



Comparison of genetic and morphological data for inferring similarity among native Dalmatian (Croatia) grapevine cultivars (*Vitis vinifera* L.)

Goran Zdunić^{1*}, Ivan Pejić², Jasminka Karoglan Kontić², Damir Vukičević³, Aleš Vokurka²,
Ivan Pezo¹ and Edi Maletić²

¹Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, HR-21000 Split, Croatia. ²Faculty of Agriculture, University of Zagreb, HR-10000 Zagreb, Croatia. ³Department of Mathematics, University of Split, HR-21000 Split, Croatia.
*e-mail: gzdunic@krs.hr

Received 13 January 2008, accepted 27 March 2008.

Abstract

Sixteen native central Dalmatian grapevine cultivars were characterised at eight SSR loci and eighteen primary morphological descriptors. The present work describes for the first time twelve native Dalmatian cultivars with morphological and molecular descriptors. One pair of synonyms was determined ('Babić'/Rogoznička'), and the other previously assumed pair ('Crljenak Kaštelanski'/Pribidrag') was verified. The remaining cultivars showed unique SSR genotype and seemed to be different from any other previously described Croatian cultivars. The results of SSR characterisation suggested the possibility of parent-offspring relationship between 'Babić' and 'Dobričić' and between 'Plavac Mali' and 'Babica'. Phenotypic relationship among cultivars based on morphological data (*d*-values) was determined according to original mathematical algorithm. The *d*-value proved to be informative and showed rather clear ability to discriminate between Dalmatian cultivars. Significant but weak correlation ($r = -0.38$) was observed by comparing the similarity matrices obtained from morphological and SSR data using the Mantel test.

Key words: Biodiversity, ampelography, SSR markers, characterisation, *Vitis vinifera*, Dalmatia.

Introduction

In the past, Dalmatia was one of the important vine growing regions of the Mediterranean. At the end of the 19th century there were more than 200 cultivars in use⁴. For geographic, social and economic reasons, the central Dalmatia was the largest grape and wine producing region in Dalmatia. Today, it is becoming the urban centre of the region of Split and Trogir where grapevine diversity is still high. The official Croatian variety list includes about 80 autochthonous cultivars, most of which are from Dalmatia¹³. Many others, which are not officially registered, are growing on small private vineyards.

During the past few years, the microsatellite analysis coupled with classical ampelographic description proved to be a powerful method for identification of cultivars and evaluation of genetic diversity^{12, 16, 18, 20}. Maletić *et al.*⁷ genotyped 22 Croatian autochthonous grapevine cultivars using SSR markers and detected several synonyms of the cultivars grown in Croatia and the neighbouring regions. Using parentage analysis at 25 microsatellite loci, Piljac *et al.*¹⁴ reconstructed the pedigree of the Dalmatian autochthonous cultivar 'Pošip Bijeli', grown on the island of Korčula. A very old cultivar 'Crljenak Kaštelanski' proved to be a synonym to the Californian 'Zinfandel' and to the Italian 'Primitivo', while together with 'Dobričić' it is a presumable parent to the most important Croatian red cultivar, 'Plavac Mali'⁸.

Although a large number of grapevine cultivars can be found in

the region of central Dalmatia, their primary characteristics and relationships and also relationships to other cultivars have not been investigated enough. Besides, their proper identification is hindered by confusion in denomination (synonyms and homonyms).

The aim of this research was (i) to characterise the most important grapevine cultivars grown in the region of central Dalmatia using SSR markers and classical ampelographic descriptors, in order to enable their reliable identification in the future and to establish germplasm collection. A further aim of this work was (ii) to evaluate and compare the phenotypic and genetic similarity among the analysed local cultivars using morphological and molecular data.

Materials and Methods

Plant material: Sixteen grapevine cultivars from central Dalmatia were analysed (Table 1). The material was sampled in 2002 and 2003 in commercial vineyards located in central Dalmatia.

SSR analysis: Twelve cultivars were genotyped by SSR markers, while the remaining four ('Crljenak Kaštelanski', 'Dobričić', 'Plavac Mali' and 'Pribidrag') had previously been genotyped by Maletić *et al.*⁸. Young leaves were collected from actively growing vines. Eight SSR loci were analysed: VVS2¹⁸, VVMD5 and VVMD7², VVMD27 and VVMD28³, ssrVrZAG62, ssrVrZAG79 and

Table 1. List of grapevine cultivars used and their economic importance in central Dalmatia, estimated as: very low=rare; low=distributed locally; medium=currenty leading in the area of Kaštela; high=widely distributed.

