Vitis **41** (2), 89–92 (2002)

Biodiversity of grapevines (*Vitis vinifera* L.) grown in the Aosta Valley

M. Labra[1)], G. Moriondo[2)], A. Schneider[3)], F. Grassi[1)], O. Failla[4)], A. Scienza[4)] and F. Sala[1)]

[1)] Dipartimento di Biologia, Università di Milano, Milano, Italia
[2)] Istituto Agricolo Regionale, Aosta, Italia
[3)] Centro per il Miglioramento Genetico della Vite, CNR, Grugliasco (Torino), Italia
[4)] Dipartimento di Produzione Vegetale, Università di Milano, Italia

Summary

The PCR-based DNA microsatellite analysis has been applied to define genetic relationships among 19 most representative grapevine cultivars in the Aosta valley. Eighteen varieties grown nearby, in the Savoy (France), Valais (Switzerland) and Piedmont (Italy) regions, were also analysed, to verify the correlations with the Aosta cultivars inferred by the analysis of synonyms and/or morphology. Results were obtained by analysing 12 DNA microsatellite loci. High biodiversity has been recorded amongst the analysed grapevines. In some cases cultivars known with different names in the Valley and in the nearby regions displayed the same SSR allele size, proving the occurrence of synonyms. In other case, identical denominations did not correspond with the genomic identity.

K e y w o r d s : SSR, microsatellite, *Vitis vinifera* L., grapevine germplasm.

A b b r e v i a t i o n s : SSR = Simple Sequence Repeat; JC = Jaccard's coefficient.

Introduction

Italian wine grapes are characterised by high biodiversity (Calò *et al.* 2001) which in recent decades is endangered by economic conveniences. An example from the Aosta Valley: The viticultural area of the valley has decreased from 3000 ha at the beginning of the 19th century to 600 ha now and some of the traditional varieties are lost.

Information on the origin and the migration of some more recent cultivars can be found in historic documents (6th to the 10th century). At the end of the 18th century, after wars and famine had devastated the land, new varieties were introduced from the nearby Piedmont region.

In the 19th century, or possibly earlier, some of the Aostan varieties, among then Petit Rouge, Vien de Nus, Prié and Cornalin, were exported to the nearby Valais region in Switzerland.

The genetic basis of today's local grapevines is considered to be the product of different intersecting events such as direct domestication from local wild vines, import, and crosses between local wild grapes and imported grapevines.

The phylogeny and genetic relationship among grapevine cultivars is of great relevance for genetic improvement, preservation of biodiversity and exploitation of traditional wines.

In recent years, simple sequence repeat (SSR) analysis has been shown to be effective to study on grapevine varietal assortments, phylogenies and pedigrees (Botta *et al.* 1995; Bowers *et al.* 1996; Karp *et al.* 1998; Sefc *et al.* 1998; Lopes *et al*. 1999; Labra *et al.* 1999; Sefc *et al*. 2000; Regner *et al*. 2000 a and b).

In the present study we applied SSR analysis to evaluate genetic relationships among 19 cultivars grown in the Aosta valley, one in Savoy (France), 10 in Valais (Switzerland) and 7 in Piedmont (Italy). Results substantially contribute to our knowledge on the biodiversity of varieties of the Aosta region and their relationship with cultivars grown in adjacent areas.

Material and Methods

P l a n t m a t e r i a l : A complete list of the 36 grapevine (*Vitis vinifera* L.) accessions investigated in this study is given in Tab. 1.

D N A e x t r a c t i o n : Young leaflets (1-2 cm long) were harvested from rooted cuttings, frozen in liquid nitrogen and ground to fine powder. Genomic DNA was extracted in 5 ml of CTAB buffer (2 % CTAB, 100 mM Tris-HCl, pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, 1 % w/v polyvinylpyrrolidone, 0.1 % v/v β-mercaptoethanol) as described by Labra *et al*. (2001).

