	193	248	258	141	167	133	139	169	197	150	158
Prosecco tondo	133	143	226	246	239	247	179	194	239	247	187	203	248	258	141	151	133	139	169	197	166	176
Raboso veronese	133	135	232	238	239	257	185	185	237	239	193	195	242	250	151	165	133	139	169	169	158	166
Refosco bianco	135	151	228	232	249	257	185	191	247	271	195	199	250	258	151	165	133	139	169	169	158	162
Refosco dal peduncolo rosso	133	133	226	232	239	247	181	189	237	239	193	193	250	258	141	165	133	139	169	177	158	168
Refosco gentile	133	155	226	226	247	263	179	185	237	247	193	203	250	258	143	165	133	139	169	169	150	168
Refoscone	151	155	226	232	249	263	185	185	237	271	193	199	242	250	165	165	133	139	169	169	162	168
Regina	133	135	226	232	239	249	185	185	237	261	185	187	242	250	143	167	139	139	177	191	150	158
Ribolla gialla	143	151	232	234	239	249	179	185	231	237	195	199	236	250	165	169	133	139	191	197	158	172
Ruacit	143	151	226	226	247	247	181	194	237	249	191	203	248	258	141	159	139	139	191	197	138	176
Sagrestana	139	151	226	232	239	247	181	191	231	249	187	191	250	258	141	169	131	139	177	177	164	164
Sangiovese	133	133	226	236	239	263	179	185	237	247	193	195	242	258	143	165	139	139	177	197	158	168
Sbulcisa	135	153	234	246	239	239	181	181	239	251	187	195	242	258	141	165	139	139	187	197	138	176
Sbulzina***	143	143	226	226	239	257	181	194	249	263	193	203	250	258	141	169	133	135	177	197	150	158
Schiava gentile	139	155	236	238	247	249	181	185	239	257	191	199	238	258	151	151	141	145	169	187	158	178
Sciaglin	133	135	228	232	247	263	179	179	231	237	191	193	250	258	141	161	131	133	169	169	150	162
Siciliana	133	143	234	**264**	243	243	181	**201**	231	**245**	187	192	242	258	**133**	165	139	139	177	187	151	170
Siora	133	139	228	232	239	249	181	181	237	239	195	199	248	258	141	165	133	139	169	197	158	178
Tazzelenghe	151	153	228	232	249	263	185	185	237	261	195	199	242	250	151	165	133	139	169	169	162	178
Terrano	135	155	226	228	247	249	189	189	221	237	191	193	238	250	141	143	139	141	169	169	150	158
Tintoria Lloyd	133	151	236	238	239	247	189	194	231	237	193	195	244	250	151	165	131	139	169	191	158	158
Trebbiano toscano	133	143	226	232	249	253	179	183	247	251	193	199	244	250	141	161	133	139	177	187	162	176
Tsaousi	135	151	228	238	247	249	179	183	261	261	187	203	246	248	143	171	131	133	177	197	138	138
Turca	151	151	228	238	249	263	189	189	231	247	193	199	250	258	141	151	133	145	169	169	162	176
Ucelut	133	143	228	236	249	263	179	189	237	237	193	199	250	254	151	159	133	139	177	177	158	176
unknown G1	133	147	236	**244**	247	251	181	**186**	251	261	193	193	242	260	151	151	137	139	177	181	160	168
unknown G2	133	151	226	236	249	249	179	183	237	251	199	201	246	250	143	151	133	139	177	191	158	178
Veltliner grün	133	151	232	232	247	257	189	194	237	251	193	203	244	248	141	161	133	145	169	183	158	176
Veltliner rot	131	133	240	246	239	253	183	194	249	271	191	195	250	250	143	151	133	139	187	197	138	150
Venere	135	151	226	232	247	263	181	185	231	239	193	203	238	258	141	169	133	139	169	177	164	168
Verduzzo trevigiano	143	151	226	228	247	263	179	189	231	237	193	203	250	258	159	167	133	141	169	169	150	158
Vinoso rosso	155	155	226	226	247	249	185	191	237	267	199	203	242	250	151	165	133	139	169	169	162	162
Vitouska	133	145	226	240	239	247	179	194	239	257	195	203	242	248	151	165	133	133	177	197	166	176
Vubola	135	143	226	228	239	257	185	194	247	251	187	195	242	248	143	167	133	139	177	187	162	176

*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 heterozygosis, Ho: observed heterozygosis, 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' (Crespan *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 (Crespan *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.

