

Vitis 49 (1), 29–38 (2010)

Molecular characterization of the autochthonous grape cultivars of the region Friuli Venezia Giulia – North-Eastern Italy

G. CIPRIANI, M. T. MARRAZZO and E. PETERLUNGER

Dipartimento di Scienze Agrarie e Ambientali, University of Udine, Udine, Italy

Summary

A sample of 48 accessions belonging to important autochthonous grapevine varieties from Friuli Venezia Giulia (North-Eastern Italy) was analyzed using two sets of microsatellite markers. One marker set, based on di-nucleotide core repeats, was compared with a recently developed set of markers based on tri-, tetra-, and penta-nucleotide repeats in order to determine genetic identities, estimate genetic diversity, and establish the power of discrimination of the two sets. A total of 20 di-nucleotide SSR markers and 19 tri-, tetra-, and penta-nucleotide SSR markers were used to screen the accessions.

All 39 primers produced PCR amplicons that were polymorphic and easily scorable in all of the accessions studied. Both datasets allowed the discrimination of all cultivars but two ('Refosco di Runcis' and 'Refoscone'). The observed heterozygosity ranged from 0.21 to 1.0 for the di-nucleotide markers, and from 0.21 to 0.88 for the tri-, tetra-, and penta-nucleotide repeat motif microsatellites, respectively.

The population structure of the 48 autochthonous accessions was evaluated, together with 8 international cultivars included as references, providing evidence for significant population structure within the sample. Microsatellites composed of tri-, tetra-, and penta-nucleotide repeats, characterized by low 'stuttering' and larger relative size differences between incremental alleles, are now available for grape fingerprinting.

Key words: SSR; long core repeat microsatellites; fingerprinting; genetic diversity.

Introduction

Grape is one of the most important horticultural crops, both for its economic relevance in many regions worldwide, and for its ancient historical connections with the development of human culture. The cultivars used for wine and table grapes belong to the Eurasian species *Vitis vinifera*, probably domesticated not later than 7000 years ago, as suggested by evidence of wine production found in Iran, in the northern Zagros mountains (THIS *et al.* 2006). From the original sites of domestication, cultivated grapes followed the ancient civilizations moving to the north-west, gradually arriving in Europe. The Romans gave names to culti-

vars, but it is difficult to correlate them to modern grape names. Only since the Middle Ages and the Renaissance have some cultivar names endured to present times. The number of different varieties held in germplasm collections is estimated at about 10,000 (ALLEWELDT and DETTWEILER 1992), with the probability of a high number of synonyms and homonyms due to the widespread practice of using vegetatively propagated cuttings in different regions, and the convergence of names for different cultivars, respectively (<http://www.genres.de/idb/vitis>).

More recently, autochthonous cultivars were neglected and substituted by the most important international varieties in many typical grape production areas. Many of them were almost lost, being maintained only in germplasm collections. These cultivars were forgotten by most viticulturists until recently, when a change in the consumer and market interests opened new opportunities to growers and winemakers.

As in many traditional grape cultivation areas, the Friuli Venezia Giulia region in North-Eastern Italy, is home to a number of autochthonous cultivars which have been recognized and harvested in past years. Some are very well known internationally, such as 'Picolit', a white dessert wine variety, and the red 'Refosco', while others are primarily enjoyed locally, such as the white wine Tocai Friulano, now renamed simply Friulano.

Some of these cultivars have previously been characterized, both morphologically and genetically using different molecular markers (CIPRIANI *et al.* 1994).

In the last decade, DNA profiling based on microsatellite markers has deeply changed grape diversity analysis and cultivar genotyping. Microsatellite markers have proven useful in parentage analysis (BOWERS and MEREDITH 1999, BOWERS *et al.* 1999 a, b, VOUILLAMOZ *et al.* 2003) and in cultivar genetic characterization (SEFC *et al.* 2000, ARADHYA *et al.* 2003, THIS *et al.* 2004). Recently, an extended list of di-nucleotide core repeat SSR markers was selected for cultivar fingerprinting and parentage analysis (DI VECCHI STARAZ *et al.* 2007).

In the present paper, a list of forty-eight local cultivars from the Friuli Venezia Giulia region have been characterized using thirty-nine microsatellite markers to determine genetic identity, estimate genetic diversity, and to identify genetic relationships. Each of the nineteen chromosomes in the grape genome was covered by two markers, one from each set: the di-nucleotide repeat SSRs (DI VECCHI STARAZ *et al.* 2007) and the recently developed longer nucleotide repeat SSRs (CIPRIANI *et al.* 2008).

Material and Methods

Plant material: Forty-eight grape accessions, believed to be of autochthonous origin based on information gathered at the time of collection, were obtained from the grapevine germplasm repository of the Friuli Venezia Giulia region, and held at University of Udine experimental farm. All accessions have been described previously and identified by standard morphological descriptors. Another 8 well-known international grape cultivars were obtained from the grapevine repository of the Vivai Cooperativi Rauscedo (PN), Italy and added to the panel as references, to infer population structure (see below). The complete list of genotypes examined in this study is presented in Tab. 1, including their recognized synonyms.

DNA isolation and amplification: Genomic DNA was extracted from 0.1 g of young leaf tissue using the CTAB method (DOYLE and DOYLE 1987). Nineteen primer pairs, one for each chromosome of the grapevine, were selected from a set of 45 markers developed from the recently sequenced grapevine genome, described by CIPRIANI *et al.* (2008). Hereafter this microsatellite set will be referred to as Microsat 1. These markers represent a suggested ‘standard set’ of microsatellites characterized by penta-, tetra-, or tri-nucleotide repeat motifs. Twenty pairs of primers were selected as suggested by DI VECCHI STARAZ *et al.* (2007), which were used to characterize the French grape repository in Domaine de Vassal, INRA (THIS, pers. comm.). This set of primers amplifies mainly di-nucleotide microsatellite markers and will be referred to as Microsat 2.

PCR was performed on each sample in 10.0 µL reaction volumes containing 0.2 mM dNTP, 10 mM Tris-HCl pH 9.0, 0.4 U *Taq* DNA polymerase, 0.2 µM FAM- or HEX dye-labelled forward primer, 0.2 µM reverse primer, and 2.5 ng template DNA. The PCR thermal profile adopted for Microsat 1, using the ‘HotMaster’ *Taq* DNA polymerase (Eppendorf, Hamburg, Germany) and the PTC-200 (MJ Research Inc., Watertown, MA, USA) thermal cycler, was: 95 °C for 2 min followed by 10 cycles of 94 °C for 20 s, 55 °C for 20 s with a -0.5 °C reduction per cycle, and 65 °C for 40 s; followed by 20 cycles of 94 °C for 20 s, 50 °C for 20 s, and 65 °C for 40 s; with a final elongation step of 65 °C for 30 min. The PCR thermal profile adopted for Microsat 2, using the same *Taq* polymerase and thermal cycler, was 95 °C for 2 min followed by 25 cycles of 94 °C for 20 s, 56 °C for 20 s, and 65 °C for 40 s, with a final elongation step of 65 °C for 30 min. PCR products were purified with 27.5 µL ethanol following standard procedures and resuspended in 30-60 µL H₂O. One µL was mixed with gel loading solution (GE HealthCare, Piscataway, NJ, USA), denatured at 95 °C for 2 min, cooled on ice, and electrophoresed using a MegaBACE 500 Capillary Sequencer (GE HealthCare).

The sizes of the dye-labelled fragments were estimated and recorded using internal size standards (Et400-R size standard, GE HealthCare) and Genetic Profiler v2.0 software (GE HealthCare, Piscataway, NJ, USA), and using a ‘binning’ approach driven by the same software, which compensated for the limits of fragment resolution. Allele

Table 1

List of cultivars and accessions analyzed with two sets of SSR markers (see text for details)

Progressive number	Cultivar	Accession	Synonym
1	Berzamin		Barzemin \ Marzamin \ Refoscón
2	Bianca Buia	n. 1	
3	Bianca Buia	n. 3	
4	Bravbama		Brambàna
5	Cividin		Porcjarùt
6	Cordenòs		Cordenòsse
7	Corvina		
8	Corvino di Nimis		Curvin
9	Forgjarin		Forzarin
10	Friularo		Raboso del piave
11	Frutignàn		Frontignàn
12	Fumât		
13	Givan		
14	Glèra	n. 1	Glère rosse
15	Glèra	vera	Glère gruesse
16	Gran rapp		
17	Malvasia Istriana	Attems	Malvasie
18	Marzemina bianca		Marzamin blanc
19	Moscato da tavola		
20	Negrât		
21	Nero Buia		
22	Nigrùz		Negrùz\Negrùc
23	Palomba		Palòmbe
24	Picolit	n. 1A	
25	Piculit neri		Picolit neri
26	Pignùl		Pignolo
27	Refosco di Rauscedo		
28	Refosco di Runcis		
29	Refosco	n. 1	Refòsc
30	Refosco	n. 2	Refòsc
31	Refosco peduncolo rosso		Refòsc
32	Refoscone		
33	Ribolla gialla	Attems	Ribùele zale
34	Ribolla nera		Ribùele nere \ Pòcalza \ Sclopetin
35	Ribolla spizàde		Ribuele spiçade
36	Ribolla gialla		Ribùele zale
37	Rossàrie Buia		
38	Schioppettino		Sclòpetin/Ribolla nera
39	Tazzelenghe		Tacelènghe
40	Terrano		Teràn
41	Tintoria		Tintòrie
42	Tocai Friulano	Attems	
43	Ucelùt		
44	Uva da tavola nera		
45	Vercluna		
46	Verdiso		Verdisòt
47	Verduzzo di Ramandolo	n. 1	Verdùc\Verdùz
48	Vitouska		
49	Chardonnay	VCR 4	
50	Sangiovese	VCR 16	
51	Riesling Renano	VCR 3	Rhein Riesling
52	Sultanina	VCR122	Thomson seedless
53	Chrupka Cervena	VCR 216	
54	Panonia Kinsce	VCR 220	
55	Silvaner Verde	VCR 186	Grüner Sylvaner
56	Tempranillo		

size was calculated as the averages of all the sizes obtained for that allele.