S S R a n a l y s i s : DNA was analysed at the following 12 microsatellite loci: VVS4, VVS29 (Thomas and Scott 1993), VVMD5, VVMD6, VVMD7 (Bowers *et al.* 1996), VVMD17, VVMD21, VVMD24, VVMD25, VVMD27 VVMD31, VVMD34 (Bowers *et al.* 1999 b)

The analysis was performed by adding 15 ng of genomic DNA to a 25 µl PCR mixture containing 0.25 µM of the DNA primer specified for each microsatellite locus, 200 µM of each of the 4 dNTPs, 0.5 U Dynazyme and Dynazyme buffer (Celbio, Italy) as specified by the supplier. PCR amplification was performed with a programmable thermal controller (PTC 100, MJ Research Inc., USA) with the following thermal cycles: 7 min at 94 °C; 35 cycles of denaturation (45 s at 94 °C), annealing (30 s at 52 °C) and extension (1 min at 72 °C); then a final step for 7 min. at 72 °C.

A n a l y s i s o f t h e D N A a m p l i f i c a t i o n p r o d u c t s : 10 µl of the PCR-amplified mixture was added to an equal volume of loading buffer (80 % formamide, 1 mg · ml^{-1} xylene cyanol FF, 1 mg · ml^{-1} bromophenol blue, 10 M EDTA, pH 8.0). A sample of 10 µl of this mixture was analysed by electrophoresis on 10 % polyacrylamide gel in TBE

Correspondence to: Dr. M. Labra, Dipartimento di Biologia, Università di Milano, Via Celoria 26, I-20133 Milano Italy. Fax: +39-226-604-330. E-mail: massimo.labra@unimi.it

Table 1

List of cultivars analysed by SSR markers, their berry colour, growing area and site of collection

Cultivars	Berry colour	Cultivation Area	Germplasm collection
Amigne	Black	Valais, Switzerland	I.A.R.
Bianc Ver	Black	Piedmont (Turin province), Italy	C.V.T.
Bonda	White	Aosta Valley, Italy	I.A.R.
Chasselas	Black	Valais, Switzerland	I.A.R.
Cornalin(Aosta Valley)	Black	Aosta Valley, Italy	I.A.R.
Cornalin (Vallais)	Black	Valais, Switzerland	S.S.C.
Corniola	Black	Aosta Valley, Italy	I.A.R.
Crovassa	Black	Aosta Valley, Italy	I.A.R.
Durize	Black	Valais, Switzerland	S.S.C.
Fumin	Black	Aosta Valley, Italy	I.A.R.
Goron	Black	Valais, Switzerland	S.S.C.
Grò Blan	White	Piedmont (Susa Valley) Italy	C.V.T
Gros Blanc	White	Aosta Valley, Italy	Commercial vineyard
Humagne Blanc	White	Valais, Switzerland	I.A.R.
Humagne Rouge	Black	Valais, Switzerland	I.A.R.
Jacquère	White	Savoia, Franche	Commercial vineyard
Luglienga	White	Piedmont, Italy	I.A.R.
Mayolet	Black	Aosta Valley, Italy	I.A.R.
Moissan	Black	Piedmont (Susa Valley) Italy	C.V.T
Ner d'Ala	Black	Aosta Valley, Italy	I.A.R.
Petit Rouge	Black	Aosta Valley, Italy	I.A.R.
Petite Arvine	White	Valais, Switzerland and Aosta Valley, Italy	I.A.R.
Premetta	Black	Aosta Valley, Italy	I.A.R.
Priè	White	Aosta Valley, Italy	I.A.R.
Provinè	Black	Piedmont (Turin province), Italy	C.V.T
Rèze	White	Valais, Switzerland	S.S.C.
Rouge du Pays	Black	Valais, Switzerland	I.A.R.
Roussien	Black	Aosta Valley, Italy	I.A.R.
Uva di Biella	Black	Piedmont (Biella province), Italy	C.V.T
Verdes	Black	Piemonte (Turin province), Italy	C.V.T
Vien de Nus	Black	Aosta Valley, Italy	I.A.R.
Vuillermin	Black	Aosta Valley, Italy	I.A.R.

I.A.R.: Collection of Institut Agricole Régional, Aosta, Italy
C.V.T.: Collection of Centro Miglioramento Vite - CNR, Grinzane, Italy
S.S.C.: Station Fédérale de Recherche en Production Végétale de Changins.

buffer (50 mM boric acid, 1 mM EDTA, pH 8.0) for 16 h at 100 mV. After staining in a 5 % ethidium bromide solution, the gel was recorded and analysed in a Gel Doc 2000 (Biorad, USA).

