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Old Hungarian grapevine cultivars and their relations characterized with microsatellite markers

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Summary: Thirty-one old Hungarian grapevine (*Vitis vinifera* L.) cultivars were investigated on 7 microsatellite loci to characterize them, to separate the cultivars from synonym names, and to confirm parent-offspring connections. Conculta (group of cultivars or bud sports) members, such as 'Gohér' and 'Bajor' representatives, were studied to find a suitable locus for the separation. Synonyms, conculta members, subcultivars and clones of 'Furmint', which was the most important cultivar of Tokaj, were also analyzed to separate the members of the different taxonomic levels. Pedigree of 'Királyleányka' was examined to find the missing ancestor, because the parent-offspring connection between the natural hybrid and 'Kövérszőlő' is questionable.

Key words: conculta, parent-offspring analysis, SSR, synonym-homonym, *Vitis vinifera* L.

Introduction

Currently more than 100 cultivars are permitted for commercial growing in Hungary, while national grapevine collections contain even more than 1000 items (cultivars, subcultivars and clones). To keep the diversity of the sort of autochthonous grapevine cultivars, it is highly important to save germplasm collections and old plantations for the future (Hajdu 2003). Evaluation and maintenance of grapevine collections are still actual tasks; several works proved that molecular genetic investigations can help these works to separate the synonyms, duplications or absences (*Grando & Frisinghelli,* 1998; *Sefc* et al., 1998; *Lopes* et al., 1999; *Lefort* et al., 2001; *Sefc* et al., 2000; *Lefort & Roubelakis-Angelakis*, 2001; *Ibanez* et al., 2003; *Lefort* et al., 2003; *Martín* et al., 2003; *Ortiz* et al., 2003; *Maul*, 2004; *Núñez* et al., 2004; *Boccacci* et al., 2005).

Conculta members: "Natural", taxonomic systems of grapevine are based on agrobiological and geographical aspects (*Bényei & Lőrincz*, 2005). *Németh* (1967) completed Negrul's classification system applying subspecific taxa. Within the species *Vitis vinifera* L. convarietas (identical with Negrul's proles), conculta (group of cultivars) and cultivar are the decisive taxa. Cultivars of a conculta (for example: 'Pinot noir', 'Pinot gris' and 'Pinot blanc') rather differ in the color of berry, the autumn coloration of leaf, and rarely in the color of matured shoot. *Németh* (1973) distinguished 44 groups of cultivars; though out of them only one member is cultivated (such as 'Sauvignon', 'Chardonnay'), the others can be found in grapevine collections. In case of the concultas, where more members are grown, for example the before mentioned 'Pinot', it is important to differentiate at any phenological stages, not only at maturation. During the period of wood propagation when the plants don't show the most characteristic patterns, it is necessary to find differences. Molecular methods could be suitable for these inspections also (*Halász* et al., 2005). In this study members of several concultas ('Furmint', 'Gohér', 'Járdovány', 'Bajor') were investigated to separate them.

Synonyms and homonyms among the old Hungarian cultivars: Most of the traditional cultivars are either native of the Carpathian Basin or present here for centuries. With the human migration the cultivars could extend not only in the place of origin, but they spread in other areas also. Cultivars could be renamed in new regions what can be a reason of the high number of synonyms. Homonymy can arise from ampelographic characteristics (typical taste or morphological specialty) which belong to cultivars with different genetic background. Several researches dealt with the autochthonous grapevine cultivars and separated them from synonyms by SSR analysis (*Maletić* et al., 1999; *Fossati*, 2000; *Schneider* et al., 2001; *Ulanowsky*, 2002).

'Furmint', which is Hungary's second most wide-spread white wine cultivar, has more than 120 synonyms. Among from these which sign individual cultivars also ('Budai gohér', 'Demjén', 'Kövérszőlő') were collected to separate them. These cultivars can be synonyms even to each other, and databases such as *Vitis International Variety Catalogue* (VIVC) also mentions them as synonyms (*Table 1*).