Cultivar	Berry colour	Economic importance	Sampling location
Babica	blue-black	medium	Kaštela area
Babić	blue-black	high	Primošten area
Crljenak Kaštelanski	blue-black	very low	Kaštela area
Crljenak Mali	blue-black	very low	Kaštela area
Dobričić	blue-black	low	Island of Šolta
Glavinuša	red	low	Kaštela area
Ljutun	blue-black	very low	Kaštela area
Maraština	green-yellow	high	Kaštela area
Mladenka	green-yellow	low	Kaštela area
Muškat Kaštelanski	red	very low	Kaštela area
Ninčuša	blue-black	low	Kaštela area
Plavac Mali	blue-black	high	Kaštela area
Pribidrag	blue-black	very low	Marušići village
Pošipica	green-yellow	very low	Kaštela area
Rogoznička	blue-black	medium	Kaštela area
Vlaška	green-yellow	medium	Kaštela area

ssrVrZAG83¹⁷. DNA extraction, PCR reactions and electrophoresis were performed as described previously¹.

The genetic similarity (GS) based on SSR data was calculated among all possible pairs of cultivars using the Dice similarity index as in Nei and Li¹⁰. Cluster analysis was based on similarity matrices obtained with the unweighted pair group method using arithmetic averages (UPGMA), and the relationship between cultivars was visualised in a dendrogram using NTSYSpc software¹⁵.

Ampelographic analysis: Ten vines from each of 16 cultivars were chosen from commercial vineyards. Cultivars were analysed in two consecutive years using 18 OIV descriptors (Codes 003, 004, 051, 053, 067, 068, 070, 079, 080, 084, 202, 204, 208, 220, 221, 223, 225 and 304)¹¹, modified by the Project GENRES 081⁶. Descriptors were selected to characterise young shoot, leaf, bunch and berry as well as time of full maturity.

Apart from their standard ampelographic description, the data of 18 OIV characteristics were used to design original mathematical algorithm for calculation of coefficients of phenotype similarities among cultivars (*d*-value). The idea was to group cultivars of similar phenotype and to estimate their possible genetic relation in a similar manner to Martinez *et al.*⁹. *d*-values were calculated as:

$$d(x, y) = \sum_{k \in NOIVK} \frac{\delta_{x(k), y(k)}}{\sum_{u, v \in S} \delta_{u(k), v(k)}} + \sum_{k \in OOIVK} \frac{|rank_k(x) - rank_k(y)|}{\sum_{u, v \in S} |rank_k(u) - rank_k(v)|}$$

where $\delta_{x(k), y(k)}$ is Kronecker symbol defined with

$$\delta_{x(k), y(k)} = \begin{cases} 0, & x(k) \neq y(k) \\ 1, & x(k) = y(k) \end{cases} \quad \text{and analogously}$$

$$\delta_{u(k), v(k)} = \begin{cases} 0, & u(k) \neq v(k) \\ 1, & u(k) = v(k), \end{cases}$$

where *x*, *y*, *u* and *v* represent cultivars and *k* is the OIV characteristic, $rank_k(x)$ is the modified rank of the cultivar *x* depending on the OIV characteristic *k*. Modified rank $rank_k(x)$ is calculated according

to the formula $rank_k(x) = p + \frac{q+1}{2}$, where *p* is the number of cultivars with lower value of the OIV characteristic *k* than the cultivar *x*, and *q* is the number of cultivars (cultivar *x* also included) that have the same value of the OIV characteristic *k* as the cultivar *x*.

The observed OIV characteristics were divided into two groups (ordinal and nominal) in order to standardize the distance between the cultivars regarding the type of characteristic. *NOIVK* is the set of nominal OIV characteristics (051, 067, 070, 080, 208, 223 and 225), while *OOIVK* is the set of ordinal OIV characteristics (003, 004, 053, 068, 079, 084, 202, 204, 220, 221, and 304). *S* denotes the set of all the observed cultivars.

Coefficients of the phenotypic similarity (*d*-value) were calculated using the programme developed in Visual C++. The final product of the analysis is a dendrogram constructed by cluster analysis applying the unweighted pair group method using arithmetic averages (UPGMA) with the NTSYSpc software package¹⁵. The comparison between ampelographic and SSR data was performed by Mantel test using the software NTSYSpc.

Results and Discussion

SSR genotypes for 16 analysed cultivars are presented in Table 2. One pair of synonyms was determined ('Babić'/'Rogoznička'), and the other ('Crljenak Kaštelanski'/'Pribidrag'), which was previously known⁸, was verified, while the other cultivars had distinct and unique SSR genotype. Genetic similarity among the analysed cultivars is shown in the dendrogram (Fig. 1). Grouping the cultivars on the basis of phenotype similarity coefficients (*d*-value) is shown in Fig. 2.