Statistical analysis: Genetic distance among accessions was measured on the basis of shared alleles (BOWCOCK *et al.* 1994) and on proximity measures based on dicotomic characters. For this purpose, each microsatellite allele was scored as a binary character for absence (0) or presence (1). Presence was scored independently of the heterozygous or homozygous state. Data were analyzed using the Jaccard's coefficient (JC):

$$JC = a/(n-d)$$

where a is the number of bands present in the two compared genotypes; n is the total number of polymorphic bands; d is the number of bands absent in both compared genotypes. The final product of the analysis was a dendrogram constructed by cluster analysis based upon UPGMA (unweighted pair-group method with arithmetic averages).

In all cases, usefulness of SSR as genomic markers was estimated by calculating gene diversity as $1-\acute{Y}\ p^2_{ij}$ (RONGWER *et al*. 1995), were p_{ij} is the frequency of the j allele for the microsatellite.

Results

Tab. 2 summarises the results showing, at each locus, the range of allele size, the number of alleles (from 2 to 10), and the values of gene diversity (from 0.243 to 0.785) among the 36 analysed grapevine cultivars. Values of gene diver-

Table 2

Range of allele size, number of alleles, and gene diversity verified at 12 microsatellite loci among the 36 grapevine accessions listed in Tab. 1. The sequence of the two nucleotide primers specific for each locus is given in the references cited in Material and Methods

Locus	Range of allele sizes (bp)	No. of alleles	Gene diversity
VVS4	162 – 178	8	0.785
VVMD5	222 – 240	8	0.776
VVMD6	172 – 208	6	0.613
VVMD7	237 – 252	10	0.756
VVMD17	212 – 222	3	0.599
VVMD21	243 – 266	5	0.725
VVMD24	208 – 219	7	0.761
VVMD25	243 – 259	4	0.738
VVMD27	175 – 189	7	0.714
VVS29	174 – 180	2	0.368
VVMD31	198 – 212	5	0.652
VVMD34	224 – 242	3	0.243

sity demonstrated the usefulness of the 12 selected genomic markers, with VVS4 and VVMD34 showing the highest and the lowest polymorphism, respectively; this agrees with observations of Bowers (1999).

Data on the microsatellite alleles produced by analysing the grapevine cultivars of Tab. 1 at the 12 microsatellite loci were used for statistical analysis. The resulting dendrogram (Figure) defines the genomic relationship among the cultivars. JC among cultivars varies from 1.0 (full genomic similarity) to 0.2 (high genomic dissimilarity) thus demonstrating the high polymorphism of the analysed genotypes.

Discussion

SSR markers have already been used to solve cases of homonymies and synonymies, to fingerprint varieties, and to search or confirm parents of prominent grapevines varieties, such as Cabernet Sauvignon, Chardonnay and Gamay (Bowers and Meredith 1997; Bowers *et al*. 1999a). Fossati *et al*. (2000) used AFLP and SSR analyses to demonstrate that the term "Schiave" refers to similar cultivation practices developed in contiguous regions rather than to a common genetic background.

In our present study the dendrogram constructed on the basis of the SSR analysis shows clear cases of synonymies, where cultivars known with different names display identical SSR alleles.

The first case is evident at the very top of the dendrogram where Ner d'Ala (grown in the lower part of the Valley), Verdes and Uva di Biella (grown in the Northern Piedmont)

