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Genetic characterization of Croatian grapevine cultivars and detection of synonymous cultivars in neighboring regions

by

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Summary: Twenty-two native Croatian grapevine varieties representing three different growing and climatic regions (Dalmatia, Istria, continental Croatia) have been genotyped at nine SSR loci. The identical genotypes of the Croatian cultivars Plavina and Brajdica confirmed the hypothesis they are the same variety. Comparing the SSR profiles of the Croatian cultivars with the profiles stored in a database containing about 300 European cultivars, further three pairs of synonyms were revealed: Teran Bijeli shares its genotype with the Italian cultivar Prosecco, Muškat Ruža Porečki corresponds to cv. Rosenmuskateller from North Italy and Moslavac is identical to the Hungarian variety Furmint. The microsatellite-based definitions of these synonyms are strongly supported by ampelographic observations. The genetic variability within the investigated Croatian cultivars was high with a genetic diversity of 75 %. A dendrogram based on allele sharing distances reflected neither common morphological features nor common geographic origins of the cultivars.

Key words: Vitis vinifera, microsatellite, SSR.

Introduction

Grapevine has been grown in Croatia since ancient times. Different climates and stresses, as well as social changes (migrations, conquests and change of frontiers) have resulted in having hundreds of grape cultivars. More than 80 native cultivars are registered in the official Croatian cultivar list, and further 40-50 varieties are extremely rare and underutilized (Pejić *et al.* 1999). However, at present Croatia is (as many other countries) faced with rapid erosion of native germplasm due to the introduction of famous European cultivars such as Chardonnay, Rheinriesling, Pinots, etc. Despite the fact that some native varieties very often give excellent wines, they are underutilized primarily due to the lack of good quality propagation material and the insufficient knowledge about their performance in different environments. As it is questionable if some varieties are really unique to Croatia, we might be able to get selected plant material of these cultivars in a neighboring country under a different name, while the local work of clonal selection is carried out.

One way to enhance the use of native varieties is a thorough characterization of the germplasm by controlling cultivar identity and determination of its uniqueness. The high number of putatively different varieties and their similar names and similar phenotypes have raised some confusion. Some different variety names might be just synonyms as a result of morphological differences caused by different environmental and sanitary conditions. The search for synonyms ought to be extended to neighboring countries, too, since in the past Croatia was part of different empires and states and a big crossroad, on the land as well as on the sea. At the end of 19th and the onset of the 20th century when the Croatian viticulture was most prosperous, Croatia was part of the Austrian-Hungarian empire whose territory included almost all neighboring countries and thus, the circulation of plant material may have been substantial. It is also possible that the name of a famous variety is used for morphologically similar but genotypically different plants.

Molecular markers, especially microsatellites, are a very powerful means for the identification of synonyms in germplasm collections (Thomas *et al.* 1994, Botta *et al.* 1995, Sefc *et al.* 1998, Lopes *et al.*, in press). Thereby, they allow the removal of duplicates and the establishment of core selections.

Objectives of this work were the determination of microsatellite profiles of 22 Croatian grapevine varieties and the search for synonymous cultivars within this sample by comparison with the available DNA profiles of grapevines from other European regions.

Material and Methods

Twenty-two Croatian grapevine varieties (Table) have been selected to represent three main production areas (continental region, Dalmatia and Istria). Leaf samples were taken from the *in situ* collections of the Institute for Agriculture and Tourism in Poreč and Faculty of Agriculture, Zagreb. Leaves were put into plastic bags and subsequently (after 24-48 hours) stored at -20 °C. DNA isolation was performed according to procedure described by Thomas *et al.* (1993).

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Table

List of the Croatian grapevine varieties and their genotypes at nine SSR loci represented by fragment lengths in base pairs (bp)

