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# Microsatellite based identification of grapevine cultivars traditional in Hungary and in the Carpathian Basin

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***Summary:*** Grapevine cultivars and clones traditional in Hungary and in the Carpathian Basin are maintained in the genetic collection of the Corvinus University ofBudapest. Mostly ancient varieties and clones were genotyped using microsatellite loci. The investigated 6 loci were sufficient to distinguish all cultivars. Some clones could also be separated based mostly on the variable VVS2 loci. For 17 out of the 31 investigated cultivars this is the first report on characterization of the polymorphism of the allele lengths by microsatellite markers on loci: VVS2, VVMD7, VVMD27, vrZAG62.

***Key words:*** Vitis, SSR, genetic analysis, Hungarian cultivars.

## Introduction

Microsatellite markers are widely used for genotyping grape cultivars (*Thomas & Scott* 1993; *Bowers* et al. 1996; *Sefc* et al. 1998a) and pedigree analysis (*Sefc* et al. 1997; Sefc et al. 1998b; *Regner* et al. 2000). Till now only a few experiments were reported, where traditional Hungarian cultivars were tested by microsatellite markers – 'Csaba gyöngye', (*Sefc* et al. 1998a, *Kozma* et al, 2002); 'Szőlőskertek királynéja' (*Sefc* et al. 1998a); 'Heunisch' (*Sefc* et al. 1998b, *Regner* et al. 2000). Recently, monitoring collections (*Balogh* et al. 2003; *Bisztray* et al. 2003; *Kiss* et al. 2003; *Kiss* et al. 2004) and attempts to investigate archaeological samples (*Bisztray* et al. 2004) have started.

In the study traditional cultivars of Hungary and of the Carpathian Basin are involved (*Csepregi & Zilai*, 1971). These were genotyped using microsatellite loci VVS2, VVMD5 VVMD7, VVMD27, VRZAG62 VRZAG 79. For 17 out of the 31 cultivars and clones investigated this is the first report on characterization of the allele lengths polymorphism by the applied set of microsatellite markers.

## Material and method

The investigated 39 genotypes belong to 31 cultivars, from the collections of the Corvinus University of Budapest and of the Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau. DNA was extracted from leaf tissue following the procedure described by *Thomas* et al. (1993), or using the protocol of the Qiagen Dneasy Plant System Minikit (*Qiagen* Inc., USA).

The cultivars were analyzed with 6 SSR markers according to the identification of grapevine cultivars (*Sefc* et al. 1998c). The probability that two random cultivars are indistinguishable is $10^{-6}$ and therefore the selected markers offer sufficient potential for individual characterization. The VVS 2 marker was developed by *Thomas & Scott* (1993) and the VVMD 5, VVMD 7, markers were gained by *Bowers* et al. (1996) as well as VVMD 27 by *Bowers* et al (1999). The VRZAG 62 and VRZAG 79 (*Sefc* et al., 1999) markers were obtained from investigations into simple sequence repeats of *Vitis riparia*.

The solution for PCR amplification contained 20 µl of the buffer solution consisting of 16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 0.01% Tween 20, 0.1 mM each dNTP (GenXpress, Vienna), 0.2µM primer, 0.7 U Biotherm Taq DNA polymerase (GenXpress, Vienna) and 50 ng genomic DNA of grapevine.

The amplification of the SSR loci was performed after 2 minutes denaturation at 94 °C followed by a two step procedure including 35 cycles with annealing phase for 30 sec. (temperature between 50 and 55 °C) and denaturation for 15 sec. at 92 °C; but with an extension step at 72 °C for 5 minutes at the end of the PCR reaction. The annealing temperature for each locus was set according to the results gained during analysis.

Yield of DNA fragments was estimated by running an aliquot of the sample on a 2% agarose gel stained with ethidium-bromide. Due to the different size range of the involved loci multiplex analysis was feasible. At least the alleles of three loci were separated in the same slot on a sequencing gel. The samples were denaturated by heating with formamide and loading together with a size standard

*Table 1.* Detected allele-lengths at the six microsatellite loci of the 39 genotypes. *denotes traditional cultivars