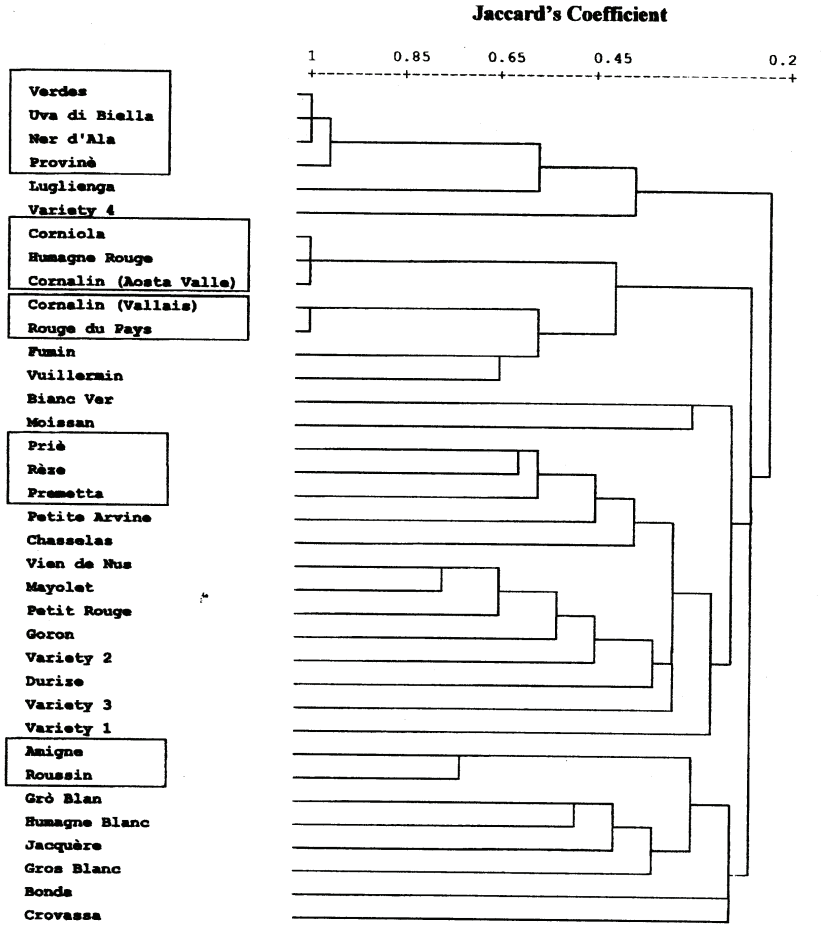

Figure: Dendrogram showing the genetic relationships of grape cultivars from the Aosta Valley and of cultivars grown in the nearby Savoy, Valais and Piedmont regions, as derived from an SSR analysis.

show full genomic similarities. Provinè grown in Central Piedmont showed high genomic similarity with this group. This suggests exchange between the Aosta valley and the neighboring areas of Northern Piedmont and the use of local synonyms to identify imported vines.

A second case of synonym is the one found among Humagne Rouge, from Valais, Corniola and Cornalin, both from the Aosta Valley. Cornalin is known to be cultivated in the Aosta Valley since 1800 (Gatta 1839; Moriondo 1999). Interestingly, a cultivar with the same name appeared more recently in Valais, but is genetically quite dissimilar from the Aosta cultivar.

Nicollier (1972) and Vaudan (1990) proposed that Cornalin and Humagne Rouge are clones selected within a heterogeneous population of Petit Rouge. This origin is now disproved by SSR analysis, which plots Petit Rouge far away from Humagne Rouge and Cornalin in the dendrogram.

A grapevine named Cornalin was also recovered in Valais (Switzerland), but this turned out to be genetically different from the Aosta Cornalin and to be identical with Rouge du Pays grown in the Swiss region.

A different situation is that of Premetta and Priè, two Aosta grapes described by Gatta (1838) and Di Rovasenda (1877) as synonymous but plotting at distinct position in the dendrogram. Though not identical, Prie, Premetta and Rèze are in nearby positions. This is interesting since Rèze is considered to be an important progenitor of the Aosta grapevine varieties being already cultivated by the Romans with the name "Retique" (Tchernia 1986). Amigne, a second important variety called Amineale by the Romans (Tchernia 1986) shows genomic similarity with Roussien, a cultivar described by Gatta (1838). Roussien is nowadays endangered since only 5-10 plants are left in the valley.

Descent from common ancestors is also suggested for the traditional Aosta grapevines Vien de Nus, Mayolet and Petit Rouge. Indeed, Vien de Nus and Petit Rouge had already been classified in a common varietal group named Oriou (Gatta 1838)

In spite of the confirmed synonyms and of the verified genomic similarities, the high genetic differences observed in the genomic structure of the grapevine cultivars grown in the Aosta valley allow to conclude that high biodiversity is still preserved in the grapevine genotypes of this small mountain region. Germplasm exchanges with the nearby regions have been documented. Data on genetic relationships will allow a solid basis to protect grapevine biodiversity and exploitation.

Acknowledgements

Research was supported by the Institut Agricole Régional, Aosta, Italy. We would like to thank D. Maigre, Centre Viticole de Candoz, Station Fédérale de Recherches en Production Végétale de Changins (Switzerland), M. L. Mascia and G. Culas from Syndicat Régional des Vins de Savoie, Chambéry (France) and D. E. Jacquin, Jongieux (France) for providing plant material. The authors are grateful to A. Grippo for figures and tables.

