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Microsatellite fingerprinting of grapevine (*Vitis vinifera* L.) varieties of the Carpathian Basin

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Summary

Altogether 101 *Vitis vinifera* L. genotypes were analysed at 6 microsatellite loci (Scu8vv, Scu10vv, VVMD21, VVMD36, ssrVRZAG64, ssrVRZAG79). Ninety-seven were autochthonous accessions of the Carpathian Basin and 4 were international cultivars. The allele composition and sizes obtained with the 6 microsatellite primer pairs were appropriate for discrimination of 95 cultivars. Berry colour-variants of cvs Gohér (Gohér fehér-white and Gohér piros-red), Lisztes (Lisztes fehér and Lisztes piros) as well as the cvs Bakator (Bakator piros and Bakator tüdőszín - light red) were exceptions.

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

Introduction

Microsatellite or SSR fingerprinting is an efficient method for molecular characterization (Sefc *et al*. 1998, 1999, Di Gaspero *et al*. 2000, Meredith 2001) since Thomas and Scott (1993) published the first microsatellite markers applicable for grapevine variety identification. Many new sequences have been described using these molecular markers (Bowers *et al*. 1996, Sefc *et al*. 1999, Di Gaspero *et al*. 2000, Scott *et al*. 2000, Lefort *et al*. 2002, Arroyo-Garcia and Martinez-Zapater 2004). Their wide-range applicability is due to the even distribution of the repetitive motifs throughout the nuclear genome, high polymorphism, frequent occurrence, co-dominant inheritance and reproducibility (Lefort and Roubelakis-Angelakis 2001, Thomas and Scott 1993, Cipriani *et al*. 1994, Sefc *et al*. 1998, 1999). The fact that they can be given as allele sizes rather than DNA bands on gels renders microsatellites particularly convenient to handle (Grando and Frisinghelli 1998, Meredith 2001). Since these markers provide a unique DNA fingerprint (Cipriani *et al*. 1994) they have been used for cultivar identification (Crespan 2004), for detection of clonal differences and verification of synonymies or homonymies (Vignani *et al.* 1996, Regner *et al.* 2000 c, Crespan and Milani 2001, Schneider *et al.* 2001, Franks *et al*. 2002, Ulanovsky *et al*. 2002).

Beside parentage and pedigree studies (Sefc *et al*. 1997, Bowers *et al*. 1999, Dettweiler *et al*. 2000, Regner *et al.* 2000 a, Piljac *et al*. 2002, Kozma *et al*. 2003), archaeological investigation concerning the origin of grapevine cultivation can also be based on microsatellite markers (Manen *et al*. 2003).

Molecular markers can assist breeding programs by means of determining the origin and genetic distance of the cultivars (Sefc *et al*. 1998, Bowers *et al*. 1999). SSR markers are also very useful tools in marker-based mapping of agronomic traits (Zyprian *et al*. 2003). More and more SSR allele size data are accumulating not only for various *Vitis* species (Lamboy and Alpha 1998, Di Gaspero *et al*. 2000), but also for varieties cultivated in various parts of the world, *e.g.* in Europe (Sefc *et al*. 2000 b). Many useful results have been gathered in the microsatellite data collections originating from the molecular genotyping of varieties in viticultural countries of Europe such as Bulgaria (Hvarleva *et al*. 2004), Croatia (Maletic *et al.* 1999), Greece (Lefort and Roubelakis-Angelakis 2001), Italy (Pellerone *et al*. 2001, Labra *et al*. 2002, Zulini *et al*. 2002), Portugal (Lopes *et al*. 1999) and Spain (Ulanovsky *et al*. 2001, Ibañez *et al*. 2003).

Conservation, characterization and sustainable utilisation of genetic resources in breeding and cultivation require the maintenance of old varieties and their precise characterization. Besides morphological traits, DNA marker systems should be involved as additional 'descriptors' for varietal identification to establish a 'DNA-based ampelographic system'. The aim of our present study was to characterize 97 ancient cultivars from the Carpathian Basin, to establish DNA fingerprints for these old Hungarian cultivars by means of microsatellite allele numbers and sizes, and to determine the discriminating power of 6 microsatellite markers. In addition to the 97 Carpathian Basin cultivars, 4 international cultivars, Csabagyöngye (Pearl of Csaba), Heunisch weiss (Weisser Heunisch), Muscat Ottonel and Pinot noir were also involved in the analyses.