Table 1. The investigated samples and their synonyms signing individual cultivars

Cultivar	Synonyms
Kövérszőlő	Bajor, Gohér
Gohér	Bajor, Budai gohér, Demjén, Török gohér
Bajor	Gohér, Kozma
Balafánt	Bajor
Demjén	Budai gohér
Furmint	Budai gohér, Demjén, Kövérszőlő
Juhfark	Budai gohér
Kozma	Bajor

Parent-offspring connections: Old, native cultivars mainly originate from natural hybridizations or mutations. Finding genetic relationship and paternity with molecular methods are useful in proving ancestry (*Magalhaes*, 2003; *Maletić* et al., 2004; *Parker* et al., 2005). The molecular genetic analysis of 'Müller Thurgau' – though it's a cross-bred cultivar – showed that incorrect parent-offspring connection had been registered (*Büscher* et al., 1994), and additional investigations were necessary to demonstrate the identity of the correct parent (*Dettweiler* et al., 2000).

Our aim was to prove the ancestry of the 'Királyleányka', which is an important cultivar in Hungary. According to *Németh* (1970) this natural hybrid is presumably the progeny of 'Leányka' and 'Kövérszőlő'.

Materials and methods

Plant material and DNA isolation: SSR analysis was carried out on 31 wine grape accessions. The samples, detailed below in *Table 2.*, were obtained from the cultivar-collections of Tarcal (T), Helvécia (H) and Pécs (P); two Furmint items were sampled from old plantations (Op) of

Table 2. Genotypes of the 31 analyzed accessions. Allele sizes are given in base pairs

Place of collecting	Locus / Cultivar	VVS2		VVMD5		VVMD7		VrZAG21		VVMD27		VrZAG62		VrZAG79	
T	Demjén	131	141	238	248	236	246	199	211	187	187	188	192	242	250
T	Kozma	133	141	224	236	238	238	199	203	177	177	200	200	242	258
T	Betyár	131	133	226	236	258	258	199	205	177	177	194	198	238	248
T	Balafánt	131	151	226	230	238	264	199	205	165	187	190	194	248	250
T	Juhfark	131	153	226	240	238	248	199	205	177	193	188	202	236	248
T	Arany furmint	131	151	224	238	236	246	201	207	177	191	188	204	236	246
T	Változó furmint	131	151	224	238	236	246	201	207	177	191	188	204	236	246
T	Red furmint	131	151	224	238	236	246	201	207	177	191	188	204	236	246
T	Furmint P.26	131	151	224	238	236	246	201	207	177	191	188	204	236	246
T	Furmint T. 85	131	151	224	238	236	246	201	207	177	191	188	204	236	246
Op	Furmint Unk. 1.	131	151	224	238	236	246	201	207	177	191	188	204	236	246
Op	Furmint Unk. 2.	131	151	224	238	236	246	201	207	177	191	188	204	236	246
T	Királyleányka	131	131	234	238	246	248	199	199	189	191	192	202	248	250
T	Kövérszőlő	131	143	226	238	238	254	199	205	177	191	196	204	248	258
T	Leányka	131	131	224	234	246	253	199	205	183	191	200	202	236	250
P	Mustos	141	141	230	238	246	248	199	205	179	191	204	204	242	258
H	Mustos fehér	141	141	230	238	246	248	199	205	179	179	204	204	242	248
T	Török gohér	131	151	236	236	238	248	201	205	179	191	188	204	248	258
P	Gohér fehér	131	151	236	236	238	248	201	205	179	191	188	204	248	258
P	Gohér piros	131	151	236	236	238	248	201	205	179	191	188	204	248	258
P	Gohér változó	131	151	236	236	238	248	201	205	179	179	188	204	248	258
T	Budai gohér	131	141	230	236	249	258	201	205	177	179	188	196	238	238
P	Bajor feketefájú	131	151	226	236	238	238	199	205	179	191	188	196	248	258
P	Bajor kék	131	151	226	236	238	238	199	205	179	179	188	196	248	258
P	Bajor szürke	131	151	226	236	238	238	199	205	179	191	188	196	248	258
T	Királyszőlő	131	131	224	230	236	236	199	199	191	191	186	194	246	246
T	Cukorszőlő	131	142	224	236	236	238	201	205	179	179	186	190	248	256
H	Sárfehér	131	151	226	230	236	244	199	205	187	191	190	198	246	248
H	Sárpiros	131	151	222	222	236	244	199	199	187	191	186	202	246	248
H	Fekete járdovány	131	151	222	232	238	248	199	205	177	191	168	200	246	248
H	Fehér járdovány	141	144	228	236	244	244	199	205	179	191	202	202	240	246