Variety	Region*)	Color of berry**)	VVS2		VVMD5		VVMD7		ssrVrZAG21		ssrVrTAG47		ssrVrZAG62		ssrVrZAG64		ssrVrZAG79		ssrVrZAG83	
Kraljevina	c	b	134	142	232	238	236	244	202	204	157	161	193	195	139	143	250	250	190	194
Mirkovača	c	b	134	143	224	238	236	246	200	204	157	159	195	203	139	159	250	250	194	194
Moslavac	c	b	132	152	224	238	236	246	200	206	157	172	187	203	159	163	236	248	188	188
Plavec Žuti	c	b	132	132	226	238	236	236	200	206	159	172	187	195	159	163	242	248	188	188
Ranfol	c	b	132	132	232	244	236	246	200	206	157	159	195	203	143	159	236	258	188	188
Škrlet	c	b	132	132	224	226	246	252	200	206	163	172	199	203	137	163	250	205	188	194
Babić	d	n	142	150	226	226	244	246	190	206	157	157	203	203	141	143	236	258	194	200
Bogdanuša	d	b	142	150	220	226	236	246	190	190	161	172	187	189	137	159	246	250	190	194
Debit	d	b	132	144	226	226	236	246	190	194	157	172	189	195	137	159	236	250	188	194
Gegić	d	b	132	144	224	226	244	250	190	214	157	172	193	199	137	141	236	250	188	194
Lasina	d	n	132	132	226	244	230	236	194	204	157	159	195	195	159	163	236	248	190	194
Plavina	d	n	132	142	230	234	236	246	200	206	157	167	187	199	143	163	236	242	188	194
Pošip Bijeli	d	b	132	132	226	238	236	236	202	204	157	159	185	187	139	143	250	258	194	194
Pošip Crni	d	n	134	134	224	238	236	236	200	202	157	159	185	187	143	159	250	258	188	194
Žilavka	d	b	132	152	224	236	236	236	200	202	157	172	187	187	143	143	248	248	188	194
Brajdica	i	n	132	142	230	234	236	246	200	206	157	167	187	199	143	163	236	242	188	194
Hrvatica	i	n	150	152	224	236	236	244	190	202	157	157	187	203	143	163	250	250	194	194
Muškat Ruža Porečki	i	n	132	134	234	238	236	246	200	206	157	172	185	187	141	159	248	254	188	188
Teran	i	n	134	154	224	226	244	246	190	200	167	167	191	193	151	163	238	250	188	194
Teran Bijeli	i	b	132	142	224	244	236	244	190	200	157	172	187	203	143	163	248	258	188	194
Vela Pergola	i	b	132	150	224	238	236	244	204	214	157	159	187	203	143	163	250	258	190	194
Žlahtina	i	b	134	138	224	226	236	244	190	200	159	163	195	203	143	159	258	258	188	190

*) Main production area: c: continental part of Croatia; d: Dalmatia and Dalmatian hinterland; i: Istria and Northern coastal region.

**) Color of berries: b: blanc (white); n: noir (red).

The samples were genotyped at nine SSR loci: VVS 2 (THOMAS and SCOTT 1993), VVMD 5, VVMD 7 (BOWERS *et al.* 1996), ssrVrZAG 21, ssrVrZAG 47, ssrVrZAG 62, ssrVrZAG 64, ssrVrZAG 79 and ssrVrZAG 83 (SEFC *et al.* in press). PCR reactions and electrophoresis were performed as described previously (SEFC *et al.* 1997).

The detected microsatellite profiles were searched for identical genotypes within the sample of Croatian cultivars as well as in combination with the SSR data of grapevines from diverse European regions stored in the SSR database of the Center of Applied Genetics (Vienna, Austria; unpublished data). The comparison of the genotypes was performed with the help of the software of H.W. WAGNER. Expected heterozygosity was calculated from the observed allele frequencies according to NEI (1973) as $(1-\Sigma p_i^2)$, p_i being the frequency of allele i.

The probability of identity PI (PAETKAU *et al.* 1995) was calculated as $\Sigma p_i^4 + \Sigma\Sigma(2p_ip_j)^2$, p_i and p_j being the frequencies of allele i and j, respectively.

A genetic distance matrix based on the proportion of shared alleles was constructed by the program MICROSAT (MINCH 1997) and was used to draw a UPGMA dendrogram with the help of the programs Neighbor (included in the PHYLIP package by J. FELSENSTEIN 1989) and Treeview (PAGE 1996). The test for genetic differentiation of grapevines from three Croatian vine growing region was carried out by the program GENEPOP (RAYMOND and ROUSSET 1995).