Material and Methods

P l a n t m a t e r i a l : Ninety-seven ancient accessions (Tab. 1) and 4 international cultivars, Pearl of Csaba, Heunisch weiss (syn.: Gouais blanc, Meredith 2001, Schneider *et al*. 2001), Muscat Ottonel and Pinot noir preserved in the Research Institute for Viticulture and Enology in Pécs (Hungary) were sampled and used in this study. The 4 well-known cultivars were included to compare allele size results with those of other laboratories.

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Table 1

Grapevine accessions of the Carpathian Basin (1-97; bold letters indicate cultivars, which are still registered) and the international cultivars (98-101) investigated

Local ancient cultivars	Berry colour	Local ancient cultivars	Berry colour	Local ancient cultivars	Berry colour
Alanttermő	white (B)	Gergely	white (B)	Lisztes fehér	white (B)
Aprófehér	white (B)	Gohér, fehér	white (B)	Lisztes piros	red (Rg)
Ágasfark	blue (N)	Gohér, piros	red (Rg)	Magyarka	white (B)
Bajor, kék	blue (N)	Gohér, változó	white (B)	Mézesfehér	white (B)
Bajor, szürke	gray (G)	Gorombaszőlő	blue (N)	Mustos	white (B)
Bakarka	white (B)	Halápi	blue (N)	Pettyesszőlő	white (B)
Bakator, piros	red (Rg)	Hamuszőlő	gray (G)	Pécsi szagos	white (B)
Bakator, kék	blue (N)	**Hárslevelű**	white (B)	Piros gránát	red (Rg)
Bakator, tüdőszínű	light red (Rs)	Hosszúnyelű	white (B)	Piros tökös	red (Rg)
Bakszem	blue (N)	**Izsáki**	white (B)	Polyhos	white (B)
Balafánt	white (B)	Járdovány	white (B)	**Pozsonyi fehér**	white (B)
Balafánt, fekete	blue (N)	Juhfark	white (B)	Purcsin	blue (N)
Bálint	white (B)	**Kadarka**	blue (N)	Rakszőlő	white (B)
Bánáti rizling	white (B)	Kéklőiros	blue (N)	Rókafarkú	white (B)
Beregi	red (Rg)	**Kéknyelű**	white (B)	Rohadó	white (B)
Betyárszőlő	white (B)	**Királyleánka**	white (B)	Sárfehér	white (B)
Bihari	white (B)	Királyszőlő	white (B)	Sárpiros	red (Rg)
Bőségszaru	white (B)	Kolontár	white (B)	Somszőlő	white (B)
Budai	white (B)	Kovácsi	white (B)	Szagos bajnár	white (B)
Cudarszőlő	white (B)	Kovácskréger	white (B)	Szeredi	red (Rg)
Cukorszőlő	white (B)	Kozma	white (B)	Szerémi	white (B)
Csíkos muskotály	white (B)	Ködös	blue (N)	Szőke szőlő	white (B)
Csókaszőlő	blue (N)	Kőporos	white (B)	Tihanyi	white (B)
Csomorika	white (B)	**Kövérszőlő**	white (B)	Tótika	blue (N)
Czeiger	white (B)	Bogdányi dinka	red (Rg)	Tökszőlő	white (B)
Demjén	white (B)	Pécsi dinka	blue (N)	Tulipiros	red (Rg)
Erdei	white (B)	**Kövidinka**	red (Rg)	Tükörszőlő	white (B)
Ezerjó	white (B)	Ürömi dinka	white (B)	Tüskéspúpú	white (B)
Fodroslevelű	white (B)	Vörösdinka	red (Rg)	Vékonyhéjú	white (B)
Furmint	white (B)	Zöld dinka	white (B)	Csabagyöngye	white (B)
Furmint, piros	red (Rg)	Kübeli	white (B)	Muscat Ottonel	white (B)
Fügér	white (B)	Lányszőlő	white (B)	Heunisch weiss	white (B)
Fügeszőlő	white (B)	Lágylevelű	white (B)	Pinot noir	blue (N)
Fürjmony	white (B)	Leányka	white (B)		

DNA isolation: DNA was extracted from young leaves with the DNeasy® Plant Mini kit (Qiagen) according to the manufacturer's protocol and to the method described by LODHI *et al*. (1994).