Data analysis: PCR and electrophoresis were repeated at least twice for all primers and samples in order to ascertain the reproducibility of the assay. The number of alleles, allele frequencies, and expected and observed heterozygosities were calculated using the computer program

CERVUS, written by Tristan Marshall, available at the web site www.fieldgenetics.com. Genotypes showing a single peak at a given locus were recorded as homozygous.

Similarity between accessions was calculated using the simple matching similarity index implemented in the SIMQUAL function (Similarity of Qualitative Data), and a cluster analysis was performed using the unweighted pair group method of analysis (UPGMA) and SHAN algorithm, and the neighbour joining algorithm (NJ). Majority consensus and strict consensus trees and clustering were done using NTSYS-pc (Exeter Software v.2.02k, Setauket, NY, USA). In addition, cophenetic correlation coefficients were calculated by comparison of the original genetic distance matrices with the cophenetic matrices obtained from the corresponding dendrograms.

Two analyses were performed separately using the genetic profiles obtained from the two sets of microsatellites, the di-nucleotide from one side, and the tri-, tetra-, and penta-nucleotide from the other side, to compare their performance in separating the genotypes analyzed.

The model-based STRUCTURE 2.2 program (PRITCHARD *et al.* 2000, FALUSH *et al.* 2003, 2007) was used to infer population structure using a burn-in of 10,000, run length of 100,000, and a model allowing for admixture and correlated allele frequencies. Five independent runs yielded consistent results. Eight international grape cultivars were added to the population analysis that was performed using only the Microsat 1 data set.

Results and Discussion

SSR polymorphism: All 39 primers produced PCR amplicons that were polymorphic and easily scorable in all of the accessions and cultivars studied. From this point of view, both of the SSR sets appeared to contain very informative markers.

The two sets of microsatellites were analyzed separately to compare their performance in fingerprinting the autochthonous cultivars included in this study.

In the Microsat 1 data set the number of alleles ranged from three to nine, the observed and expected heterozygosities ranged from 0.21 to 0.88 and from 0.41 to 0.79, respectively, and the polymorphism index content (PIC) ranged from 0.33 to 0.76 (Tab. 2). The frequencies of the least and the most represented alleles were 0.01 and 0.8, respectively. The non-exclusion probability between two unrelated individuals (NE-I) and between two hypothetical full siblings (NE-SI) ranged between 0.07 to 0.44 and 0.38 to 0.69, respectively. These values define the probability that the genotypes at a single locus do not differ between two randomly-chosen individuals. This probability may be calculated in two ways. The basic formula assumes that the two individuals are unrelated, while a more conservative formula assumes the two individuals are full siblings (WAITS *et al.* 2001). The combined non-exclusion probabilities for all 19 loci in Microsat 1 for two unrelated individuals and for full siblings were 6.02×10^{-15} and 9.09×10^{-7} , respectively. Null alleles are a common cause of apparent deviations from Hardy-Weinberg equilibrium at microsat-

Table 2

Summary of statistics for the 39 SSR markers developed for grape fingerprinting and used in the present work

	N° alleles	LG	hObs	hExp	PIC	NE-I	NE-SI	F(Null)
Microsat 1 Loci								
VChr1a	8	1	0.69	0.63	0.59	0.18	0.49	-0.05
VChr2a	3	2	0.67	0.53	0.44	0.32	0.57	-0.13
VChr3a	8	3	0.71	0.78	0.74	0.09	0.39	0.05
VChr4a	7	4	0.75	0.69	0.64	0.15	0.45	-0.06
VChr5c	6	5	0.81	0.76	0.71	0.11	0.40	-0.05
VChr6a	4	6	0.60	0.56	0.47	0.29	0.54	-0.04
VChr7b	4	7	0.77	0.72	0.66	0.13	0.43	-0.04
VChr8a	9	8	0.71	0.79	0.75	0.08	0.38	0.05
VChr9a	7	9	0.88	0.79	0.76	0.07	0.38	-0.05
VChr10a	6	10	0.23	0.35	0.33	0.44	0.69	0.19
VChr11a	3	11	0.46	0.55	0.48	0.27	0.55	0.11
VChr12a	6	12	0.77	0.74	0.69	0.11	0.41	-0.03
VChr13a	7	13	0.60	0.66	0.62	0.16	0.46	0.05
VChr14a	4	14	0.79	0.63	0.55	0.22	0.49	-0.13
VChr15a	8	15	0.58	0.70	0.64	0.15	0.44	0.09
VChr16b	7	16	0.52	0.49	0.46	0.29	0.58	-0.05
VChr17a	3	17	0.21	0.41	0.33	0.42	0.65	0.34
VChr18a	6	18	0.56	0.59	0.55	0.21	0.51	0.01
VChr19b	4	19	0.52	0.64	0.57	0.20	0.48	0.10
Microsat 2 Loci								
VVIP60	11	1	0.65	0.76	0.73	0.08	0.39	0.10
VVIB01	5	2	0.79	0.66	0.59	0.18	0.47	-0.11
VVMD28	14	3	0.90	0.88	0.86	0.03	0.32	-0.01
VVMD32	7	4	0.96	0.84	0.81	0.05	0.35	-0.08
VVMD27	7	5	0.81	0.82	0.78	0.06	0.36	0.00
VVMD21	5	6	0.60	0.56	0.51	0.25	0.53	-0.04
VVMD7	11	7	0.83	0.81	0.78	0.07	0.37	-0.02
VVC1B11	7	8	0.85	0.77	0.72	0.10	0.40	-0.06
VVIQ52	5	9	0.69	0.68	0.61	0.17	0.46	-0.02
VVIV37	9	10	0.85	0.78	0.74	0.09	0.39	-0.06
VVMD25	9	11	0.83	0.81	0.78	0.06	0.36	-0.02
VVS2	11	11	0.83	0.84	0.81	0.05	0.35	0.00
VVC4F3	12	12	0.83	0.76	0.72	0.09	0.40	-0.05
VVIH54	11	13	0.88	0.80	0.76	0.08	0.38	-0.05
VVMD24	7	14	0.69	0.75	0.71	0.10	0.41	0.04
VVIV67	8	15	0.88	0.84	0.81	0.05	0.35	-0.03
VVMD5	9	16	0.90	0.82	0.78	0.06	0.36	-0.05
VVIN73	6	17	0.21	0.21	0.20	0.63	0.80	0.03
VVIN16	5	18	0.67	0.64	0.59	0.18	0.48	-0.03
VVIP31	11	19	1.00	0.87	0.84	0.04	0.33	-0.08

LG = linkage group; hObs, hExp = observed and expected heterozygosity; PIC = polymorphism index content; NE-I, NE-SI = non-exclusion probability between two unrelated individuals and between two hypothetical full siblings, respectively; null allele = frequency of null allele.

ellite loci (PEMBERTON *et al.* 1995) and may interfere with pedigree reconstructions. In the absence of null alleles, the estimated frequency of null alleles will be close to zero (Tab. 2), and may be slightly negative in the presence of an excess of observed heterozygous genotypes. The software Cervus uses an iterative algorithm based on the observed and expected frequencies of the various genotypes. Cervus also provides estimates of the frequencies of the visible alleles taking account of the presence of any null allele. Ten iterations are used to generate null allele frequencies. A locus with a large positive estimate of null allele frequencies indicates an excess of homozygotes but does not necessarily imply that a null allele is present. VChr17a had the lowest number of alleles (three) and the highest estimate of null alleles (0.34) suggesting some care using this marker.