Results and Discussion

In this work, a set of 22 cultivars from Croatia were genotyped at nine SSR loci in order to detect synonymous cultivars and to describe the genetic structure of the Croatian gene pool. The cultivars were sampled from the three vine-growing regions Dalmatia, Istria and continental Croatia. The SSR genotypes of the 22 Croatian native grapevine varieties are presented in the Table. Microsatellite profiles collected in the SSR database of the Center of Applied Genetics, Vienna, Austria (data not published) were included in the search for identical genotypes. One pair of synonyms was revealed within the Croatian sample, and further three pairs of synonyms were detected among cultivars from Croatia on the one hand and from Italy and Hungary on the other hand.

The two Croatian synonyms are the cultivars Plavina and Brajdica, showing the same genotype at the 9 SSR loci. It has previously been suspected by viticulturists that these two varieties might be identical (see also the ampelography of BULIĆ 1949). However, these two different names are still in use today. Our data provide strong evidence that Plavina and Brajdica are actually synonyms for the same variety.

The variety Teran Bijeli, considered as a rare native variety of the region of Istria, shares its genotype with the Italian variety Prosecco, a well known cultivar grown in the north-east of the Country. A comparison of the Italian ampelographic data on Prosecco (COSMO and POLSINELLI 1958) and our own ampelographic data of Teran Bijeli (data not published) further indicates possibility that these cultivars are synonymous.

Furthermore, Muškat Ruža Porečki displays the same SSR genotype as Rosenmuskateller. Muškat Ruža Porečki is known to be a low yielding and high quality grapevine and has physiologically female flowers. According to AMBROSI *et al.* (1998), this rare characteristic is a feature of cv. Rosen-muskateller, too. Besides, they refer the cv. Rosenmuskateller as an old cultivar from South Tyrol (a region in North Italy), while BABO and MACH (1909) as well as TURKOVIĆ and TURKOVIĆ (1963) state that this variety was introduced to South Tyrol from Dalmatia at the end of 19th century. This might be an additional evidence that Muškat Ruža Porečki and Rosenmuskateller are the same variety, as well as that it is probably native in Dalmatia.

Cv. Moslavac, known as old native variety of the region Moslavina, has been shown to have the same genotype as cv. Furmint, which is spread in Hungary and considered as a Hungarian variety. However, according to other ampelographers, e.g. TRUMMER (1841) and GOETHE (1887) Moslavac is referred to as a Croatian variety. Thus, the geographic origin of this cultivar remains an open question.

The name of the cultivar Hrvatica means "Croatian girl" in Croatian language, which is also the translation of the name of the Italian cultivar Croatina, which is grown in the north-west of Italy. As, additionally, these two cultivars have a similar phenotype, they have sometimes been considered to be synonymous. Microsatellite profiles reject this assumption, as the two cultivars are clearly distinguishable at each of the analyzed SSR loci. This finding is in accordance with the statement of an experienced Croatian ampelographer that Hrvatica is most likely endemic to Croatia and not identical with the Italian cultivar Croatina (SOKOLIĆ 1992).

The probability to obtain identical genotypes from different cultivars at all nine loci within the Croatian cultivars was estimated as 7.6×10^{-8}, and has been shown to be in the order of 10^{-9} in cultivars from different European locations (SEFC *et al.*, submitted). Therefore, it is highly unlikely to detect false synonyms with these markers. Furthermore, the ampelographic information on the involved cultivars as well as some historical records highly support the microsatellite data.

The genetic variability in the Croatian cultivars was rather high with a gene diversity (expected heterozygosity) of 75 %. Observed heterozygosity was generally higher than expected with random union of alleles, with a mean of 83 % across loci. The lowest heterozygosity (53 %) was observed in the cv. Žilavka.

Analysis of allele sharing proportions between all pairs of cultivars showed a mean of 38 % shared alleles. This is close to the value of 36 % observed in a set of Portuguese cultivars (LOPES *et al.*, in press).