different well-known plots of land in the Tokaj wine-region. The DNA was extracted with the DNEasy® Plant Mini Kit (Qiagen- Biomarker Ltd. Gödöllő), according to the protocol of the manufacturer. Following the extraction, concentration and purity of the DNA were measured with NanoDrop™ ND-1000 spectrophotometer (NanoDrop Ltd.).

PCR conditions and SSR analysis: Peltier PTC-200 DNA Engine Thermal Cycler (MJ Research) was used to perform the PCR with 35 cycles of 45 sec. denaturation at 95 °C, 30 sec. annealing at 55 °C and 45 sec. extension at 72 °C. The following 7 fluorescent labeled microsatellite primer pairs (Biomi Ltd. Gödöllő) were used in this study: VrZAG62, VrZAG79, VVMD5, VVMD7, VVMD27, VvS2 and VrZAG21 (*Regner* et al., 2000). The fragment lengths analysis was carried out with ABI Prism 3000 fragment length analysator.

Statistical analysis: The statistical analyses of the data were carried out with the Identity 1.0 program, freely available on http://www.boku.ac.at/zag/forsch/identity.htm.

Pair wise distance matrices were obtained from allele lengths of the investigated loci based on the proportion of shared alleles using the Msat2 software with 1000 bootstrap replicates. From the replicated distance matrices UPGMA trees were drawn with the neighbor package and a consensus tree was calculated with the consense package of PHYLIP. The tree was finally drawn using drawtree of PHYLIP.

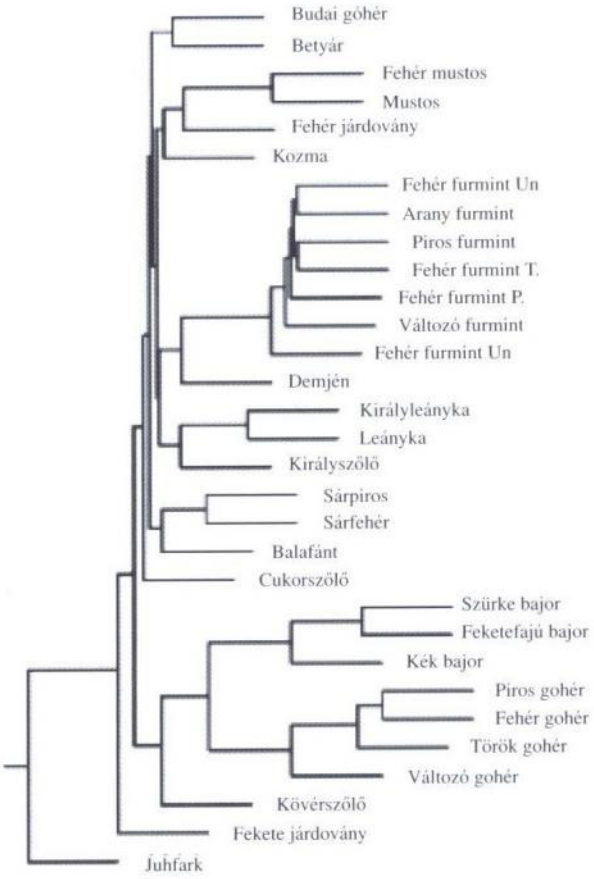

Figure 1 Dendrogram generated by the PHYLIP, depicting the genetic relationship among the 31 grapevine cultivars.