PCR conditions: PCR was performed in a GeneAmp 9700 thermal cycler (ABI Perkin-Elmer) in a 25 µl volume. The reaction mixture contained 20 ng DNA template 1 µM of each primer, 75 µM of each dNTP, 2 mM $MgCl_2$, 1 x PCR buffer and 1 unit Taq polymerase (Promega). The following PCR profile was applied: precycle: 4 min at 95 °C; 36 cycles of denaturation 20 s at 95 °C; 30 s annealing at 56 °C and 1 min extension at 72 °C; postcycle: 5 min at 72 °C.

SSR analysis: Six microsatellite loci were selected, Scu08vv, Scu10vv, VVMD21, VVMD36, ssrVrZAG64 and ssrVrZAG79 (Tab. 2), partly according to the recommendation of the GENRES081 EU project (DETTWEILER and THIS 2000) and partly on the basis of our preliminary primer test results (KISS *et al*. 2003). Each forward primer was labelled with Cy-5 (IDT Inc., BioSciences). The amplification products were separated on 8 % denaturing polyacrylamide gel (Amersham Biosciences, Uppsala, Sweden). The allele sizes were estimated with ALFexpress II DNA analyser (Amersham Biosciences). ALFexpress™ sizer™ 50-500 (Amersham Biosciences) was applied as standard. Allele frequencies, expected (He) and observed (Ho) heterozygosity and probability of identity (PI) were calculated according to the Identity 1.0 software (WAGNER and SEFC 1999).

Results and Discussion

Unique microsatellite fingerprints have been obtained for 95 out of 101 genotypes (Tab. 3). Only berry colour-variants of cvs Gohér (white and red), Lisztes (white and

Table 2

Name, sequences and allele size range of the 6 microsatellite loci

No.	Primer name	Sequence	Allele size range (bp)	Reference	Allele size range in th present study (bp)
1.	Scu8vv	f: cga gac cca gca tcg ttt caag r: gca aaa tcc tcc ccg tac aag tc	180	Scott *et al.* 2000	185-192
2.	Scu10vv	f: tac ccc cac aac cct ttt r: ttc tcc gcc acc tcc ttt tcac	205-307	Scott *et al.* 2000	202-217
3.	VVMD21	f: ggt tgt cta tgg agt tga tgt tgc r: gct tca gta aaa agg gat tgc g	243-266	Bowers *et al.* 1999	244-267
4.	VVMD36	f: gaa aat taa taa tag ggg gac acg gg r: gca act gta aag gta aga cac agt cc	244-315	Bowers *et al.* 1999	244-296
5.	ssrVrZAG64	f: tat gaa aga aac cca acy cgg cacg r: tgc aat gtg gtc agc ctt tga tgg g	137-197	Sefc *et al.* 1999	139-165
6.	ssrVrZAG79	f: aga ttg tgg agg agg gaa caa accgr r: tgc ccc cat ttt caa act ccc tcc c	236-260	Sefc *et al*. 1999	240-262