| | Cultivar/clone | VVS2 | MD 5 | MD 7 | MD 27 | ZAG 62 | ZAG 79 |
|---|---|---|---|---|---|---|---|
| 1 | Pirosszlanka* | 136:144 | 226:236 | 239:239 | 183:189 | 191:191 | 244:252 |
| 2 | Csomorika* | 133:153 | 238:240 | 239:249 | 179:181 | 189:205 | 238:260 |
| 3 | Budaizöld* | 144:153 | 226:226 | | 189:194 | 201:205 | 252:252 |
| 4 | Tramini | 151:151 | 232:238 | 243:257 | 189:189 | 189:195 | 246:252 |
| 5 | Mézesfehér* | 134:144 | 226:232:236 | 243:247 | 181:194 | 190:206 | 252:260 |
| 6 | Kövidinka | 133:144 | 234:240 | 239:249 | 181:187 | 189:195 | 252:260 |
| 7 | Hárslevelű 1 | 133:146 | 226:232 | 239:247 | 180:180 | 191:205 | 238:250 |
| 8 | Hárslevelű 2 | 133:146 | 226:232 | 239:247 | 179:179 | 189:205 | 238:252 |
| 9 | Hárslevelű P41 | 133:146 | 226:232 | 239:247 | 179:179 | 191:205 | 238:250 |
| 10 | Hárslevelű 921 | 133:146 | 226:232 | 239:247 | 179:179 | 191:205 | 238:250 |
| 11 | Hárslevelű P311 | 133:146 | 226:232 | | 179:179 | 191:205 | 238:250 |
| 12 | Királyleányka 1* | 133:135 | 226:238 | 247:257 | 185:189 | 196:196 | 238:250 |
| 13 | Királyleányka 2* | 133:133 | 236:240 | 247:249 | 194:194 | 196:206 | 250:252 |
| 14 | Furmint | 133:133 | 226:240 | 239:249 | 179:194 | 189:205 | 238:250 |
| 15 | Furmint:T85 | 138:153 | 226:240 | 239:249 | 179:194 | 189:205 | 238:250 |
| 16 | Furmint T92 | 133:153 | 226:240 | 239:249 | 179:194 | 189:205 | 238:250 |
| 17 | Ezerjó 1* | 133:133 | 234:240 | 239:249 | 187:201 | 190:196 | 248:260 |
| 18 | Ezerjó 2* | 133:133 | 226:232 | 239:239 | 179/185 | 191:191 | 238:252 |
| 19 | Sárfeher* | 133:133 | 232:232 | 247:249 | 185:189 | 191:205 | 248:260 |
| 20 | Juhfark* | 135:146 | 226:240 | 239:247 | 179:194 | 197:205 | 238:250 |
| 21 | Bánáti* | 133:144 | 240: 248 | 239:247 | 179/181 | 190:206 | 252:260 |
| 22 | Leányka | 133:133 | 226:236 | 247:253 | 185:194 | 193:193 | 238:252 |
| 23 | Kövérszőlő* | 133:146 | 228:240 | 239:255 | 179:194 | 197:205 | 238:252 |
| 24 | Budai Góhér* | 133:133 | 226:232 | 228:228 | 179:179 | 190:196 | 252:260 |
| 25 | Sárgamuskotály | 133:133 | 226:236 | 233:249 | 179:181 | 196:196 | 252:256 |
| 26 | Török Góhér* | 133:133 | 240:240 | 239:249 | 181:194 | 190:205 | 250:260 |
| 27 | Kékfrankos | 144:144 | 226:240 | 239:249 | 179:194 | 195:205 | 238:252 |
| 28 | Szürkebarát | 137:151 | 228:238 | 239:243 | 185:189 | 189:195 | 240:246 |
| 29 | Olaszrizling | 135:151 | 226:238 | 247:257 | 185:189 | 195:197 | 252:252 |
| 30 | Nemeskadarka | 133:135 | 226:226 | 247:255 | 185:194 | 189:205 | 250:250 |
| 31 | Bíborkadarka | 135:153 | 226:234 | 249:255 | 181:194 | 205:205 | 252:252 |
| 32 | Creaza* | 135:153 | 226:234 | 247:253 | 181:194 | 205:205 | 252:252 |
| 33 | Gloria Hungariae | 133:149 | 232:238 | 247:259 | 183:194 | 187:205 | 248:258 |
| 34 | Katonatelep | 135:153 | 226:228 | 239:247 | 185:194 | 189:195 | 250:260 |
| 35 | Muskat Piros | 133:149 | 228:232 | 227:227 | 181:181 | 189:195 | 256:260 |
| 36 | Csókaszőlő* | 133:146 | 228:232 | 247:253 | 179:185 | 205:205 | 238:238 |
| 37 | Csaba Gyöngye* | 133:155 | 236:236 | 247:249 | 179:181 | 187:205 | 256:260 |
| 38 | Zalagyöngye | 133:155 | 226:236 | 239:249 | 181:189 | 187:195 | 260:262 |
| 39 | Zefír | 151:151 | 228:238 | 233:243 | 189:194 | 189:197 | 240:252 |

(Genescan 350 Tamra, Appl. Biosys.) to a 6% polyacrylamid gel. Detection of the SSR fragments labeled with 6-FAM, HEX and TET was carried out by an automated sequencer (ABI 373, Perkin-Elmer, Vienna). Labeling with different fluorescent coloring agents facilitated the application of multiplex analysis. Detected allele sizes were recorded in base pairs for each locus.

## Results and discussion

All varieties can be clearly distinguished using the applied microsatellite markers *(table 1.)*. These varieties are traditional in Hungary and in the Carpathian Basin and share many common alleles, some of them identical with alleles found by *Regner* et al. (2000) in the 'Heunisch' genotype and its close relatives.

However, besides these representative samples a wide survey on the existing gene pools of these varieties will be necessary. It can reveal intervarietal polymorphism as found in 'Hárslevelű 2'. Some of the 'Hárslevelű' types differ clearly in morphological characteristics and that was the reason for involving them in this study. A different situation is found in cultivars 'Királyleányka' and 'Ezerjó'. It seems that the true variety is not given. Especially in ancient autochthonous varieties variability is extended and therefore even outcrossed seedlings or individual genotypes could become a homonymous variety. More genetic information would be necessary to define the relationship among these varieties. For the purpose of identification it will be necessary to randomise the alleles of all 6 loci which were obtained (*This* et al.2004). We identified for the VVS2 allele n+10=133bp (BA1) length, for the VVMD 5 allele n+12= 234 bp (CH1), for the VVMD7 allele n+8= 239 bp (CF1), for the VVMD 27 allele n+6=181bp, for the VRZAG 62 allele n+14= 189bp (CH1) and at least for the VRZAG 79 allele n= 238 bp (RO1). Due to this STMS marker-key all data are easily comparable.

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