Acknowledgements

This research was carried out as part of the programme "Characterization of ancient Friuli varieties" and was financed by the Regional Agency for Agricultural Development of Friuli Venezia Giulia, with funds made available by the FVG Regional Law 22 April 2002, n. 11 "Preservation of autochthonous genetic resources of agricultural and forestry interest". The authors thank

M. Giust and E. Peterlunger for access to the "Rinascita" Farm and S. Osvaldo collections, respectively.

References

Aradhya, M. K.; Dangl, G. S.; Prins, B. H.; Boursiquot, J. M.; Walker, M. A.; Meredith, C.; Simon, C. J;. 2003: Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L. Genet. Res. **81**, 179-192.

Belkhir, K.; Borsa, P.; Chikhi, L.; Raufaste, N.; Bonhomme, F.; 2003: GENTIX 4.04, Logiciel sous Windows TM pour la Génétique des Populations. Laboratoire Génome, Populations, Interactions, Adaptations, UMR 5171. Univ. Montpellier 2, Montpellier, France.

Bowers, J. E.; Dangl, G. S.; Vignani, R.; Meredith, C. P.; 1996: Isolation and characterisation of the new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome **39**, 628-633.

Bowers, J. E.; Dangl, G. S.; Meredith, C. P.; 1999: Development and characterisation of additional microsatellite DNA markers for grape. Am. J. Enol. Vitic. **50,** 243-246.

Calò, A.; Costacurta, A.; 1991: Delle viti in Friuli. Ed. Arti Grafiche Friulane.

Cancellier, S.; Giannetto, S.; Meneghetti, S.; Milani, N.; Crespan, M.; 2006: Approfondimenti sulle popolazioni delle Corbine e delle Corvine presenti nel Veneto. Proceedings of the National Congress "I varietà autoctoni minori: aspetti tecnici, normativi e commerciali", Villa Gualino, Torino 30th November - 1st December 2006. Testo negli Atti su CD, sessione 2, 57-63.

Cancellier, S.; Giannetto, S.; Crespan, M.; 2009: Groppello di Breganze e Pignola sono lo stesso varietà. Groppello di Breganze and Pignola are the same cultivar. Riv. Vit. Enol. **2-3**, 3-9.

Cipriani, G.; Spadotto, A.; Jurman, I.; Di Gaspero, G.; Crespan, M.; Meneghetti, S.; Frare, E.; Vignani, R.; Cresti, M.; Morgante, M.; Pezzotti, M.; Pè, E.; Policriti, A.; Testolin R.; 2010: The SSR-based molecular profile of 1005 grapevine (*Vitis vinifera* L.) accessions uncovers new synonymy and parentages, and reveals a large admixture among varieties of different geographic origin. Theor. Appl. Genet. (DOI: 10.1007/s00122-010-1411-9, in press).

Costacurta, A.; Calò, A.; Carraro, R.; Giust, M.; Aggio, L.; Borsa, D.; Di Stefano, R.; Of the Zan, F.; Fabbro, A.; Crespan, M.; 2005: L'identificazione e la caratterizzazione dei Refoschi, 25-45. In: Proc. Conference "dei Refoschi". Ed. Lloyd of San Dorligo della Valle, Trieste.

Costacurta, A.; Giannetto, S.; Meneghetti, S.; Crespan, M.; 2006: Does it exist a Greek ampelographical heredity in South Italy? SSR profiles comparison of cultivars growing in both Countries, 9-14. Proc. "Ampelos 2006" 2nd Int. Symp. Evaluation and Exploitation of Grapes of Corresponding Terroir through Winemaking and Commercialization of Wines, Santorini, Greece, 1-3 June 2006.

Costantini, E.; Mattaloni, C.; Petrussi, C.; 2007: La vite nella storia e nella cultura del Friuli. Ed. Forum, Udine.

Crespan, M.; Milani, N.; 2001: The Muscats: a molecular analysis of synonyms, homonyms and genetic relationships within a large family of grapevine cultivars. Vitis **40**, 23 - 30.

Crespan, M.; 2003. The parentage of Muscat of Hamburg. Vitis **42**, 193-197.

Crespan, M.; Calò, A.; Giannetto, S.; Sparacio, A.; Storchi, P.; Costacurta, A.; 2008: 'Sangiovese' and 'Garganega' are two key varieties of the Italian grapevine assortment evolution. Vitis **47,** 97-104.