Considering the Microsat 2 data set, the number of alleles ranged from five to fourteen, the observed and ex-

pected heterozygosities ranged from 0.21 to 1.0 and from 0.21 to 0.88 respectively, and the PIC ranged from 0.20 to 0.86 (Tab. 2). The frequencies of the least and the most represented alleles were 0.01 and 0.88, respectively. The non-exclusion probability between two unrelated individuals (NE-I) and between two hypothetical full siblings (NE-SI) ranged between 0.03 to 0.63 and 0.32 to 0.80, respectively. The combined non-exclusion probabilities for all 20 loci in Microsat 2 for two unrelated individuals and for full siblings were 7.84×10^{-22} and 1.22×10^{-8} , respectively.

Both data sets are very informative; Microsat 2, based on di-nucleotide-type microsatellites, showed a higher number of alleles, and consequently, a potentially higher power for discriminating close genotypes. On the other hand, Microsat 1, based on tri-, tetra-, and penta-nucleotide microsatellites, has the advantage of ease in scorability, while maintaining a very high power of discrimination for successful fingerprinting of the grape varieties. Allele sizes of all 48 cultivars or accessions analyzed with the Microsat 1 and 2 primer sets are presented in the Tabs 3 and 4, respectively.

Characterization of the autochthonous grape germplasm: In the second column of Tab. 1 the cultivar names are reported as recorded during the sampling campaign devoted to rescuing the old germplasm of the region. The possible synonyms are reported in the last column. Synonymous cultivars can arise due only to a possible transliteration between the cultivar name and its synonym, or substitution of a vowel. In other cases, the names or synonyms assigned may be confusing and can lead to a misidentification. For example 'Refoscón', which is synonymous with 'Berzamìn', could suggest that this genotype was or was related to 'Refosco' or 'Refoscone', however, the molecular analysis presented here indicates that it is a different cultivar (Tabs 3 and 4 and Fig. 1). Most of the cultivars in this sample are well known, and records are reported in the literature. For a recent review see COSTANTINI *et al.* (2007). Some cultivar names have not been reported in any publication (e.g. 'Bianca di Buia', 'Moscato da tavola', 'Nero di buia', 'Uva da tavola nera') and their names were assigned as locally known or recognized, often referring only to the color of the bunches ("Bianca" or "nero" for white or black berries respectively) or to the use ("uva da tavola" for table grape). It is not the aim of this publication to identify these germplasms or to assign these samples to known national or international cultivars.

Of the 48 accession analysed, all gave different allelic profiles using both sets of microsatellites except for the cultivars 'Refosco di Runcis' and 'Refoscone' (Fig. 1).

Two genetic similarity matrices, based on a simple matching coefficient, were obtained with both data sets of microsatellites (Microsat 1 and 2). These matrices were used for grouping all of the accessions using the UPGMA and NJ algorithms. The cophenetic correlations between the similarity matrices and the cophenetic matrices obtained from the dendrograms were low ($r < 0.7$) for both the Microsat 1 and 2 data sets produced, indicating a poor fit of the cluster analysis. Nevertheless, to summarize these data for ease of interpretation, majority and strict consensus trees were obtained from 32 and 1040 UPGMA trees

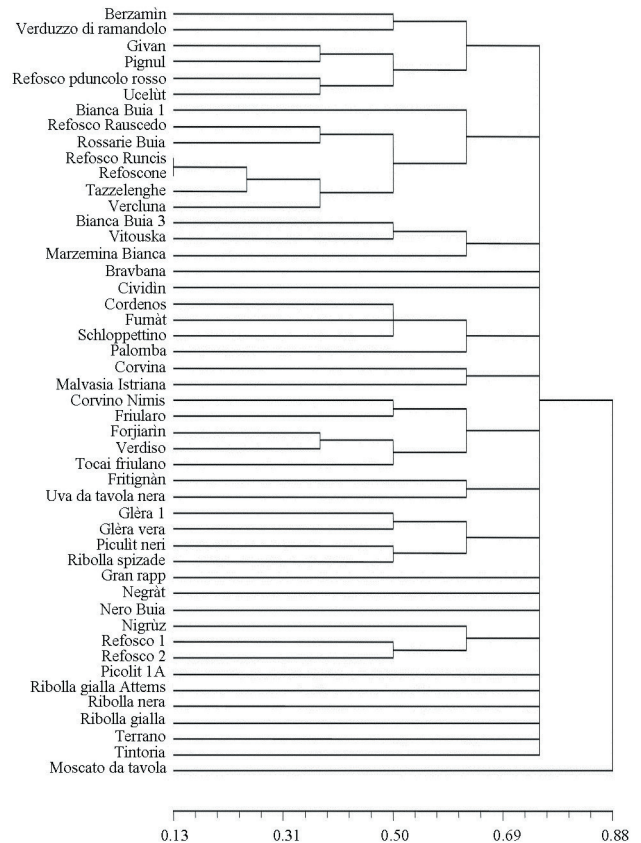


Fig. 1: Majority consensus tree obtained from the analysis of 48 autochthonous cultivars from the Friuli Venezia Giulia region (North-Eastern Italy). The tree was obtained from 32 possible topologies constructed with the UPGMA method using the tri-, tetra-, and penta-nucleotide microsatellite data set.

for the data sets originated by Microsat 1 and 2, respectively. Both data sets produced identical consensus tree topology; Fig. 1 shows the majority consensus tree obtained from the analysis of the 48 autochthonous cultivars. The NJ dendrograms were much more branched, but again, the cophenetic correlations were very low (data not shown). As a result, any speculation about the relationships among the cultivars and accessions is unrealistic and not conclusive. It is worth mentioning that 'Schioppettino' and 'Ribolla Nera', previously considered to be synonymous, were identified by this study as two different cultivars. Microsat 1 and 2 data sets showed a large difference in the genotypes of these two cultivars, with only 21 % and 10 % of alleles shared respectively (Tabs 3 and 4). The two plant phenotypes also look quite different, with 'Ribolla nera' appearing quite dissimilar to the ampelographic description of 'Schioppettino' (COSTANTINI *et al.* 2007).

A model-based clustering method for inferring population structure was used on the Microsat 1 data set including the 48 autochthonous cultivars and 8 international cultivars as reported in the methods section (Tab. 1). The 19 markers selected for genotyping meet the conditions for a correct analysis of the genetic structure of the samples (CIPRIANI *et al.* 2008). Analysis of the 56 grape genotypes considered in this study provided evidence of significant population structure within the sample. Although the small sample size used in this study suggests careful consideration of any conclusions, the data analyzed with STRUC-

Table 3
Allele sizes of 48 autochthonous grape cultivars analyzed with 19 tri-, tetra-, and penta-nucleotide repeat motif SSR markers. Allele sizes were calculated as the average of all sizes obtained for that allele