Table 3

Microsatellite profile of the 101 grapevine varieties

No.	Variety name	Allele size (bp) in locus Scu8vv	Scu10vv	VVMD21	VVMD36	VrZag64	VrZag79
1.	Alanttermő	185:185	202:208	250:259	254:276	161:165	254:260
2.	Aprófehér	185:185	208:214	250:250	264:266	141:145	246:254
3.	Ágasfark	185:192	202:202	244:250	254:264	145:165	252:262
4.	Bajor, kék	185:192	202:208	250:257	252:252	145:165	252:262
5.	Bajor, szürke	185:192	202:208	250:257	254:254	145:165	252:262
6.	Bakarka	185:185	214:214	244:250	264:266	141:145	254:254
7.	Bakator, kék	185:185	202:208	250:250	264:264	141:165	252:262
8.	Bakator, piros	185:185	202:208	244:257	266:288	145:165	254:254
9.	Bakator, tüdőszínű	185:185	202:208	244:257	266:288	145:165	254:254
10.	Bakszem	185:192	202:208	250:250	252:264	141:165	240:262
11.	Balafánt	185:192	202:208	244:259	276:288	145:165	240:254
12.	Balafánt, fekete	185:192	202:202	250:250	254:276	161:165	252:252
13.	Bálint	185:192	208:214	250:259	264:276	141:145	252:254
14.	Bánáti rizling	185:185	208:211	250:257	254:288	161:161	254:262
15.	Beregi	185:185	208:214	244:250	254:288	139:145	254:262
16.	Betyárszőlő	185:185	202:214	250:257	264:266	139:165	262:262
17.	Bihari	185:185	202:205	250:250	264:264	141:161	250:262
18.	Bogdányi dinka	185:185	214:214	244:250	264:266	139:145	254:262
19.	Bőségszaru	185:185	202:205	244:250	276:296	145:165	248:252
20.	Budai	185:192	208:214	250:250	244:254	141:165	252:252
21.	Cudarszőlő	185:185	208:214	250:250	244:254	145:145	242:254
22.	Cukorszőlő	185:185	202:208	257:257	254:276	141:161	254:262
23.	Csíkos muskotály	185:185	208:217	250:267	244:264	143:161	254:258
24.	Csókaszőlő	185:185	202:208	257:257	288:288	161:165	240:254
25.	Csomorika	185:185	208:211	257:257	288:288	141:145	240:262
26.	Czeiger	185:185	202:208	250:250	264:288	139:165	254:254
27.	Demjén	185:185	202:202	244:257	254:288	141:165	254:262
28.	Erdei	185:185	202:214	244:250	264:264	145:165	246:254
29.	Ezerjó	185:185	202:202	244:250	258:276	139:139	240:254

Tab. 3, continued

No.	Variety name	Allele size (bp) in locus Scu8vv	Scu10vv	VVMD21	VVMD36	VrZag64	VrZag79
30.	Fodroslevelű	185:192	202:214	250:257	264:266	139:165	262:262
31.	Furmint	185:192	202:208	250:259	254:276	161:165	240:252
32.	Furmint, piros	185:192	202:208	250:257	254:276	161:165	240:252
33.	Fügér	185:192	208:208	244:244	254:264	141:145	252:252
34.	Fügeszőlő	185:192	208:208	244:244	264:288	145:145	240:252
35.	Fürjmony	185:192	205:208	250:257	254:264	141:161	250:254
36.	Gergely	185:185	208:214	244:250	266:276	159:165	240:254
37.	Gohér, fehér	185:192	202:208	244:257	254:288	141:145	252:262
38.	Gohér, piros	185:192	202:208	244:257	254:288	141:145	252:262
39.	Gohér, változó	185:192	202:208	244:257	254:288	145:145	252:262
40.	Gorombaszőlő	185:185	208:214	250:259	254:266	139:145	252:252
41.	Halápi	188:188	208:217	244:267	244:254	141:157	252:258
42.	Hamuszőlő	185:185	208:208	250:250	264:276	139:141	248:254
43.	Hárslevelű	185:185	202:208	244:259	264:276	145:165	240:254
44.	Hosszúnyelű	185:185	208:214	244:257	254:288	141:145	240:254
45.	Izsáki	185:185	208:214	244:250	254:276	139:161	240:246
46.	Járdovány	185:185	208:214	244:250	266:276	141:161	240:254
47.	Juhfark	185:185	208:208	250:257	264:276	141:165	240:252
48.	Kadarka	185:185	208:214	250:250	266:276	145:165	252:252
49.	Kéklőpiros	185:185	202:208	250:257	264:270	159:165	252:262
50.	Kéknyelű	185:185	202:208	244:250	252:264	159:165	252:254
51.	Királyleányka	185:185	208:214	244:250	254:266	161:161	252:254
52.	Királyszőlő	185:185	202:208	250:259	266:288	145:165	254:262
53.	Kolontár	185:192	202:208	244:250	254:264	141:145	252:262
54.	Kovácsi	185:192	208:208	257:257	264:288	161:161	254:254
55.	Kovácskréger	185:185	202:211	250:257	254:264	145:161	252:254
56.	Kozma	185:185	202:208	257:267	254:264	141:145	262:262
57.	Ködös	185:185	208:208	257:259	254:276	145:165	252:252
58.	Kőporos	185:185	208:214	257:259	264:266	145:165	254:260
59.	Kövérszőlő	185:185	208:208	250:259	264:266	145:161	240:254
60.	Pécsi dinka	185:185	202:208	244:244	254:288	141:145	252:254
61.	Kövidinka	185:185	208:214	244:250	264:264	139:141	254:262
62.	Ürömi dinka	185:185	214:214	250:250	266:276	145:161	246:254
63.	Vörösdinka	185:185	208:214	244:250	254:264	139:145	254:262
64.	Zöld dinka	185:185	202:208	244:257	264:264	145:145	254:254
65.	Kübeli	185:185	208:214	257:259	264:266	161:165	254:260
66.	Lányszőlő	185:185	208:211	250:257	254:276	161:161	252:254
67.	Lágylevelű	185:185	202:214	250:250	254:254	165:165	252:254
68.	Leányka	185:185	202:208	250:250	266:276	161:165	240:254
69.	Lisztes fehér	185:185	208:208	250:257	276:288	141:161	240:262
70.	Lisztes piros	185:185	208:208	250:257	276:288	141:161	240:262
71.	Magyarka	185:192	208:208	244:250	264:288	145:165	248:254
72.	Mézesfehér	185:192	208:214	250:257	266:276	141:165	254:262
73.	Mustos	185:185	208:214	244:250	254:276	145:161	246:252
74.	Pettyesszőlő	185:185	202:208	244:244	254:288	145:165	250:252
75.	Pécsi szagos	185:185	208:211	257:267	264:288	161:161	254:258
76.	Piros gránát	185:185	208:214	244:250	254:264	139:145	250:254
77.	Piros tökös	185:185	202:214	244:250	276:288	145:165	252:254
78.	Polyhos	185:185	202:202	244:259	254:288	145:161	252:262
79.	Pozsonyi	185:192	202:214	244:259	264:264	139:145	254:254
80.	Purcsin	185:185	208:214	250:250	254:276	161:165	250:258
81.	Rakszőlő	185:185	208:214	244:244	254:266	139:161	254:254
82.	Rókafarkú	185:185	208:214	250:250	264:276	141:165	240:246

