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The parentage of Pos ip bijeli, a major white wine cultivar of Croatia

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Summary

Fourteen Croatian grape cultivars (*Vitis vinifera* L.) were analyzed at 25 polymorphic microsatellite loci and the alleles were analyzed for possible parent-progeny relationships. Alleles at 24 out of the 25 loci are consistent with Pos ip bijeli (hermaphrodite) being the progeny of two other varieties, Zlatarica blatska bijeli and Bratkovina bijeli. A further search in a microsatellite database of over 300 cultivars revealed no other possible parents for Pos ip. Likelihood analysis with observed allele frequencies indicated that the probability of the observed Pos ip alleles is more than 10^{15} times higher for the presumptive parents than two random varieties and at least 300 times more likely than close relatives of the presumptive parents. The likelihoods were 10^{13} and at least 200, respectively, when the 95 % upper confidence limits of the allele frequencies were used in the analysis. Pos ip and its presumptive parents have all been grown on the Dalmatian island of Korc ula for many years, suggesting that this island is its origin. A comparison of representative groups of Croatian, Italian and Greek cultivars indicated that 11 out of the 13 Pos ip alleles are more frequent in the Croatian gene pool than in the other two. Pos ip and the two presumptive parental cultivars were characterized with 58 ampelographic and ampelometric descriptors. Pos ip shares 50 out of the 58 characteristics with one or both of the parents and combines several desirable characteristics from both parents. Pos ip has become increasingly popular in Dalmatian viticulture and has replaced some other white wine cultivars.

K e y w o r d s : grape, *Vitis vinifera*, microsatellite, SSR, parentage, descriptors, ampelography, Dalmatia.

Introduction

Vine cultivation in the Croatian coastal region of Dalmatia, notably on the islands of Korc ula and Vis, dates back to the 4th century BC (Fazinic 1981). Since these early beginnings many grape varieties have been grown in this area. At the end of the 19th century, it was possible to find several hundred cultivars in Dalmatia alone (Bulić 1949) and most of them were considered to be autochthonous. Besides favorable climatic conditions, history and good connections with other countries where grapes were grown had a strong influence on the number of cultivars. It is probable that some of them arose in this area and that others were introduced long ago. Unfortunately, many cultivars have been lost since the beginning of the 20th century, the victims of introduced pests and pathogens (phylloxera, *Plasmopara, Uncinula*) as well as modern economic pressure. Today, more than 80 native cultivars are registered in the official Croatian cultivar list, while an additional 50 rare genotypes remain underutilized (Pejić *et al.* 2000). Because native Croatian cultivars are threatened with extinction as a consequence of preferential planting of popular international varieties, we have established a program to collect and genetically characterize the most important Croatian varieties.

Microsatellite DNA markers are the method of choice for studying poorly characterized varieties such as these. While ampelography cannot always differentiate similar varieties, microsatellite markers can not only separate them but also detect genetic relationships with other cultivars (Thomas *et al.* 1994).

Pos ip bijeli is one of the most important white wine cultivars in Croatia, producing the first white wine with a protected geographic origin. Recent results have demonstrated its uniqueness (Maletić *et al.* 1999) but its origin is still unknown. Some viticulturists have speculated that Pošip originated on the island of Korc ula (Jelaska 1967), however its morphology (*Proles orientalis*) has led to the conclusion that it originated further east and was brought to the island later (Maletić 1993). Our results support the former hypothesis.

The aims of this research were to generate microsatellite profiles of major grape varieties of the Dalmatian Coast of Croatia and to look for close relatives of Pos ip bijeli among cultivars growing in the same area.

Material and Methods

M i c r o s a t e l l i t e a n a l y s i s : Young leaves of 13 varieties were collected from vines growing on the island of Korcula, Croatia (Tab. 1). Desiccated or lyophylized material was sent to the Department of Viticulture and Enology, University of California, Davis, USA, where DNA was extracted according to the protocol outlined in the Qiagen DNeasy Plant Mini Handbook (Anonymous 2000). DNA of Zinfandel and duplicate DNA samples of Vranac crni, Plavac mali and Plavina crna were obtained from the DNA collection in Davis.

Microsatellite loci were PCR amplified as described previously (Bowers *et al.* 1996), separated on denaturing 6 % polyacrylamide sequencing gels and visualized by silver

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Table 1

Cultivars analyzed

Babica	Plavac mali
Brac ki Crljenak n.	Dobrićić
Bratkovina b.	Plavina crna
Bratkovina n.	Vranac crni
Cetinka b.	Vugava b.
Grk	Zinfandel
Pos ip b.	Zlatarica blatska b.

staining using a Promega kit (Promega, Madison, Wisconsin, USA).