Sample name	Locus									
	VChr1a	VChr2a	VChr3a	VChr4a	VChr5c	VChr6a	VChr7b	VChr8a	VChr9a	VChr10a
Berzamin	223/227	147.8/147.8	189.4/198.7	196.8/196.8	118.5/126.7	182/182	182/190.1	183.3/208.2	87.9/109.7	109.2/109.2
Bianca Buia 1	223/243.4	139.7/147.8	189.4/201.9	181.6/196.8	102/118.5	182/186	190.1/190.1	183.3/198.9	116.1/119	109.2/109.2
Bianca Buia 3	218.9/227	139.7/147.8	183.2/189.4	196.8/196.8	86.1/86.1	174.1/186	182/186	183.3/208.2	87.9/109.7	109.2/137.3
Bravbama	223/243.4	139.7/155.9	189.4/223	170.1/192.9	86.1/102	182/186	182/186	198.9/198.9	97.1/109.7	109.2/109.2
Cividin	223/243.4	147.8/155.9	180.1/201.9	181.6/181.6	86.1/126.7	182/186	173.8/182	195.5/208.2	87.9/112.9	109.2/109.2
Cordenos	231.2/243.4	139.7/147.8	180.1/189.4	181.6/204.3	102/126.7	182/186	182/190.1	208.2/208.2	100/109.7	109.2/109.2
Corvina	223/223	139.7/147.8	180.1/180.1	196.8/196.8	86.1/102	182/186	173.8/190.1	198.9/208.2	109.7/112.9	109.2/118.8
Corvino Nimis	223/223	147.8/147.8	183.2/189.4	196.8/204.3	126.7/126.7	186/186	173.8/186	183.3/186.5	116.1/119	109.2/109.2
Forgiatin	223/223	139.7/147.8	180.1/180.1	181.6/204.3	105.9/126.7	182/186	173.8/186	173.9/198.9	87.9/97.1	109.2/109.2
Friularo	223/243.4	147.8/155.9	180.1/189.4	181.6/196.8	102/105.9	182/186	182/186	183.3/208.2	87.9/119	106/109.2
Fruignan	223/227	139.7/147.8	183.2/201.9	196.8/204.3	86.1/86.1	182/186	173.8/182	183.3/208.2	87.9/116.1	109.2/131.1
Fumat	223/223	139.7/147.8	180.1/189.4	196.8/204.3	86.1/102	182/186	182/190.1	198.9/208.2	97.1/109.7	109.2/109.2
Givan	223/243.4	147.8/147.8	180.1/180.1	196.8/204.3	102/126.7	182/186	173.8/190.1	183.3/195.5	109.7/116.1	109.2/109.2
Glèra 1	223/231.2	139.7/147.8	183.2/189.4	181.6/196.8	86.1/126.7	174.1/182	182/186	183.3/208.2	87.9/112.9	109.2/109.2
Glèra vera	223/243.4	139.7/147.8	180.1/189.4	181.6/196.8	86.1/86.1	182/186	182/186	183.3/183.3	87.9/100	109.2/140.4
Gran rapp	198.7/243.4	139.7/139.7	183.2/186.4	181.6/196.8	86.1/102	182/182	182/186	186.5/195.5	87.9/100	109.2/137.3
Malvasia Istriana Attems	176.8/243.4	139.7/147.8	180.1/180.1	196.8/196.8	102/102	182/182	173.8/190.1	208.2/208.2	109.7/112.9	118.8/118.8
Marzemina bianca	223/243.4	139.7/147.8	183.2/189.4	160/181.6	86.1/102	182/186	182/186	183.3/208.2	87.9/116.1	140.4/140.4
Moscato da tavola	194.6/223	147.8/147.8	183.2/268.2	192.9/192.9	102/118.5	186/186	182/182	183.3/195.5	87.9/100	137.3/137.3
Negràt	223/243.4	139.7/147.8	201.9/201.9	181.6/196.8	86.1/102	186/186	182/190.1	198.9/208.2	87.9/112.9	109.2/109.2
Nero Buia	223/227	147.8/147.8	189.4/268.2	181.6/204.3	86.1/102	169.5/186	182/190.1	200/200	87.9/87.9	109.2/131.1
Nigrùz	176.8/223	139.7/147.8	186.4/201.9	181.6/181.6	86.1/102	186/186	182/182	208.2/208.2	87.9/97.1	109.2/109.2
Palomba	231.2/243.4	147.8/147.8	189.4/201.9	196.8/204.3	102/126.7	182/182	182/182	186.5/208.2	97.1/109.7	109.2/109.2
Picolit 1A	223/231.2	139.7/139.7	201.9/201.9	173.3/196.8	118.5/126.7	182/186	173.8/190.1	186.5/208.2	87.9/112.9	109.2/118.8
Piculit neri	223/227	147.8/147.8	180.1/183.2	181.6/204.3	86.1/102	182/182	182/190.1	173.9/208.2	100/112.9	109.2/109.2
Pignùl	223/243.4	147.8/147.8	180.1/180.1	196.8/204.3	102/126.7	182/186	173.8/190.1	183.3/189.5	109.7/116.1	109.2/109.2
Refosco di Rauscedo	223/223	147.8/147.8	180.1/201.9	196.8/204.3	102/118.5	182/186	173.8/182	198.9/198.9	87.9/87.9	109.2/109.2
Refosco di Runciis	223/227	139.7/147.8	189.4/201.9	173.3/196.8	86.1/118.5	182/186	182/190.1	198.9/208.2	87.9/87.9	109.2/109.2
Refosco n. 1	176.8/223	139.7/147.8	180.1/201.9	181.6/196.8	86.1/126.7	182/186	182/190.1	208.2/208.2	87.9/109.7	109.2/109.2
Refosco n. 2	227/231.2	147.8/147.8	189.4/201.9	181.6/204.3	86.1/86.1	182/186	186/190.1	208.2/208.2	87.9/109.7	109.2/109.2
Refosco peduncolo rosso	223/223	139.7/147.8	180.1/201.9	173.3/196.8	102/126.7	182/186	182/190.1	183.3/198.9	97.1/116.1	109.2/109.2
Refoscone	223/227	139.7/147.8	189.4/201.9	196.8/196.8	86.1/102	182/186	190.1/190.1	198.9/208.2	87.9/87.9	109.2/109.2
Ribolla gialla Attems	223/243.4	139.7/147.8	183.2/201.9	196.8/196.8	102/126.7	182/186	173.8/182	186.5/186.5	97.1/116.1	109.2/109.2
Ribolla nera	223/223	139.7/147.8	180.1/183.2	181.6/196.8	86.1/102	182/186	182/182	183.3/183.3	87.9/109.7	109.2/118.8
Ribolla spizade	223/243.4	147.8/147.8	201.9/201.9	181.6/196.8	102/118.5	174.1/182	182/182	183.3/208.2	112.9/116.1	140.4/140.4
Ribolla gialla	223/223	139.7/147.8	180.1/183.2	181.6/196.8	86.1/102	186/186	182/190.1	198.9/198.9	87.9/116.1	109.2/137.3
Rossarie Buia	223/223	147.8/147.8	201.9/201.9	181.6/196.8	102/118.5	182/186	182/190.1	173.9/198.9	87.9/97.1	109.2/109.2
Schioppettino	223/227	139.7/147.8	189.4/201.9	196.8/204.3	102/110.2	182/186	182/190.1	177/208.2	97.1/109.7	109.2/109.2
Tazzelenghe	223/227	147.8/147.8	189.4/201.9	173.3/196.8	86.1/118.5	182/182	190.1/190.1	198.9/208.2	87.9/109.7	109.2/109.2
Terrano	223/223	147.8/155.9	201.9/201.9	181.6/196.8	102/118.5	182/182	182/190.1	173.9/186.5	97.1/109.7	109.2/109.2
Tintoria	223/223	147.8/155.9	183.2/201.9	181.6/181.6	86.1/102	182/186	173.8/173.8	195.5/198.9	97.1/112.9	109.2/109.2
Tocai Friulano Attems	223/223	139.7/147.8	198.7/201.9	173.3/196.8	118.5/118.5	c/c	173.8/186	186.5/198.9	87.9/97.1	109.2/109.2
Ucelùt	223/223	139.7/147.8	201.9/201.9	196.8/196.8	102/102	182/186	173.8/190.1	183.3/186.5	109.7/116.1	109.2/109.2
Uva da tavola nera	198.7/243.4	139.7/147.8	189.4/189.4	181.6/196.8	86.1/102	182/182	173.8/182	198.9/198.9	87.9/112.9	109.2/109.2
Verceluna	227/243.4	147.8/147.8	201.9/201.9	181.6/196.8	86.1/118.5	182/182	190.1/190.1	198.9/208.2	87.9/87.9	109.2/109.2
Verdisio	223/223	139.7/155.9	198.7/201.9	173.3/196.8	105.9/126.7	186/186	186/186	198.9/198.9	87.9/97.1	109.2/109.2
Verduzzo di Ramandolo 1	223/223	139.7/147.8	180.1/198.7	181.6/196.8	102/126.7	182/182	190.1/190.1	198.9/208.2	87.9/87.9	109.2/109.2
Vitouska	223/227	139.7/147.8	183.2/183.2	196.8/196.8	86.1/86.1	174.1/182	182/186	183.3/208.2	109.7/112.9	109.2/140.4