Crespan, M.; Cancellier, S.; Chies, R.; Gianetto, S.; Meneghetti, S.; Costacurta, A.; 2006a. Molecular contribution to the knowledge of two ancient varietal populations: Rabosi and Glere. Proc. 9th Int. Conference on Grape Genetics and Breeding. Udine, 2-6 luglio 2006. Acta Hortic. **827,** 217-220.

Crespan, M.; Cancellier, S.; Chies, R.; Giannetto, S.; Meneghetti, S.; 2006 b: Individuati i genitori del Raboso veronese: una nuova ipotesi sulla sua origine (The parents of Raboso veronese were discovered: a new hypothesis on its origin). Riv. Vit. Enol. **1**, 3-12.

Crespan, M.; Crespan, G.; Giannetto, S.; Meneghetti, S.; Costacurta, A.; 2007: 'Vitouska' is the progeny of 'Prosecco tondo' and 'Malvasia bianca lunga'. Vitis **46**, 192-194.

Crespan, M.; Meneghetti, S.; Cancellier, S.; 2009: Identification and genetic relationship of the principal rootstocks cultivated in Italy. Am. J. Enol. Vitic. **60**, 349-356.

Dangl, G. S.; Mendum, M. L.; Prins, B. H.; Walker, M. A.; Meredith, C. P.; Simon, C. J.; 2001: Simple sequence repeat analysis of a clonally propagated species: a tool for managing a grape germplasm collection. Genome **44**, 432-438.

Falush, D.; Stephens, M.; Pritchard, J. K.; 2003. Inference of population structure: Extensions to linked loci and correlated allele frequencies. Genetics **164**, 1567-1587.

Maletić, E.; Sefc, M. K.; Steinkellner, H.; Karoglan Kontić, J.; Pejić, I.; 1999: Genetic characterization of Croatian grapevine cultivars and detection of synonymous cultivars in neighboring regions. Vitis **38,** 79-83.

Martín, J. P.; Borrego, J.; Cabello, F.; Ortiz, J. M.; 2003: Characterization of Spanish grapevine cultivar diversity using sequence-tagged microsatellite site markers. Genome **46**, 10-18.

Pritchard, J. K; Stephens, M; Donnelly, P.; 2000. Inference of population structure from multi-locus genotype data. Genetics **155,** 945-959.

Schneider, A.; Torello Marinoni, D.; Crespan, M.; 2008. Genetics and ampelography trace the origin of Muscat fleur d'oranger. Am. J. Enol. Vitic. **59**, 200-204.

Sefc, K. M.; Regner, F.; Turetschek, E.; Glössl, J.; Steinkellner, H.; 1999: Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome **42**, 367-373.

Sefc, M. K.; Lopes, M. S.; Lefort, F.; Botta, R.; Roubelakis-Angelakis, K. A.; Ibáñez, J.; Pejíc, I.; Wagner, H. W.; Glössl, J.; Steinkellner, H.; 2000: Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. Theor. Appl. Genet. **100**, 498-505.

Štajner, N.; Korošec-Koruza, Z.; Rusjan, D.; Javornik, B.; 2008. Microsatellite genotyping of old Slovenian grapevine varieties (*Vitis vinifera* L.) of the Primorje (coastal) winegrowing region. Vitis **47**, 201-204.

This, P.; Jung, A.; Boccacci, P.; Borrego, J.; Botta, R.; Costantini, L.; Crespan, M.; Dangl, G.S.; Eisenheld, C.; Ferreira-Monteiro, F.; Grando, S.; Ibáñez, J.; Lacombe, T.; Laucou, V.; Magalhães, M.; Meredith, C.P.; Milani, N.; Peterlunger, E.; Regner, F.; Zulini, L.; Maul, E.; 2004: Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theor. Appl. Genetics **109**, 1448-1458.

Thomas, M. R.; Scott, N. S.; 1993: Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). Theor. Appl. Genet. **86**, 985-990.

Vouillamoz, J. F.; Magre, D.; Meredith, C. P.; 2004: Identity and parentage of two alpine grape cultivars from Switzerland (*Vitis vinifera* L. Lafnetscha and Himbertscha). Vitis **43**, 81-87.

Welter, L. J.; Göktürk-Baydar, N.; Akkurt, M.; Maul, E.; Eibach, R.; Töpfer, R.; Zyprian, E. M.; 2007: Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis vinifera* L.). Mol. Breed. **20**, 359-374.

Received May 4, 2010