A UPGMA dendrogram was constructed based on the allele sharing distances between the cultivars (Figure). Unexpectedly, we did not observe clustering of varieties with similar ampelographic features. For example, cv. Škrlet and cv. Ranfol both belong to the conv. Pontica, subconv. Balcanica, and thus sharing some similar morphological traits such as bunch and berry shape, density of leaf hairs, etc. (MIROŠEVIĆ 1986). Nevertheless, they were placed quite apart in the dendrogram. On the other hand, some varieties were joined together in the dendrogram (e.g. Moslavac and

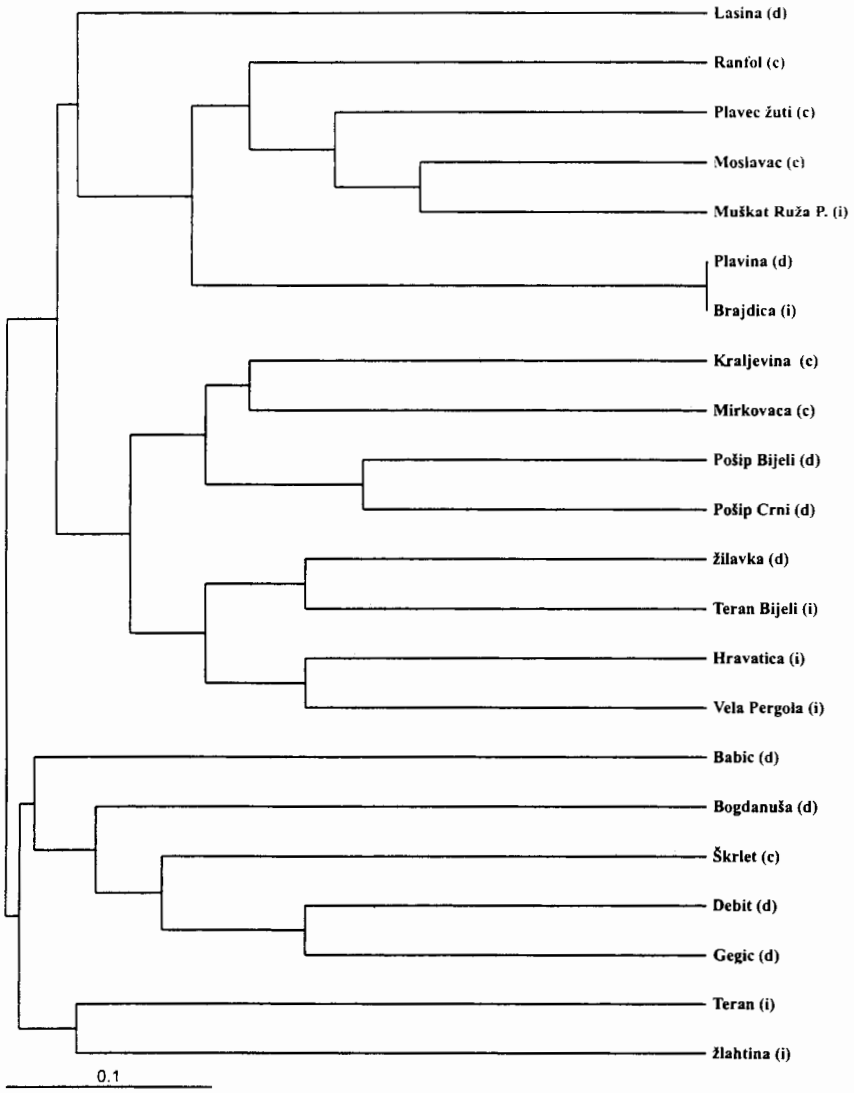

Figure: Dendrogram showing the genetic relationship among 22 Croatian grapevine varieties based on UPGMA clustering of allele sharing distances at nine SSR loci. Cultivar names are followed by abbreviations (**c**), (**i**), and (**d**) which refer to their geographical origins, continental Croatia, Istria and Dalmatia, respectively.

Muškat Ruža Porečki) which are obviously very different in morphological traits such as structure of flower, berry color, leaf shape, or the climatic zone of growing.

The grapevines in this study were sampled from three growing regions (continental Croatia, Istria and Dalmatia). Like the morphological similarities, the common geographical origins were not reflected by the grouping of the cultivars in the phenogram. An exception was the clustering of Pošip Bijeli and Pošip Crni, which are both native to the island of Korčula. Another test for the differentiation of the grapevine cultivars from continental Croatia, Istria and Dalmatia was carried out by comparing the allele frequencies in the three regional groups, and again, no indication of genetic differentiation among the Croatian grapevines was detected.

This work was a first step towards the genetic characterization of the Croatian grapevine germplasm. The preliminary results indicate the uniqueness of the major part of the investigated cultivars and reveal a substantial level of genetic variation within the Croatian grapevines. The maintenance of the valuable genetic resources represented by these cultivars should be considered as highly important.

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