Twenty-five polymorphic microsatellite loci were tested (Tab. 2). Alleles were analyzed with a computer program (B.H. Prins, unpubl.) in order to detect possible parent-progeny relationships among the genotypes.

Statistical analyses: In order to estimate the probability of the proposed parentage, cumulative likelihood ratios were calculated as described previously for the parentage of Cabernet Sauvignon (BOWERS AND MEREDITH 1997) and Chardonnay (BOWERS *et al.* 1999). For each locus, the likelihood ratio is the ratio of the probability of the observed Pos ip alleles if it had the putative parents to the probability of those alleles if two random or related cultivars were the parents. The cumulative likelihood ratio is the product of the ratios for each locus. Allele frequencies and their upper 95 % confidence limits were calculated from over 300 wine cultivars of *Vitis vinifera*.

Data for 5 out of the 25 loci were not used in likelihood calculations because 3 loci, VMC5H2, VMC5H5, and VMC5A1, are strongly biased to northeastern France and allele frequencies were not available for VMC5C1 and VMC2B3.

Ampelographic analysis: The cultivars Bratkovina bijeli, Zlatarica blatska bijeli and Pos ip bijeli were described according to the OIV descriptor list (OIV 1983) as modified by EU project GENRES 081 (ANONYMOUS 2001 a and b). Twenty-four descriptors were used, encompassing leaf, shoot, inflorescence, cluster and berry morphology, fruit composition and phenology.

Table 2

SSR genotypes of Pos ip bijeli and its presumptive parents Bratkovina b. and Zlatarica blatska b.

Locus	Bratkovina		Pošip		Zlatarica	
VVMD5	234	240	228	240	226	228
VVMD6	212	212	212	214	212	214
VVMD7	239	247	239	239	239	239
VVMD21	249	256	249	249	249	258
VVMD24	210	219	210	219	210	214
VVMD25	243	259	243	245	245	245
VVMD26	249	251	249	249	249	251
VVMD27	179	181	179	181	181	194
VVMD28	247	249	247	249	247	251
VVMD31	204	212	204	204	204	212
VVMD32	253	253	253	257	257	273
VVMD36	266	276	266	266	254	266
VVS2	133	135	133	133	133	153
VrZAG62	187	205	187	189	189	189
VrZAG79	249	251	251	259	257	259
VrZAG83	197	197	197	197	193	197
VrZAG93	199	199	189	199	189	189
VMC2C3	165	165	165	165	165	165
VMC5G6.1	129	142	129	139	139	155
VMC2H4	218	224	202	224	202	224
VMC5A1	172	172	162	172	162	172
VMC5H2	194	194	194	209	194	209
VMC2B3	166	170	164	170	166	166
VMC5H5	176	192	192	194	176	194
VMC5C1	147	190	183	190	147	183

Results and Discussion

All 14 cultivars have distinct microsatellite genotypes (data not shown). Alleles at 24 out of 25 loci (Tab. 2) are consistent with Pos ip bijeli (hermaphrodite) being the progeny of two other autochthonous varieties grown on the island of Korc ula, Zlatarica blatska bijeli (hermaphrodite) and Bratkovina bijeli (female). The exception is a 2-base pair discrepancy at locus VMC2B3 that may be attributable to a somatic mutation at that locus in either Pos ip or Zlatarica subsequent to the time of the cross. We previously observed a similar discrepancy at one locus between Pinot and one of its progeny, Romorantin (BOWERS *et al.* 1999). and we have detected several such mutations at microsatellite loci among clones of Pinot noir and Chardonnay (RIAZ *et al.* 2002).

Among the cultivars analyzed in this experiment, no other pair could have been the parents, the nearest possibilities being excluded by the results obtained for the first 19 loci. Furthermore, a search within the existing UC Davis microsatellite database of over 300 cultivars resulted in no other remotely possible parental combination for Pos ip.

Based on observed allele frequencies, the likelihood ratio of the probability of the observed Pos ip bijeli alleles if Bratkovina and Zlatarica blatska are the parents versus two random cultivars is $>10^{15}$ (Tab. 3). Likelihood ratios calculated with 95 % upper confidence limits of allele frequencies are more conservative because they assume that the Pos ip alleles are more common than has been observed. In this case, the Pos ip alleles are 4.18×10^{13} times more likely if its parents were Zlatarica and Bratkovina versus two random cultivars (Tab. 3). For the 5 loci that were not included in the likelihood calculations, the alleles are all consistent with the proposed parentage (Tab. 2).