Tab. 3, continued

Sample name	Locus									
	VChr11a	VChr12a	VChr13a	VChr14a	VChr15a	VChr16b	VChr17a	VChr18a	VChr19b	
Berzamin	200.5/200.5	132.8/136.9	142.4/142.4	135.1/190.7	150.4/154.4	179.2/191.4	178.8/186.6	153/165.3	164.3/168.4	
Bianca Buia 1	200.5/200.5	136.9/145.1	151.9/151.9	135.1/135.1	154.4/154.4	166.8/191.4	186.6/186.6	153/165.3	168.4/168.4	
Bianca Buia 3	200.5/200.5	132.8/144	142.4/145.3	135.1/135.1	150.4/154.4	166.8/191.4	186.6/186.6	153/165.3	160.3/160.3	
Bravbama	187.9/208.9	136.9/145.1	151.9/156.7	135.1/135.1	142.3/142.3	166.8/191.4	186.6/186.6	161.2/165.3	168.4/168.4	
Cividin	187.9/187.9	136.9/144	145.3/151.9	135.1/190.7	150.4/154.4	186.3/191.4	186.6/186.6	165.3/165.3	164.3/168.4	
Cordenos	187.9/208.9	144/144	147.2/151.9	135.1/190.7	142.3/146.3	186.3/191.4	186.6/186.6	165.3/165.3	164.3/164.3	
Corvina	187.9/200.5	124.6/145.1	147.2/156.7	135.1/190.7	142.3/154.4	191.4/191.4	178.8/186.6	161.2/165.3	168.4/172.4	
Corvino Nimis	187.9/187.9	124.6/144	137.7/151.9	128/135.1	150.4/150.4	191.4/191.4	186.6/186.6	165.3/169.4	168.4/168.4	
Forjinaro	200.5/208.9	136.9/144	142.4/147.2	128/135.1	154.4/162.5	179.2/191.4	186.6/186.6	165.3/165.3	164.3/168.4	
Friularo	187.9/187.9	144/144	151.9/151.9	128/135.1	142.3/150.4	191.4/191.4	186.6/186.6	165.3/165.3	168.4/168.4	
Fruignàn	200.5/200.5	144/145.1	151.9/151.9	130.9/190.7	142.3/154.4	191.4/191.4	178.8/178.8	161.2/165.3	168.4/168.4	
Fumât	187.9/208.9	136.9/145.1	142.4/151.9	135.1/190.7	150.4/162.5	191.4/191.4	186.6/186.6	165.3/165.3	164.3/164.3	
Givan	200.5/200.5	124.6/144	147.2/147.2	135.1/135.1	150.4/154.4	186.3/191.4	186.6/186.6	161.2/165.3	164.3/164.3	
Glèra 1	187.9/200.5	144/144	142.4/151.9	190.7/190.7	146.3/150.4	186.3/191.4	186.6/186.6	194.1/194.1	164.3/172.4	
Glèra vera	187.9/200.5	144/145.1	142.4/151.9	135.1/190.7	150.4/150.4	191.4/191.4	186.6/186.6	165.3/165.3	164.3/164.3	
Gran rapp	187.9/200.5	136.9/144	147.2/151.9	135.1/190.7	146.3/154.4	182.1/191.4	186.6/186.6	153/169.4	160.3/164.3	
Malvasia Istriana Attems	200.5/200.5	139.9/144	151.9/166.2	135.1/190.7	150.4/150.4	166.8/191.4	178.8/178.8	153/165.3	160.3/164.3	
Marzemina bianca	200.5/200.5	136.9/139.9	151.9/151.9	130.9/135.1	138.3/154.4	191.4/191.4	186.6/186.6	153/161.2	164.3/164.3	
Moscato da tavola	200.5/200.5	139.9/145.1	147.2/151.9	128/135.1	154.4/154.4	191.4/191.4	178.8/178.8	165.3/165.3	168.4/172.4	
Negrât	200.5/200.5	136.9/139.9	142.4/142.4	135.1/190.7	150.4/150.4	191.4/191.4	186.6/186.6	165.3/165.3	164.3/164.3	
Nero Buia	200.5/200.5	136.9/144	137.7/151.9	128/135.1	150.4/150.4	191.4/191.4	186.6/186.6	165.3/165.3	164.3/168.4	
Nigrûz	200.5/208.9	144/144	147.2/166.2	135.1/190.7	142.3/142.3	191.4/191.4	186.6/186.6	161.2/165.3	160.3/168.4	
Palomba	187.9/200.5	124.6/145.1	151.9/151.9	130.9/190.7	150.4/154.4	186.3/191.4	186.6/186.6	165.3/165.3	164.3/172.4	
Picolit 1/A	187.9/187.9	144/144	142.4/147.2	135.1/190.7	142.3/150.4	191.4/191.4	186.6/186.6	165.3/165.3	164.3/168.4	
Piculit neri	200.5/200.5	124.6/144	147.2/147.2	128/135.1	150.4/154.4	186.3/191.4	186.6/186.6	165.3/165.3	164.3/168.4	
Pignûl	187.9/200.5	136.9/144	147.2/151.9	135.1/190.7	150.4/154.4	191.4/191.4	186.6/186.6	161.2/165.3	164.3/164.3	
Refosco di Rauscedo	187.9/200.5	136.9/144	151.9/151.9	128/135.1	150.4/154.4	186.3/191.4	186.6/186.6	165.3/173.4	168.4/168.4	
Refosco di Runcis	200.5/200.5	136.9/139.9	151.9/151.9	190.7/190.7	154.4/154.4	191.4/191.4	178.8/186.6	165.3/173.4	168.4/168.4	
Refosco n. 1	200.5/200.5	136.9/144	142.4/151.9	190.7/190.7	142.3/154.4	166.8/191.4	178.8/186.6	165.3/165.3	160.3/160.3	
Refosco n. 2	200.5/200.5	136.9/144	142.4/151.9	190.7/190.7	150.4/154.4	186.3/191.4	178.8/186.6	165.3/165.3	164.3/164.3	
Refosco peduncolo rosso	200.5/200.5	136.9/145.1	137.7/151.9	130.9/190.7	162.5/166.5	191.4/191.4	186.6/186.6	165.3/165.3	164.3/164.3	
Refoscone	200.5/200.5	136.9/139.9	151.9/151.9	190.7/190.7	154.4/154.4	191.4/191.4	178.8/186.6	165.3/173.4	168.4/168.4	
Ribolla gialla Attems	187.9/200.5	139.9/139.9	142.4/151.9	135.1/190.7	150.4/154.4	166.8/191.4	186.6/186.6	153/173.4	160.3/164.3	
Ribolla nera	187.9/187.9	124.6/144	151.9/156.7	135.1/190.7	150.4/154.4	186.3/191.4	186.6/186.6	153/153	164.3/168.4	
Ribolla spizade	200.5/208.9	144/144	151.9/151.9	135.1/190.7	150.4/150.4	191.4/191.4	186.6/186.6	165.3/165.3	164.3/172.4	
Ribolla gialla	187.9/200.5	139.9/139.9	151.9/151.9	135.1/190.7	130.1/154.4	166.8/191.4	178.8/186.6	165.3/173.4	164.3/164.3	
Rossarie Buia	200.5/208.9	136.9/144	151.9/151.9	135.1/190.7	150.4/154.4	191.4/191.4	186.6/186.6	165.3/169.4	168.4/168.4	
Schioppetuno	187.9/208.9	136.9/144	147.2/151.9	130.9/135.1	142.3/142.3	191.4/191.4	186.6/186.6	165.3/165.3	164.3/168.4	
Tazzelenghe	200.5/200.5	136.9/145.1	147.2/151.9	135.1/190.7	154.4/154.4	191.4/191.4	178.8/178.8	165.3/173.4	168.4/168.4	
Terrano	187.9/208.9	136.9/144	151.9/151.9	130.9/190.7	150.4/150.4	191.4/191.4	178.8/186.6	161.2/165.3	164.3/168.4	
Tintoria	187.9/208.9	144/144	151.9/151.9	135.1/190.7	150.4/154.4	166.8/166.8	178.8/178.8	153/165.3	168.4/172.4	
Tocai Friulano Attems	200.5/208.9	136.9/144	137.7/142.4	128/135.1	150.4/154.4	191.4/191.4	186.6/186.6	153/173.4	164.3/168.4	
Ucclut	200.5/200.5	124.6/136.9	151.9/151.9	135.1/190.7	150.4/150.4	191.4/191.4	178.8/186.6	165.3/165.3	164.3/164.3	
Uva da tavola nera	200.5/200.5	136.9/145.1	142.4/151.9	135.1/190.7	142.3/142.3	191.4/191.4	178.8/178.8	161.2/165.3	164.3/168.4	
Verceluna	200.5/200.5	136.9/144	150.4/151.9	135.1/190.7	150.4/154.4	166.8/191.4	178.8/178.8	165.3/173.4	164.3/168.4	
Verdiso	200.5/208.9	136.9/144	142.4/151.9	128/135.1	142.3/150.4	166.8/191.4	178.8/178.8	165.3/165.3	164.3/168.4	
Verduzzo di Ramandolo 1	200.5/200.5	124.6/136.9	142.4/147.2	135.1/135.1	150.4/150.4	179.2/191.4	186.6/186.6	153/165.3	164.3/168.4	
Vitouska	200.5/200.5	144/144	142.4/145.3	135.1/190.7	150.4/150.4	191.4/191.4	186.6/186.6	153/153	160.3/164.3	

TURE produced the highest log likelihood scores when the number of populations was set to six (Fig. 2). As a result of this analysis, some general observations have been made: a) all of the international cultivars were included in populations 2 and 5 (Fig. 2); b) all of the autochthonous cultivars related to table grapes were included in population 2; c) the autochthonous cultivars are distributed among the 6 populations, showing a quite diverse genetic background; and d) the ‘refosco group’ is distributed into three populations (3, 4, and 6). The 8 international cultivars were chosen on the basis of their different areas of cultivation across Europe, with the aim of introducing a large allelic diversity into the analysis in order to infer possible relationships among the autochthonous cultivars and the international varieties. The genetic diversity of the local cultivars appears to be very large considering that a similar analysis of population structure performed on 48 international cultivars, mainly known as Italian and French varieties, showed a separation in only two populations (CIPRIANI *et al.* 2008). This sub-structure of the local cultivars demonstrated that the introduction and cultivation of grape into the Friuli Venezia Giulia region originated from a plurality of centers of diversification.

The ‘refosco group’ could be a case of cultivar homonymy or of mislabelling, considering that they were distributed into three populations, showing evidence of a diverse genetic background.

Conclusions

The present work confirms the usefulness of SSR markers for studying genetic diversity in grape. High levels of polymorphism, and a high number of alleles per locus were expected because of the high level of heterozygosity observed in the grape genome. Although a relatively small number of cultivars were analyzed and the genetic diversity was under-represented, the analysis of similarity showed the genetic richness of the local germplasm. It was not possible to compare the accessions analyzed in the present work with SSR profiles of other grape cultivars reported in the literature, because of the heterogeneity of markers used

and the difficulty of comparing allelic patterns obtained for the same cultivar with different protocols and equipment. A subset of microsatellite markers have been proposed and extensively used during the European Union funded project GENRES081 that focused on the compilation, standardization, and exchange of information concerning grapevine genetic resources (THIS and DETTWEILER 2003). However, the number of available microsatellites has been increased in recent years as a consequence of an agreement among different institutions that developed hundreds of new SSR markers within the Vitis Microsatellite Consortium. These markers have been used to develop a new set of SSRs devoted to grape fingerprinting (DI VECCHI STARAZ *et al.* 2007).