Tab. 3, continued

No.	Variety name	Allele size (bp) in locus Scu8vv	Scu10vv	VVMD21	VVMD36	VrZag64	VrZag79
83.	Rohadó	185:185	208:208	250:257	264:276	145:161	250:258
84.	Sárfehér	185:192	202:208	244:250	264:264	139:165	252:254
85.	Sárpiros	185:185	202:208	244:244	264:288	145:165	254:260
86.	Somszőlő	185:185	202:214	244:250	252:256	139:153	252:254
87.	Szagos bajnár	185:185	205:208	250:250	264:288	139:161	250:262
88.	Szeredi	185:185	202:202	250:257	254:276	145:161	252:252
89.	Szerémi	185:185	202:208	250:250	276:276	161:165	252:258
90.	Szőke szőlő	185:185	202:208	244:250	272:276	139:145	254:260
91.	Tihanyi	185:185	208:208	257:267	254:264	145:145	252:262
92.	Tótika	185:185	202:214	250:257	254:276	145:165	252:254
93.	Tökszőlő	185:185	208:214	257:257	264:276	161:165	240:262
94.	Tulipiros	185:185	208:208	244:244	254:288	145:145	252:254
95.	Tükörszőlő	185:185	202:214	250:250	254:264	161:165	246:262
96.	Tüskéspúpú	185:185	208:211	257:257	254:288	145:161	254:262
97.	Vékonyhéjú	185:185	202:208	250:250	264:276	161:165	246:262
98.	Csabagyöngye	185:185	205:214	244:267	264:296	161:161	258:262
99.	Muscat Ottonel	185:185	208:214	267:267	264:276	139:161	258:262
100.	Heunisch weiss	185:185	208:214	250:250	264:276	161:161	240:246
101.	Pinot noir	185:192	205:217	250:250	254:254	141:165	242:248

red) and Bakator (red and light-red) gave identical SSR patterns with the selected 6 primer pairs. Therefore, in additional, highly polymorphic microsatellite loci ssrVrZAG62 (Sefc *et al*. 1999) and VVMD5 (Bowers *et al*. 1996) were included into the analyses for these questionable cases; however, these genotypes remained indistinguishable (data not shown). Sefc *et al.* (2000 a) were also able to detect unique genotypes for 100 cultivars with 10 SSR markers except for coloured variants.