Equivalence of 'Babić' and 'Rogoznička' is suggested in the name 'Rogoznička', which indicates the area of Rogoznica as the origin of the cultivar, where 'Babić' is the predominant one. Results in Table 2 confirm the equivalence of the genotype at 8 SSR loci and verify with high probability the synonym 'Babić'/'Rogoznička'. Phenotypic similarity of 'Babić' and 'Rogoznička' is relatively high (Fig. 2), but at the same time its value is similar to two other pairs that have been confirmed to be different cultivars ('Ljutun'/'Crljenak Mali' and 'Dobričić'/'Plavac Mali'). This clearly indicates the limitation of ampelographic evaluation when two samples from vineyards with different environmental conditions and viticultural practices are compared. These results are not in accordance with the observations of Bulić⁴, who considered 'Rogoznička' as a separate cultivar.

'Babić' (syn. 'Rogoznička') and 'Dobričić' always share at least one mutual allele at all 8 SSR loci, as well as on the other 8 SSR loci (data not shown), which indicates the possibility that these two cultivars are genetically closely related, i.e., as parent and offspring.

'Babica' and 'Plavac Mali' also have a close genetic relationship. They share at least one mutual allele at all 8 SSR loci, as well as at 17 other SSR loci (data not shown), which, regarding the confirmed parenthood of 'Plavac Mali'⁸, indicates a large probability that 'Babica' is its direct offspring. Since 'Babica' is not widely spread (only in the Kaštela valley) and is probably a „new” cultivar because Bulić⁴ did not mention it in his work although he made a thorough ampelographic inventory in the Dalmatian region and described more than 200 cultivars, the other parent should be found among the cultivars of this area. So far it has not been discovered, in spite of the thorough genotyping of the cultivars

Table 2. Genetic profiles of 16 grapevine cultivars from central Dalmatia analysed at 8 SSR loci. Allele sizes are given in base pairs (bp).

Cultivar	VVS-2	VVMD-5	VVMD-7	VVMD-27	VrZAG-62	VrZAG-79	VrZAG-83	VVMD-28
Babica	143	151	226	228	239	249	179	179
Babić	143	151	228	228	247	249	179	179
Crljenak Kaštelanski*	133	143	226	236	247	249	179	181
Crljenak Mali	145	151	226	226	247	251	181	194
Dobričić*	145	151	228	228	247	249	179	179
Glavinuša	133	145	228	240	239	249	179	181
Ljutun	145	151	226	228	249	253	181	194
Maraština	143	147	226	240	239	253	179	179
Mladenka	145	153	226	226	249	253	181	194
Muškat Kaštelanski	133	147	234	240	243	243	179	185
Ninčuša	145	145	226	228	249	249	179	194
Plavac Mali*	143	145	226	228	247	249	179	179
Pribidrag*	133	143	226	236	247	249	179	181
Pošipica	133	133	234	238	239	245	179	181
Rogoznička	143	151	228	228	247	249	179	179
Vlaška	143	153	226	238	239	253	179	179

* previously published ⁸.

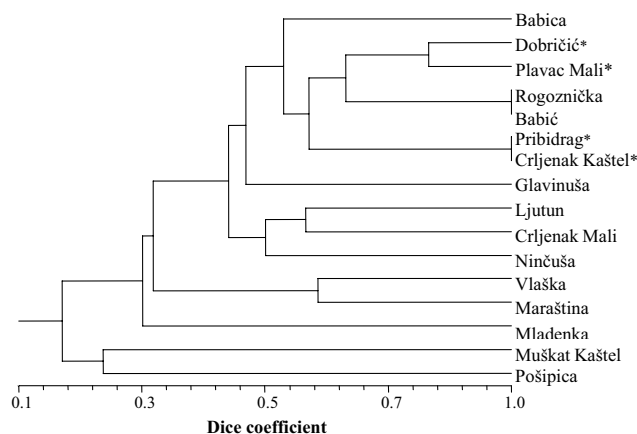


Figure 1. Genetic relationship within 16 grapevine cultivars from central Dalmatia based on the Dice genetic similarity values computed from SSR data generated from 8 SSR loci. Data of genotypes marked with symbol * previously published ⁸.