The available grape genome sequence (<http://www.appliedgenomics.org/vitis8x/>) enabled the recovery of thousands of microsatellite markers with long repeats, namely, tri-, tetra-, and penta-nucleotides, which are the most recommended marker type for individual genotyping.

The two microsatellite sets, based on di-nucleotide repeats (DI VECCHI STARAZ *et al.* 2007) and tri-, tetra-, and penta-nucleotide repeats (CIPRIANI *et al.* 2008), respectively, were compared in a case study of autochthonous cultivars and both appeared to be very effective in cultivar fingerprinting. Both sets cover the 19 haploid chromosome number of the grape genome and therefore their segregations were completely independent.

The tri-penta-nucleotide repeat markers, which are the “marker of choice” in human DNA analysis due to their low ‘stuttering’ and larger relative size differences between incremental alleles, are now available in grape.

Acknowledgements

This research was supported with funds from the Regional Government of Friuli Venezia Giulia, Grape Breeding project. M.T. MARRAZZO has been supported by a grant of the Fondo Sociale Europeo 2000-2006, Asse D, Misura D4, Miglioramento delle risorse umane nel settore della ricerca e sviluppo tecnologico, Regione Autonoma Friuli Venezia Giulia. The authors thank C. COLEMAN for having kindly reviewed the manuscript.



Fig. 2: Frequency assignment of the 48 autochthonous cultivars of the Friuli Venezia Giulia region (North-Eastern Italy) and 8 international cultivars to different populations. Six different levels of grey correspond to the 6 sub populations obtained from the analysis with the software STRUCTURE.

Table 4

Allele sizes of 48 autochthonous grape cultivars analyzed with 20 di-nucleotide repeat motif SSR markers. Allele sizes were calculated as the average of all the sizes obtained for that allele

Sample name	Locus									
	VMC1B11	VMC4F3	VVIB01	VVIH54	VVIN16	VVIN73	VVIP31	VVIP60	VVIQ52	VVIV37
Berzamin	173/184.7	174.1/174.1	292.8/296.7	165.1/169	154.2/160.2	265.1/265.1	181/184.9	320.7/320.7	81.1/81.1	161.6/163.6
Bianca Buia 1	173/182.8	167.9/174.1	290.7/300.8	165.1/167.1	158.2/160.2	265.1/265.1	181/196.7	320.7/320.7	87.2/87.2	161.6/163.6
Bianca Buia 3	173/196.6	167.9/208	296.7/300.8	167.1/167.1	152.3/154.2	257.4/265.1	175/179.3	316.4/319.4	81.1/91.5	163.6/171.8
Bravbama	173/173	167.9/180.2	292.8/296.7	142.1/178.5	152.3/160.2	265.1/265.1	181/184.9	320.7/320.7	87.2/87.2	153.3/163.6
Cividin	167.1/184.7	174.1/174.1	292.8/296.7	169/188.3	152.3/160.2	265.1/265.1	184.9/190.7	316.6/319.4	83.1/87.2	153.3/163.6
Cordenos	167.1/169.1	167.9/180.2	290.7/296.7	165.1/169	152.3/160.2	265.1/265.1	190.7/196.7	305.4/325.1	81.1/83.1	153.3/163.6
Corvina	167.1/173	167.9/174.1	290.7/292.8	165.1/169	152.3/160.2	265.1/265.1	184.9/196.7	320.7/320.7	81.1/83.1	159.6/163.6
Corvino Nimis	167.1/184.7	167.9/174.1	292.8/296.7	169/169	152.3/158.2	265.1/265.1	181/184.9	319.4/324.1	81.1/81.1	153.3/163.6
Forjatin	169.1/173	174.1/190.5	292.8/292.8	167.1/167.1	152.3/154.2	259.3/265.1	190.7/196.7	317.6/320.7	81.1/83.1	153.3/163.6
Friularo	184.7/184.7	170.2/174.1	292.8/292.8	165.1/167.1	152.3/160.2	265.1/265.1	181/184.9	320.7/320.7	87.2/87.2	159.6/163.6
Fruignàn	184.7/184.7	165.8/204.9	292.8/296.7	167.1/169	152.3/160.2	257.4/263.2	190.7/196.7	320.7/330.8	81.1/85.1	153.3/161.6
Fumât	169.1/173	167.9/174.1	296.7/296.7	167.1/169	152.3/154.2	265.1/265.1	184.9/190.7	305.4/320.7	81.1/83.1	161.6/165.5
Givan	173/184.7	174.1/180.2	290.7/296.7	165.1/174.8	152.3/152.3	265.1/265.1	177/184.9	319.4/325.1	81.1/87.2	161.6/163.6
Glèra 1	167.1/184.7	167.9/174.1	296.7/296.7	165.1/169	150.5/152.3	265.1/265.1	179.3/190.7	316.6/325.1	83.1/87.2	163.6/163.6
Glèra vera	167.1/182.8	167.9/174.1	296.7/296.7	169/188.3	150.5/152.3	265.1/265.1	179.3/192.6	319.4/325.1	81.1/83.1	163.6/171.8
Gran rapp	167.1/184.7	167.9/184.4	290.7/292.8	167.1/169	152.3/154.2	265.1/265.1	177/190.7	320.7/320.7	81.1/87.2	161.6/171.8
Malvasia Istriana Attems	167.1/184.7	167.9/184.4	296.7/296.7	178.5/180.6	152.3/154.2	257.4/265.1	184.9/196.7	316.6/320.7	81.1/87.2	163.6/180.1
Marzemina bianca	182.8/184.7	174.1/174.1	290.7/292.8	178.5/188.3	152.3/152.3	265.1/265.1	184.9/196.7	316.6/319.4	81.1/81.1	163.6/171.8
Moscato da tavola	167.1/184.7	174.1/175.7	292.8/296.7	167.1/167.1	150.5/158.2	255.6/265.1	179.3/188.9	316.6/321.8	83.1/85.1	165.5/171.8
Negrât	167.1/167.1	167.9/174.1	292.8/296.7	165.1/169	152.3/158.2	265.1/265.1	181/196.7	317.6/320.7	81.1/81.1	161.6/163.6
Nero Buia	169.1/173	174.1/182.9	296.7/300.8	149.6/165.1	150.5/150.5	265.1/267.1	177/190.7	316.6/321.8	83.1/83.1	151.2/161.6
Nigrûz	182.8/184.7	184.4/210	290.7/296.7	167.1/169	152.3/160.2	265.1/265.1	192.6/194.7	320.7/320.7	83.1/87.2	153.3/163.6
Palomba	167.1/182.8	170.2/174.1	296.7/296.7	165.1/178.5	152.3/152.3	257.4/265.1	184.9/196.7	317.6/317.6	81.1/81.1	153.3/163.6
Picolot 1A	167.1/184.7	180.2/184.4	290.7/296.7	167.1/180.6	152.3/152.3	257.4/265.1	181/184.9	325.1/328.9	81.1/83.1	153.3/159.6
Picullit neri	167.1/184.7	167.9/180.2	292.8/296.7	151.7/169	152.3/152.3	265.1/265.1	190.7/196.7	317.6/325.1	81.1/87.2	153.3/163.6
Pignûl	173/184.7	174.1/180.2	290.7/296.7	165.1/174.8	152.3/152.3	265.1/265.1	177/184.9	319.4/325.1	81.1/87.2	161.6/163.6
Refosco di Rauscedo	169.1/173	174.1/174.1	292.8/296.7	167.1/169	152.3/152.3	265.1/265.1	181/196.7	320.7/320.7	81.1/87.2	161.6/163.6
Refosco di Runcis	173/184.7	174.1/184.4	292.8/296.7	167.1/169	152.3/160.2	265.1/265.1	177/181	320.7/320.7	81.1/83.1	161.6/161.6
Refosco n. 1	167.1/184.7	167.9/174.1	292.8/296.7	178.5/180.6	152.3/160.2	265.1/265.1	179.3/196.7	317.6/317.6	81.1/87.2	153.3/153.3
Refosco n. 2	169.1/184.7	167.9/174.1	296.7/296.7	165.1/169	150.5/160.2	265.1/265.1	177/190.7	316.6/320.7	83.1/83.1	161.6/163.6
Refosco peduncolo rosso	173/184.7	167.9/174.1	292.8/296.7	161.3/167.1	158.2/160.2	265.1/265.1	177/184.9	317.6/320.7	81.1/81.1	161.6/161.6
Refoscone	173/184.7	174.1/184.4	292.8/296.7	167.1/169	152.3/160.2	265.1/265.1	177/181	320.7/320.7	81.1/83.1	161.6/163.6
Ribolla gialla Attems	167.1/184.7	174.1/174.1	296.7/300.8	165.1/169	152.3/152.3	265.1/265.1	190.7/196.7	316.6/320.7	81.1/83.1	161.6/163.6
Ribolla nera	167.1/184.7	167.9/174.1	292.8/296.7	169/169	150.5/152.3	265.1/265.1	181/190.7	320.7/325.1	83.1/83.1	159.6/163.6
Ribolla spizade	182.8/184.7	174.1/174.1	296.7/296.7	165.1/174.8	152.3/152.3	265.1/265.1	179.3/192.6	319.4/325.1	81.1/87.2	163.6/171.8
Ribolla gialla	173/184.7	174.1/190.5	292.8/296.7	165.1/169	152.3/152.3	265.1/265.1	177/190.7	320.7/320.7	81.1/83.1	161.6/171.8
Rossarie Buia	167.1/173	174.1/180.2	290.7/296.7	165.1/169	152.3/160.2	265.1/265.1	181/184.9	317.6/319.4	87.2/87.2	153.3/163.6
Schioppettino	167.1/167.1	165.8/170.2	290.7/296.7	165.1/169	152.3/152.3	265.1/265.1	181/184.9	320.7/320.7	81.1/87.2	153.3/163.6
Tazzelenghe	167.1/173	174.1/174.1	292.8/296.7	165.1/169	152.3/152.3	265.1/265.1	177/184.9	320.7/320.7	81.1/83.1	161.6/163.6
Terrano	167.1/167.1	165.8/184.4	290.7/296.7	167.1/169	152.3/160.2	257.4/265.1	184.9/194.7	315.3/320.7	81.1/87.2	153.3/153.3
Tintoria	173/182.8	174.1/180.2	292.8/296.7	165.1/167.1	160.2/160.2	265.1/265.1	184.9/190.7	317.6/317.6	81.1/83.1	163.6/163.6
Tocai Friulano Attems	173/184.7	174.1/174.1	292.8/296.7	165.1/180.6	152.3/154.2	265.1/265.1	190.7/194.7	316.6/319.4	87.2/87.2	163.6/165.5
Ucellut	184.7/182.8	174.1/180.2	296.7/300.8	165.1/167.1	152.3/152.3	265.1/265.1	177/190.7	316.6/320.7	81.1/83.1	153.3/165.5
Uva da tavola nera	184.7/184.7	174.1/208	296.7/300.8	167.1/167.1	152.3/154.2	265.1/265.1	182.9/192.6	320.7/324.1	81.1/87.2	153.3/175.8
Verceluna	173/184.7	174.1/208	292.8/296.7	167.1/188.3	152.3/152.3	265.1/265.1	181/192.6	320.7/320.7	81.1/83.1	165.5/165.5
Verdiso	173/188.6	180.2/190.5	292.8/292.8	165.1/167.1	154.2/160.2	265.1/265.1	190.7/194.7	315.3/320.7	81.1/83.1	165.5/165.5
Verduzzo di Ramandolo 1	173/184.7	180.2/190.5	290.7/296.7	165.1/167.1	154.2/160.2	265.1/265.1	190.7/196.7	315.3/320.7	83.1/83.1	153.3/163.6
Vitouska	167.1/196.6	167.9/208	290.7/300.8	167.1/174.8	152.3/154.2	265.1/265.1	175/192.6	316.6/325.1	81.1/87.2	163.6/171.8