Comparing the results on allele size with the literal allele size ranges (Scott *et al*. 2000) it can be concluded, that in case of Scu8vv and Scu10vv new allele sizes were identified in the Carpathian Basin cultivars. Scu08vv and Scu10vv represent 5'UTR regions of EST sequences (Scott *et al*. 2000) and have intermediate variability, while, in our study Scu10vv amplified 6 different alleles, more than VVMD21. The microsatellite VVMD21 resulted in a very similar allele size range, while in case of VVMD36 the interval was narrower than expected.

None of the samples gave an amplified fragment corresponding to the upper limit reported for ssrVrZAG64. Both, the minimum and maximum values obtained with the ssrVrZAG79 locus were higher than the allele size limits found in the Greek Vitis Database (Sefc *et al*. 1999).

As for the international standard cultivars their allele sizes were in the same intervals as the Carpathian Basin cultivars at each microsatellite locus. The allele sizes observed in our study for Heunisch weiss with VVMD36 were exactly the same as those reported by Regner *et al*. (2000 b, d) (Tab. 4). Our results on Pinot noir obtained with VVMD36 are also identical with those of Bowers *et al*. (1999) and Regner *et al*. (2000 b). In case of Muscat Ottonel in our investigation VVMD36 resulted in the same size as reported by Crespan and Milani (2001) despite a different methodology. The values obtained with VVMD21, VVMD36, ssrVrZag64 and ssrVrZag79 for Csabagyöngye (Pearl of Csaba), Heunisch weiss, Muscat Ottonel and Pinot noir proved to be 1-4 bp higher than the international results (Bowers *et al*. 1999, Regner *et al*. 2000 b, d, Sefc *et al*. 1998, 2000 b, Lefort and Roubelakis-Angelakis 2000). Similar differences were also observed in other laboratories (Crespan and Milani 2001, Regner *et al*. 2000 b, d). It is not obvious to explain the reason for this; according to This *et al*. (2004) such fragment size alterations might be explained with the stutter or the extra base additions of certain types of Taq polymerases. We have repeated the analyses three times with the whole sample set (101 accessions), and the results proved to be consistent.

Most alleles (12) were obtained at the VVMD36 locus (Tab. 5), while the lowest number of alleles (3) were detected with Scu8vv primers. The frequency of different alleles showed variability at the investigated loci. The rank of microsatellite markers in informativeness and discriminating power is the following: ssrVrZAG79 (PI 0.11 / 10 alleles) > VVMD36 (PI 0.12 / 12 alleles) > ssrVrZAG64 (PI 0.14 / 9 alleles) > VVMD21 (PI 0.24 / 5 alleles) > Scu10vv (PI 0.27 / 6 alleles) > Scu8vv (PI 0.67 / 3 alleles). VVMD36 and ssrVrZAG64 had the highest heterozygosity among the tested cultivars.

The number of primers sufficient for reliable varietal identification depends on the nature and the discriminating power of each primer (Tessier *et al*. 1999). Generally 6 primer pairs are sufficient to differentiate between genotypes (Zulini *et al*. 2002, This *et al*. 2004), but closely related cultivars require a higher number (Meredith 2001). In our study this concerns the varieties Gohér, Lisztes and Bakator, whose berry colour-variants were undistinguishable. All the other accessions could be successfully genotyped with the 6 micro-satellites.