It is highly likely that the origin of Pos ip bijeli is Korc ula. All three cultivars have been grown in the vineyards of Korc ula for a very long time and are still grown there. Isolation and ideal growing conditions on Korc ula are probable reasons why Pos ip did not significantly spread from this island for years.

If Pos ip did not originate in Dalmatia, Italy or Greece would be the next most likely places because of their geographic proximity to Dalmatia and their long viticultural

Table 3

Parentage analysis of Pos ip bijeli (P) and its presumptive parents Bratkovina (B) and Zlatarica blatska (Z)

	Genotype			Frequency of Pos ip alleles		Likelihood ratio of B x Z[a] versus alternative parents (observed allele frequencies)					Likelihood ratios of B x Z versus alternative parents (upper confidence limits of allele frequencies)				
Locus	B	P	Z	Observed[b]	Upper 95% confidence limit	X x Y[c,d]	B x X[e]	Z x X[e]	B x Z[f] relative	Z x B[f] relative	X x Y	B x X	Z x X	B x Z relative	Z x B relative
VVMD32	253	253	257	0.13	0.15	18.39	4.66	7.89	1.65	1.78	13.39	3.95	6.78	1.60	1.74
	—	257	273	0.11	0.13										
VVMD36	266	266	254	0.04	0.05	163.47	12.79	12.79	1.86	1.86	95.26	9.76	9.76	1.81	1.81
	276	—	266												
VrZAG62	187	187	189	0.06	0.07	15.08	3.34	9.03	1.54	1.80	10.94	3.05	7.18	1.51	1.76
	205	189	—	0.30	0.33										
VMC5G6.1	129	129	139	0.02	0.03	51.08	4.00	25.54	1.60	0.96	29.89	3.41	17.51	1.55	0.95
	142	139	155	0.13	0.15										
Cumulative product for these 4 loci						$2.32x10^{6}$	796	$2.33x10^{4}$	7.52	5.71	$4.17x10^{5}$	401	8313	6.74	5.25
Cumulative product for all 24 loci						$7.12x10^{15}$	$8.00x10^{7}$	$1.77x10^{10}$	391	718	$4.18x10^{13}$	$7.62x10^{6}$	$1.07x10^{9}$	204	407

[a] No direction is implied by the order of the parents in each cross.
[b] Based on 332 to 359 cultivars.
[c] Where X and Y are random unrelated cultivars.
[d] The identity of both parents is unknown.
[e] The identity of one parent is assumed but that of the other is unknown.
[f] The identity of one parent is assumed but the possibility is considered that the other parent is actually a close relative of the cultivar proposed as the second parent.

history. We compared the frequencies of the Pos ip alleles among groups of Italian, Greek, and Croatian cultivars. Allele frequencies were calculated for 7 informative SSR loci from previously reported genotypes of 32 Greek, 30 Italian, and 30 Croatian cultivars primarily from the coast (16 plus the 14 in this study) (MALETIĆ *et al.* 1999, SEFC *et al*. 2000). (The allele data are available at http://www.boku.ac.at/zag/forsch/grapeSSR2.htm)

All but two of the Pos ip alleles are more frequent in the Croatian gene pool than in the Italian or Greek groups (Tab. 4). This supports the connection of Pos ip bijeli with the Croatian gene pool of *Vitis vinifera* L. and provides further evidence that it is an autochthonous Croatian cultivar.

Table 4

The frequency of Pos ip alleles in groups of Italian, Greek and Croatian cultivars. The cultivar names and individual cultivar genotypes can be found in SEFC *et al.* (2000). The Croatian group consists of 16 cultivars from SEFC *et al.* (2000) plus the 14 cultivars analyzed in this work Numbers in **bold** indicate allele frequencies that are higher in the Croatian group than in the Italian or Greek groups