Tab. 4, continued

Sample name	Locus											VV52
	VV167	VVMD5	VVMD7	VVMD21	VVMD24	VVMD25	VVMD27	VVMD28	VVMD32	VV52		
Berzamin	358.5/374	230.6/239	255.7/261.5	243.1/248.9	208/208	257.1/262.9	177.9/179.9	219.9/247.4	251.8/261.5	152.2/156.3		
Bianca Buia 1	360.6/365.7	224.7/224.7	246.1/248	243.1/248.9	211.8/215.6	239.7/241.6	183.6/187.7	268.7/235.6	249.9/271.2	139.8/156.3		
Bianca Buia 3	358.5/376.9	224.7/239	238.5/248	248.9/248.9	208/208	239.7/241.6	193.1/177.9	268.7/249.6	251.8/255.7	146/146		
Bravbama	360.6/374	230.6/235	246.1/255.7	248.9/248.9	211.8/215.6	249.4/255.3	183.6/187.7	255.2/259	271.2/271.2	135.7/152.2		
Cividin	362.7/368.6	235/237.1	246.1/246.1	243.1/248.9	208/215.6	255.3/257.1	179.9/183.6	268.7/254.4	240.2/271.2	142.1/43.9		
Cordenos	358.5/358.5	224.7/226.7	238.5/261.5	248.9/248.9	211.8/211.8	255.3/255.3	179.9/187.7	259.2/49.6	240.2/263.5	135.7/156.3		
Corvina	358.5/374	230.6/230.6	248/255.7	243.1/248	208/215.6	255.3/257.1	183.6/187.7	265/237.6	261.5/271.2	135.7/156.3		
Corvino Nimis	362.7/365.7	230.6/235	246.1/255.7	248.9/248.9	213.7/216.8	249.4/255.3	179.9/179.9	265/237.6	251.8/271.2	135.7/152.2		
Forjinarin	362.7/368.6	230.6/239	246.1/255.7	248.9/248.9	206/213.7	239.7/249.4	179.9/187.7	259/245.4	255.7/271.2	133.6/142		
Friularo	365.7/374	224.7/230.6	246.1/246.1	243.1/248.9	216.8/216.8	255.3/255.3	177.9/187.7	265/237.6	240.2/271.2	135.7/135.7		
Fruignàn	362.7/362.7	224.7/237.1	238.5/251.8	248.9/248.9	208/208	241.6/241.6	183.6/193	268.7/237.6	249.9/251.8	133.6/133.6		
Fumât	358.5/368.6	226.7/235	248/259.6	243.1/248	208/216.8	241.6/253.4	183.6/189.6	237.6/229.8	251.8/271.2	133.6/152.2		
Givan	362.7/374	224.7/230.6	246.1/257.6	243.1/248.9	208/215.6	243.7/255.3	177.9/189.6	268.7/237.6	240.2/271.2	133.6/156.3		
Glèra 1	358.5/365.7	226.7/245.2	238.5/238.5	248.9/248.9	211.8/216.8	239.7/253.3	179.9/193	245.4/237.6	255.7/263.5	133.6/135.7		
Glèra vera	358.5/360.6	224.7/245.2	238.5/246.1	248.9/255.9	211.8/215.6	243.7/255.3	183.6/193	249.6/245.4	255.7/271.2	135.7/156.3		
Gran rapp	358.5/358.5	224.7/245.2	242.3/246.1	248.9/255.9	208/208	241.6/241.6	187.7/189.6	249.6/259	261.5/271.2	133.6/146		
Malvasia Istriana Attems	358.5/362.7	220.9/239	238.5/238.5	248.9/248.9	208/209	255.3/255.3	177.9/177.9	259/255.2	255.7/271.2	143.9/143.9		
Marzemina bianca	360.6/366.7	224.7/233.1	238.5/246.1	248.9/255.9	208/215.6	241.6/243.7	177.9/193	278.4/245.4	249.9/271.2	154.2/156.3		
Moscato da tavola	374/374	230.6/237.1	238.5/248	255.9/255.9	211.8/211.8	249.4/249.4	177.9/187.7	249.6/237.6	255.7/271.2	135.7/150.1		
Negrât	358.5/374	230.6/230.6	246.1/255.7	248.9/248.9	208/215.6	249.4/255.3	183.6/187.7	239.6/229.8	240.2/251.8	133.6/135.7		
Nero Buia	365.7/374	226.7/230.6	238.5/250	248.9/255.9	208/208	239.7/241.6	179.9/183.6	265/237.6	240.2/263.5	133.6/156.3		
Nigrûz	360.6/365.7	224.7/224.7	248/251.8	243.1/248.9	208/216.8	239.7/255.3	183.6/187.7	235.6/235.6	251.8/271.2	133.6/135.7		
Palomba	360.6/368.6	224.7/230.6	246.1/246.1	248.9/255.9	211.8/215.6	255.3/255.3	187.7/193	261.1/237.6	240.2/263.5	135.7/143.9		
Picolit 1A	362.7/365.7	230.6/237.1	246.1/246.1	243.1/248.9	206/216.8	239.7/255.3	179.9/183.6	235.6/235.6	240.2/249.9	135.7/139.8		
Piculit neri	358.5/368.6	230.6/245.2	238.5/261.5	248.9/248.9	211.8/211.8	255.3/255.3	177.9/193	235.6/237.6	240.2/263.5	135.7/152.2		
Pignûl	362.7/374	224.7/230.6	246.1/255.7	243.1/248.9	208/215.6	243.7/255.3	177.9/189.6	265/237.6	240.2/271.2	133.6/156.3		
Refosco di Rauscedo	358.5/365.7	224.7/224.7	246.1/261.5	248.9/255.9	208/211.8	241.6/257.1	177.9/183.6	245.4/235.6	251.8/261.5	133.6/156.3		
Refosco di Runcis	358.5/374	224.7/230.6	248/261.5	243.1/248.9	208/208	257.1/262.9	183.6/183.6	245.4/235.6	251.8/261.5	152.2/156.3		
Refosco n. 1	362.7/366.7	224.7/226.7	246.1/246.1	248.9/255.9	208/208	239.7/255.3	182.1/193	268.7/229.8	251.8/261.5	139.8/143.9		
Refosco n. 2	365.7/365.7	224.7/226.7	238.5/248	248.9/248.9	208/211.8	239.7/257.1	183.6/193	278.4/235.6	263.5/271.2	133.6/156.3		
Refosco peduncolo rosso	358.5/368.6	224.7/230.6	238.5/246.1	248.9/248.9	208/216.8	241.6/255.3	179.9/187.7	249.6/235.6	249.9/261.5	133.6/133.6		
Refoscone	358.5/374	224.7/230.6	248/261.5	243.1/248.9	208/208	257.1/262.9	183.6/183.6	237.6/235.6	251.8/263.5	152.2/156.3		
Ribolla gialla Attems	366.7/374	224.7/230.6	238.5/248	248.9/248.9	208/208	241.6/262.9	179.9/179.9	268.7/235.6	249.9/249.9	133.6/152.2		
Ribolla nera	365.7/374	224.7/230.6	238.5/248	243.1/248.9	208/211.8	239.7/255.3	179.9/187.7	247.4/235.6	255.7/271.2	135.7/139.8		
Ribolla spizade	358.5/360.6	226.7/230.6	238.5/246.1	243.1/248.9	211.8/216.8	239.7/255.3	177.9/193	237.6/237.6	255.7/271.2	133.6/143.9		
Ribolla gialla	365.7/374	230.6/233.1	238.5/248	243.1/248.9	208/208	255.3/262.9	177.9/183.6	245.4/229.8	249.9/251.8	143.9/152.2		
Rossarie Buia	365.7/374	226.7/237.1	238.5/242.3	248.9/248.9	213.7/215.6	239.7/249.4	183.6/187.7	235.6/219.9	240.2/271.2	137.8/152.2		
Schioppettino	358.5/362.7	226.7/230.6	232.7/261.5	248.9/248.9	211.8/215.6	249.4/255.3	183.6/187.7	237.6/237.6	240.2/249.9	152.2/152.2		
Tazzelenghe	358.5/374	226.7/230.6	248/261.5	243.1/248.9	208/215.6	262.9/266.9	183.6/183.6	259/235.6	251.8/271.2	152.2/154.2		
Terrano	365.7/374	224.7/226.7	246.1/248	248.9/255.9	211.8/216.8	241.6/255.3	187.7/187.7	259/235.6	249.9/271.2	135.7/156.3		
Tintoria	365.7/365.7	235/237.1	238.5/246.1	248.9/255.9	208/211.8	249.4/255.3	187.7/193	229.8/235.6	251.8/271.2	133.6/152.2		
Tocai Friulano Attems	362.7/365.7	226.7/237.1	238.5/255.7	248.9/255.9	216.8/216.8	241.6/249.4	183.6/193	235.6/249.6	240.2/257.7	133.6/152.2		
Ucellût	358.5/362.7	226.7/235	248/261.5	248.9/248.9	206/215.6	241.6/255.3	177.9/187.7	261.5/271.2	261.5/271.2	133.6/143.9		
Uva da tavola nera	374/376.9	224.7/226.7	246.1/250	255.9/265.5	211.8/216.8	241.6/249.4	177.9/183.6	268.7/268.7	261.5/263.5	133.6/133.6		
Verecluna	358.5/374	230.6/235	246.1/261.5	243.1/243.1	206/208	255.3/257.1	183.6/183.6	229.8/235.6	261.5/271.2	143.9/156.3		
Verdiso	362.7/374	226.7/235	246.1/246.1	248.9/255.9	208/215.6	239.7/249.4	179.9/183.6	235.6/247.4	240.2/251.8	133.6/152.2		
Verduzzo di Ramandolo 1	362.7/368.6	224.7/235	238.5/261.5	248.9/248.9	208/211.8	239.7/255.3	179.9/189.6	237.6/247.4	240.2/255.7	133.6/152.2		
Vitouska	365.7/376.9	224.7/239	238.5/246.1	243.1/248.9	208/211.8	239.7/241.6	177.9/193	237.6/255.2	251.8/261.5	133.6/146		