Table 4

Comparison of the allele sizes of the international cultivars observed in different laboratories

Cultivar	Reference	Method	VVMD21	VVMD36	ssrVrZag64	ssrVrZag79
Csabagyöngye	Greek Vitis	ALFexpress, Pharmacia	-	-	159:159	254:258
Pearl of Csaba	Database	6 % Acrylamide 7M urea	-	262:294	-	-
	SEFC *et al*. 1998					
	Present study	ALFexpress, 8 % Acrylamide (ReproGel™, Amersham)	244:267	264:296	161:161	258:262
Heunisch weiss	REGNER *et al*. 2000 d	ALFexpress, Pharmacia	248:248	262:274	159:159	236:242
	REGNER *et al*. 2000 b	6 % Acrylamide 7M urea	249:249	264:276	160:160	238:244
		373 ABI CE 6 % Polyacrylamide				
	Present study	ALFexpress, 8 % Acrylamide (ReproGel™, Amersham)	250:250	264:276	161:161	240:246
Ottonel muskotály	CRESPAN and	GE 5 % Polyacrylamide	266:266	264:276	137:159	254:258
Muscat Ottonel	MILANI 2001	7M urea	-	262:274	-	-
	SEFC *et al*. 1998	ALFexpress, Pharmacia 6 % Acrylamide 7M urea				
	Present study	ALFexpress, 8 % Acrylamide (ReproGel™, Amersham)	267:267	264:276	139:161	258:262
Pinot noir / Pinot	REGNER *et al*. 2000 d	ALFexpress, Pharmacia	248:248	252:252	139:163	238:244
	REGNER *et al*. 2000 b	6 % Acrylamide 7M urea	249:249	254:254	140:164	240:246
	SEFC *et al*. 2000 b	373 ABI CE 6 %	-	-	139:163	238:244
	BOWERS *et al*. 1999	Polyacrylamide	249:249	254:254	-	-
		ALFexpress, Pharmacia 6 % Acrylamide 7M urea				
		GE 6 % acrylamide 7M urea				
	Present study	ALFexpress, 8 % Acrylamide (ReproGel™, Amersham)	250:250	254:254	141:165	242:248

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Table 5

Allele sizes and frequencies (**a**); number of alleles, probability of identity (PI), expected and observed heterozygosity (**b**) obtained for 101 grapevine cultivars

(**a**)

Locus	Allele frequencies			Locus	Allele frequencies:		
	Allele size (bp)	Observed	Upper 95 % confidence limit		Allele size (bp)	Observed	Upper 95 % confidence limit
Scu8vv	185	0.8713	0.9052	ssrVrZag64	139	0.0990	0.1390
	188	0.0099	0.0295		141	0.1386	0.1834
	192	0.1188	0.1613		143	0.0049	0.0218
Scu10vv	202	0.2722	0.3265		145	0.2722	0.3265
	205	0.0297	0.0564		153	0.0049	0.0218
	208	0.4505	0.5083		157	0.0049	0.0218
	211	0.0297	0.0564		159	0.0148	0.0365
	214	0.2029	0.2533		161	0.2227	0.2744
	217	0.0148	0.0365		165	0.2326	0.2849
VVMD21	244	0.2475	0.3005	ssrVrZag79	240	0.1039	0.1446
	250	0.4356	0.4935		242	0.0099	0.0294
	257	0.2128	0.2638		246	0.0445	0.0750
	259	0.0643	0.0989		248	0.0198	0.0433
	267	0.0396	0.0689		250	0.0346	0.0627
VVMD36	244	0.0198	0.0433		252	0.2277	0.2796
	252	0.0247	0.0499		254	0.3069	0.3625
	254	0.2277	0.2796		258	0.0396	0.0689
	256	0.0049	0.0218		260	0.0247	0.0499
	258	0.0049	0.0218		262	0.1881	0.2373
	264	0.2673	0.3213				
	266	0.0990	0.1390				
	270	0.0049	0.0218				
	272	0.0049	0.0218				
	276	0.1831	0.2320				
	288	0.1485	0.1943				
	296	0.0099	0.0294				

(**b**)

Locus	Sample size	Number of alleles	Probability of identity (PI)	Expected heterozygosity (He)	Observed heterozygosity (Ho)
Scu8vv	101	3	0.66	0.23	0.24
Scu10vv	101	6	0.27	0.68	0.78
VVMD21	101	5	0.24	0.69	0.65
VVMD36	101	12	0.12	0.81	0.86
ssrVrZag64	101	9	0.14	0.79	0.85
ssrVrZag79	101	10	0.11	0.80	0.82

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