Locus	Pos ip alleles	Italian n=30	Greek n=32	Croatian n=30	UC Davis database n>300
VVS2	133	0.333	0.141	**0.383**	0.399
	133				
VVMD5	228	0.117	0.063	**0.283**	0.127
	240	0.083	0.219	0.167	0.104
VVMD7	239	0.150	0.250	**0.467**	0.350
	239				
VVMD27	179	0.317	0.250	**0.450**	0.216
	181	0.117	0.203	**0.267**	0.154
VrZAG62	187	0.033	0.047	**0.067**	0.054
	189	0.133	0.469	0.283	0.301
VrZAG79	251	0.283	0.313	**0.317**	0.301
	259	0.167	0.156	**0.333**	0.132
VrZAG83	197	0.467	0.500	**0.517**	0.280
	197				

The ampelographic analysis (Tab. 5) provides further support for the proposed parentage of Pos ip bijeli. Pos ip shares 8 out of the 24 characteristics with both presumptive parents, 12 with Zlatarica blatska b. and one with Bratkovina b. For 34 additional ampelographic characteristics (data not shown), Posip shares 19 with both presumptive parents, 4 with Zlatarica and 6 with Bratkovina. A complete table of ampelographic data can be found at http://www.agr.hr/zavodi/genetics.

The shoot tips and young leaves of Pos ip are similar to those of Zlatarica blatska but the overall appearance of the mature leaves does not resemble either parent. All three cultivars share similar cluster and berry morphology (Tab. 5).

Zlatarica blatska b. has long been known to be a high quality variety. Its drawback, however, is low and irregular yield. The yield of Bratkovina b., on the other hand, is high but the quality is regarded as only intermediate and it lacks distinctive flavor. It is interesting to note here that, as for Chardonnay and 15 other varieties of northeastern France (BOWERS *et al.* 1999), our parentage also demonstrates that excellent wine cultivars may arise from crosses containing one mediocre parent.

Pos ip bijeli combines several desirable viticultural characteristics from both parents (high and regular yield, high sugar content, acceptable acid level and distinctive variety flavor). Furthermore, the early ripening of Pos ip bijeli (as well as Zlatarica blatska b. according to PULLIAT *et al.* 1897) makes this cultivar suitable for the production of high quality wine even in less favorable environments. These positive attributes explain the popularity of Pos ip bijeli, which has now replaced some other white wine cultivars in Dalmatian viticulture.

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Table 5

Ampelographic description of Pos ip b, Zlatarica blatska b. and Bratkovina b. (selected primary, secondary and ampelometric characteristics) according to the OIV descriptor list (OIV 1983) modified by EU project GENRES 081 (2001). Secondary descriptors are indicated by an asterisk (*). A complete table of ampelographic data can be found at http://www.agr.hr/zavodi/genetics

OIV Code	Characteristic	Bratkovina bijela	Pos ip bijeli	Zlatarica Blatska bijela
003	Young shoot: intensity of anthocyanin coloration of the prostrate hairs of the tip	weak	very weak	very weak
004	Young shoot: density of prostrate hairs of the tip	sparse	very sparse	very sparse
051	Young leaf: color of the upper side (leaves 1 to 4)	green	bronze	bronze
053	Young leaf: density of prostrate hairs between veins (lower side, leaf 4)	medium	none	very weak
067	Mature leaf: shape of blade	circular	wedge-shaped	pentagonal
068	Mature leaf: number of lobes	3 lobes	3 to 5 lobes	5 lobes
072	Mature leaf: goffering of the blade	weak	very weak	absent
079	Mature leaf: degree of petiole sinus opening	overlapping	open	slightly open
080	Mature leaf: shape of base of petiole sinus	U-shaped	U-shaped	U-shaped
084	Mature leaf: density of prostrate hairs on the lower side	weak	none	none
087	Mature leaf: density of erect hairs on the lower side	weak	weak	weak
151	Inflorescence: sex of the flower	female with upright stamina	hermaphrodite	hermaphrodite
202	Bunch: length	20,18 cm	22,94 cm	22,18 cm
204	Bunch: density	loose	loose	loose
208	Bunch: shape	conical or pyramidal	conical or pyramidal	conical or pyramidal
209	Bunch: presence of a wing	2 wings	2 wings	2 wings
223	Berry: shape	obtuse-ovate	obtuse-ovate	obtuse-ovate
236	Berry: particular flavor	none	other	other
301*	Time of bud burst	medium	early	early
303*	Beginning of berry ripening (veraison)	medium	early	early
351*	Vigor of shoot growth	medium	weak	weak
505*	Sugar content of must	18,1 %	21,3 %	20,2 %
506*	Total acid content (tartaric acid)	7,3 g/l	7,6 g/l	6,8 g/l
507*	Yield per vine	high	high	medium

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