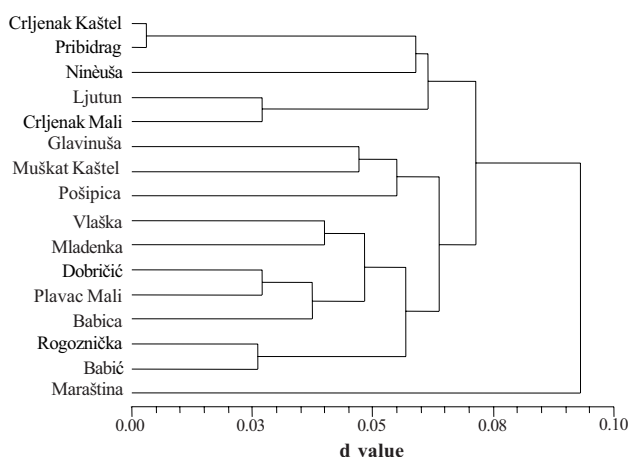


Figure 2. Phenotypic relationship within 16 grapevine cultivars from central Dalmatia based on the *d*-values obtained from ampelographic observation at 18 OIV descriptors.

from this region. However, it could be possible to determine a parent-offspring relationship in the absence of one parent by analysing local cultivars on a larger number of SSR loci (>50) ¹⁹.

According to Bulić ⁴, the cultivar 'Crljenak Mali' is a synonym for 'Plavac Mali'. However, based on the ampelographic and SSR analyses, 'Crljenak Mali' clearly differs from 'Plavac Mali'. According to the SSR analysis, 'Crljenak Mali' belongs to the same group with the other analysed cultivars (Fig. 1), and shows closest genetic similarity to 'Ljutun' (GS = 0.65). This suggests that 'Crljenak Mali' could be of local origin, either an old forgotten cultivar or a generative offspring of some Dalmatian cultivar.

In viticultural practice 'Babić' and 'Babica' have often been considered synonyms, but their SSR profiles clearly show that they are two different cultivars. Several phenotypical similarities between 'Babić' and 'Babica' (shape of bunch and berry, colour of berry skin, time of full maturity) could be the reason for similarity in their names, but the phenotypical similarity based on 18 characteristics is relatively small (Fig. 2). Although genetic similarity coefficient is above average (GS = 0.64), it does not indicate greater genetic similarity, and the genotype at the VrZAG 62 locus eliminates direct relationship.

Genotypes of 'Muškat Kaštelanski' and 'Pošipica' stand apart from the other analysed cultivars (Fig. 1), which indicates the possibility that these two cultivars belong to some other genetic pool and have most likely been introduced here from another area.

Comparing the similarity matrices obtained from ampelographic and SSR data using the Mantel test, a significant but weak and negative correlation was observed ($r = -0.38$, $P = 0.0078$). Considering that values of the index of genetic similarity (identity = 1) computed out of molecular data, were correlated with the index of phenotypic similarity (identity = 0) computed out of ampelographic data, this correlation was actually positive. Even though the correlation was significant and proves that both methods generally differentiate related and unrelated pairs of cultivars in a similar way, it was too weak to have a practical relevance. Genetic correlation among 'Crljenak Kaštelanski', 'Dobričić' and 'Plavac Mali' has been established earlier ⁸, which is confirmed by the high coefficient of phenotypic similarity of 'Dobričić' and 'Plavac Mali', but 'Crljenak Kaštelanski' is, according to the same phenotype, quite apart from these two

cultivars (Fig. 2). This indicates that phenotypic similarity coefficients (*d*-value), at least on the basis of the characteristics used in this work, are not reliable enough to determine a genetic relationship. Comparison between morphology and DNA fingerprinting technique in grape has been recently demonstrated by Martinez *et al.*⁹, who did not find significant correlation between AFLP and 53 morphological characters ($r = 0.33$) using simple matching coefficient.

A possible explanation for low reliability of phenotypic characters in genetic similarity analysis is inheritance. *d*-Value can be significantly encumbered with segregation of qualitative characteristics to highly contrastive forms (e.g., presence or absence of hairs, round vs. oval shape of the berries, etc.), which has as a consequence maximal differences on the level of descriptors, with segregated genotypes being directly related (half- or full-sib). However, morphological descriptors are not always sufficient for precise identification of grapevine cultivars but coupled with microsatellite analysis, as has been demonstrated recently^{5 12 20}, proved to be very useful as complementary technique for evaluation of genetic diversity.

In this study, new *d*-value presented a rather clear ability to discriminate between cultivars on a low number of cultivars and limited number of OIV descriptors. It could be applied, due to a number of advantages of classical ampelographic identification (speed, simplicity, cost-effectiveness) and the already existing international ampelographic bases, for preliminary analysis of a possible genetic connection among accessions.