References

- ALLEWELDT, G.; DETTWEILER, E.; 1992: The Genetic Resources of *Vitis*, 3rd ed. Siebeldingen, Institut für Rebenzüchtung Geilweilerhof.
- BOWERS, J. E.; BOURSIQUOT, J. M.; THIS, P.; CHU, K.; JOHANSSON, H.; MEREDITH, C. P.; 1999 a: Historical genetics: the parentage of Chardonnay, Gamay, and other wine grapes of northeastern France. *Science* **285**, 1562-1565.
- BOWERS, J. E.; DANGL, G. S.; MEREDITH, C. P.; 1999 b: Development and characterization of additional microsatellite DNA markers for grape. *Am. J. Enol. Vitic.* **50**, 243-246.
- BOWERS, J. E.; MEREDITH, C. P.; 1996: The parentage of a classic wine grape: Cabernet Sauvignon. *Nat. Genet.* **16**, 84-87.
- CIPRIANI, G.; FRAZZA, G.; PETERLUNGER, E.; TESTOLIN, R.; 1994: Grapevine fingerprinting using microsatellite repeats. *Vitis* **33**, 211-215.
- CIPRIANI, G.; MARRAZZO, M. T.; DI GASPERO, G.; PFEIFFER, A.; MORGANTE, M.; TESTOLIN, R.; 2008: A set of microsatellite markers with long core repeat optimized for grape (*Vitis* spp.) genotyping. *BMC Plant Biol.* **8**, 127.
- COSTANTINI, E.; MATTALONI, C.; PETRUSSI, C.; 2007: La vite nella storia e nella cultura del Friuli. Forum editions, Vol 2.
- DI VECCHI STARAZ, M.; BANDINELLI, R.; BORSELLI, M.; THIS, P.; BOURSIQUOT, J.M.; LAUCOU, V.; LACOMBE, T.; 2007: Genetic structuring and parentage analysis for evolutionary studies in grapevine: kin group and origin of the cultivar Sangiovese revealed. *J. Am. Soc. Hortic. Sci.* **132**, 514-524.
- DOYLE, J. J.; DOYLE, J. L.; 1990: Isolation of plant DNA from fresh tissue. *Focus*, **12**, 13-15.
- FALUSH, D.; STEPHENS, M.; PRITCHARD, J. K.; 2003: Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567-1587.
- FALUSH, D.; STEPHENS, M.; PRITCHARD, J. K.; 2007: Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol. Ecol. Notes* doi:10.1111/j.1471-8286.2007.01758.x
- PEMBERTON, J. M.; SLATE, J.; BANCROFT, D. R.; BARRETT, J. A.; 1995: Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. *Mol. Ecol.* **4**, 249-252.
- PRITCHARD, J. K.; STEPHENS, M.; DONNELLY, P.; 2000: Inference of population structure from multilocus genotype data. *Genetics* **155**, 945-959.
- SEFC, K. M.; LOPES, M. S.; LEFORT, F.; BOTTA, R.; ROUBELAKIS-ANGELAKIS, K. A.; IBANEZ, J.; PEJIC, I.; WAGNER, H. W.; GLOSSL, 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.
- THIS P.; DETTWEILER, E.; 2003: EU-project GENRES ct96 no81: European *Vitis* database and results regarding the use of a common set of microsatellite markers. *Acta Hortic.* **603**, 59-66.
- THIS P.; JUNG, A.; BOCCACCI, P.; BORREGO, J.; BOTTA, R.; COSTANTINI, L.; CRESPIAN, M.; DANGL, G. S.; EISENHELD, C.; FERREIRA-MONTEIRO, F.; GRANDO, S.; IBÁÑEZ, J.; LACOMBE, T.; LAUCOU, V.; MAGALHÃES, R.; 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. Genet.*, **109**, 1448-1458.
- THIS P.; LACOMBE, T.; THOMAS, M.R.; 2006: Historical origins and genetic diversity of wine grapes. *Trends Genet.* **22**, 511-519.
- VOUILLAMOZ J.; MAIGRE, D.; MEREDITH, C. P.; 2003: Microsatellite analysis of ancient alpine grape cultivars: pedigree reconstruction of *Vitis vinifera* L. Cornalin du Valais. *Theor. Appl. Genet.* **107**, 448-454.
- WAITS L.P.; LUIKART, G.; TABERLET, P.; 2001: Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol. Ecol.* **10**, 24925

Received July 21, 2009