References

Botta, R.; Scott, N. S.; Eynard, I.; Thomas, M. R.; 1995: Evaluation of microsatellite sequence-targeted site markers for characterizing *Vitis vinifera* cultivar. Vitis **34**, 99-102.

Bowcock, A. M.; Ruiz-Linares, A.; Tomfohrde, J.; Minch, E.; Kidd, R.; Cavalli-Sforza, L.; 1994: High resolution of human evolutionary trees with polymorphic microsatellites. Nature **368**, 455-457.

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

Bowers, J. E.; Meredith, C. P.; 1997: The parentage of a classic wine grape, Cabernet Sauvignon. Nat. Genet. **16**, 84-87.

Calò, A.; Scienza, A.; Costacurta, A.; 2001: I Vitigni d'Italia. Edagricole, Bologna, Italy.

Fossati , T.; Labra, M.; Castiglione, S.; Failla, O.; Scienza, A.; Sala, F.; 2001: The use of AFLP and SSR molecular marker to decipher homonyms and synonyms in grapevine cultivars: The case of the varietal group known as "Schiave". Theor. Appl. Gen. **102**, 200-205.

Gatta, L. F.; 1838: Saggio Intorno alle Viti ed ai Vini della Valle d'Aosta. Filli Enrico ed., Aosta, Italy.

Karp, A.; Isaac, P.; Ingram, D.; 1998: Molecular Tools for Screening Biodiversity, 1st ed. Chapman & Hall, London.

Labra, M.; Carreno-Sanchez, E.; Bardini, M.; Basso, B.; Sala, F.; Scienza, A.; 2001: Extraction and purification of DNA from grapevine leaves. Vitis **40**, 101-102.

Labra, M.; Failla, O.; Fossati, T.; Castiglione, S.; Scienza, A.; Sala, F.; 1999: Phylogenetic analysis of cv. Ansonica growing on the island of Giglio, Italy, by AFLP and SSR markers. Vitis **38**, 161-166.

Lopes, M. S.; Sefc, K. M.; Eires Dias, E.; Steinkellner, H.; Laimer da Camara Machado, M.; Camara Machado, A.; 1999: The use of microsatellites for germplasm management in a Portuguese grapevine collection. Theor. Appl. Gen. **99**, 733-739.

Moriondo, G.; 1999: Vini e Vitigni Autoctoni della Valle d'Aosta. Ed. IAR, Imprimerie E. DUC, Aosta, Italy.

Nicollier, J.; 1972: Un dossier relatif aux cépages dits rouges du pays en Valais et rouge indigenes en Vallée d'Aoste. Revue Suisse de Viticulture et d'Arboriculture **4**, 125-128.

Regner, F.; Elisabeth, W.; Stadlbauer, A.; 2000 a: Differentiation and identification of White Riesling clones by genetic markers. Vitis **39**, 103-107.

Regner, F.; Stadlbauer, A.; Eisenheld, C.; Kaserer, H.; 2000 b: Genetic relationships among Pinots and related cultivars. Am. J. Enol. Vitic. **51**, 7-14.

Rongwer, J.; Akkaya, M. S.; Bhagwat, A. A.; Lavi, U., Cregan, P. B.; 1995: The use of microsatellite DNA markers for soybean genotype identification. Theor. Appl. Gen. **90**, 43-48.

Sefc, K. M.; Lopes, M. S.; Lefort, F.; Botta, R.; Roubelakis-Angelakis, K. A.; Ibanez, I.; Pejiè, H. W.; Wagner, J.; Glossl, H.; Steinkellner, H.; 2000: Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivar. Theor. Appl. Gen. **100**, 489-505.

Sefc, K. M.; Regner, F.; Glossl, J.; Steinkellner, H.; 1998: Genotyping of grapevine and rootstock cultivars using microsatellite markers. Vitis **37**, 15-20.

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

Tchernia, A.; 1986: Le Vin de l'Italie Romaine. Ecole Française de Rome. Roma, Italy.

Vaudan, J.; 1990: Speciale Vitini. I Quaderni dell'Informatore Agrario. Industrie Grafiche Editoriali Musumeci, Quart, Aosta, Italy.

Received November 15, 2001