The present study also provides the new and necessary data for distinguish twelve autochthonous Dalmatian grapevine cultivars. This would help prevent disappearing local cultivars and establishing germplasm collection. For complete characterisation and evaluation of the studied cultivars, and especially for the analysis of parenthood, it is necessary to complete the collection of all the remaining Dalmatian cultivars, and to continue genotyping at a larger number of SSR loci.

Acknowledgements

This work was supported by Croatian Ministry of Science, Education and Sports. We are grateful to local vine growers for help. We also thank Mr. Ante Vuletin for technical assistance.

References

¹Benjak, A., Ercisli, S., Vokurka, A., Maletić, E. and Pejić, I. 2005. Genetic relationship among grapevine cultivars native to Croatia, Greece and Turkey. *Vitis* **44**:73-77.

²Bowers, J.E., Dangl, G.S., Vignani, R. and Meredith, C.P. 1996. Isolation and characterisation of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome* **39**:628-633.

³Bowers, J.E., Dangl, G.S. and Meredith, C.P. 1999. Development and characterization of additional microsatellite DNA markers for grape. *Am. J. Enol. Vitic.* **50**:243-246.

⁴Bulić, S. 1949. Dalmatinska ampelografija. Poljoprivredni nakladni zavod, Zagreb, Croatia.

⁵Dettweiler, E., Jung, A., Zyprian, E. and Töpfer, R. 2000. Grapevine cultivar Müller-Thurgau and its true to type descent. *Vitis* **39**:63-65.

⁶European Union Project GENRES 081. 2001. Primary and secondary descriptor list for grapevine cultivars and species (*Vitis* L.). Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Germany.

⁷Maletić, E., Sefc, K.M., Steinkellner, H., Karoglan Kontić, J. and Pejić, I. 1999. Genetic characterization of Croatian grapevine cultivars and detection of synonymous cultivars in neighboring regions. *Vitis*

38:79-83.

⁸Maletić, E., Pejić, I., Karoglan Kontić, J., Piljac, J., Dangl, G.S., Vokurka, A., Lacombe, T., Mirošević, N. and Meredith, C.P. 2004. Zinfandel, Dobričić and Plavac mali: The genetic relationship among three cultivars of the Dalmatian coast of Croatia. *Am. J. Enol. Vitic.* **55**:174-180.

⁹Martinez, L., Cavagnaro, P., Masuelli, R. and Rodriguez, J. 2003. Evaluation of diversity among Argentine grapevine (*Vitis vinifera* L.) varieties using morphological data and AFLP markers. *Electronic Journal of Biotechnology* **6** [online], Available from: <http://www.ejbiotechnology.info/content/vol6/issue3/full/11/#17> (accessed August 2007)

¹⁰Nei, M. and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.* **76**:5269-5273.

¹¹OIV (Organisation Internationale de la Vigne et du Vin) 1983. Code of descriptive characteristics of the varieties and species of *Vitis*. OIV, Paris.

¹²Ortiz, J.M., Martín, J.P., Borrego, J., Chávez, J., Rodríguez, I., Munoz, G. and Cabello, F. 2004. Molecular and morphological characterization of a *Vitis* gene bank for the establishment of a base collection. *Genet. Resour. Crop Evol.* **51**:403-409.

¹³Pejić, I., Maletić, E., Karoglan Kontić, J., Kozina, B. and Mirošević, N. 2000. Diversity of autochthonous grapevine genotypes in Croatia. *Acta. Hortic.* **528**:67-73.

¹⁴Piljac, J., Maletić, E., Karoglan Kontić, J., Dangl, G.S., Pejić, I., Mirošević, N. and Meredith, C.P. 2002. The parentage of Pošip bijeli, a major white cultivar of Croatia. *Vitis* **41**:83-87.

¹⁵Rohlf, F.J. 1993. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Version 1.80. New York.

¹⁶Sefc, K.M., Steinkellner, H., Glössl, J., Kampfer, S. and Regner, F. 1998. Reconstruction of a grapevine pedigree by microsatellite analysis. *Theor. Appl. Genet.* **97**:227-231.

¹⁷Sefc, K.M., Regner, F., Turetschek, E., Glössl, J. and Steinkellner, H. 1999. Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. *Genome* **42**:367-373.

¹⁸Thomas, M.R. and Scott, N.S. 1993. Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). *Theor. Appl. Genet.* **86**:985-990.

¹⁹Vouillamoz, J.F. and Grando, M.S. 2006. Genealogy of wine grape cultivars: Pinot is related to Syrah. *Heredity* **97**:102-110

²⁰Zulini, L., Fabro, E. and Peterlunger, E. 2005. Characterisation of the grapevine cultivar Picolit by means of morphological descriptors and molecular markers. *Vitis* **44**:35-38.