Results

Conculta members: Seven different 'Furmint' samples – three conculta-members (Változó, Piros and Fehér), one subcultivar of 'Furmint' (Arany), two clones (P.26, T.85) and two items from old plantations (marked as Unk. 1 and Unk. 2) – were collected for the separation and identification. The samples show no difference in any loci (*Table 2*). It means that this conculta differ from each other in the color of the berry, but no in these loci. The randomly-collected 'Furmint' samples proved the usefulness of the method, since these were identical to the other 'Furmint' samples and to each other. These results confirmed while samples of old plantations may differ in morphological characteristics, but can be identified with these molecular markers. The seven examined microsatellite loci were not variable enough to separate the different taxonomy clusters of 'Furmint' from each other (*Figure 1*).

Conculta members of 'Gohér' show homology at all loci except the 'Változó gohér', which was homozygote at the locus VVMD27. Interestingly the 'Blue bajor' – member of the Bajor conculta – was distinct from the other conculta members at the same locus (*Table 2*).

'Sárfehér' and 'Sárpiros'; 'Fekete járdovány' and 'Fehér járdovány' are not conculta members because several differences were found. They are probably homonyms with different berry color.

However *Regner* and colleagues (2000) could not separate the 'Pinot' conculta members from each other with 34 SSR. Our results suggest that the investigated bud sports differ only in the color of the berry. The investigated color variations could be differing in other region of the genome because of the mutation. These mutations could cause deletion/insertion or other movements in the coding and non-coding region. Our bud sports are mutated in the coding region, it is seen by the different berry color, and in the non-coding region, proved it the different fragment lengths of the microsatellite regions which are in the non-coding region as it can be known.

Hypothetically it can be concluded that all of the conculta members are bud sports of an ancient cultivar. In case of 'Pinot' conculta it was proved that 'Pinot noir' was the source of the mutation which originates the 'Pinot blanc' (*Yakushi* et al., 2006) for example. In some cases only the color of the berry mutated in such cases other part of the genome also.

Synonys among the old Hungarian cultivars: Our results suggest that 'Gohér fehér' and 'Török gohér' are synonyms; these two names refer one cultivar. 'Gohér piros' also has the same genetical background, but it can be differentiated morphologically with the berry color. 'Budai gohér' is an individual cultivar; it differed at several loci from the 'Gohér' group of cultivars.

All of the cultivars with the synonym "Budai gohér", which was the most frequent name (*Table 1*), can be separated from the individual cultivar 'Budai gohér'. This result confirmed our morphological examinations, that this

was not the member of the 'Gohér' conculta. It is only an identity of names without significant genetical similarity.

Parent-offspring connections: Inconsistent with the morphology-based theory (*Németh*, 1970) the SSR results showed that 'Királyleánka' could not be the offspring of 'Kövérszőlő', but the paternity of the 'Leánka' is still possible. These results were reported also by *Kiss* et al. (2006), but possible parents were not given by the authors. Because of the morphological characteristics of the 'Királyleánka' the missing ancestor had to be chosen from the convarietas (proles) pontica. With the application of databases and results of earlier investigations (*Halász* et al., 2005) only one possible parent ('Mustos' a. k. a. 'Mustoasa de Maderat') was found which is already investigated. Two samples, 'Mustos' and 'Fehér mustos' (which were mentioned as synonyms), were analysed. The result of our investigations at the 7 loci showed, that neither of the samples had the correct genetic background to be considered as a parent of 'Királyleánka'. Autochthon Transylvanian cultivars can be the objects of the further studies, because it was the place of origin of the natural hybrid.

The two 'Mustos' samples did not match at the loci VVMD27 and VrZAG79. Interestingly the members inside 'Bajor' and 'Gohér' concultas were separated at the same locus (VVMD27). Probably it refers that 'Mustos' samples also form a group of cultivars. Additional morphological and molecular investigations are needed to prove it.

Results showed that 'Gohér' and 'Bajor' cultivars can be at close relation with each other. According to the fragment lengths of the two cultivars at the seven loci the parent-offspring connection is probable.

Results of statistical analysis: The statistical analysis clears questions which were questionable before. According to *Balassa* (1991) ancestry can be between the *cv.* Demjén first mentioned at 1632 – and Kozma. The names of the cultivars were given by St. Kosma and his twin brother Damian. Results show that (Figure) these two cultivars are far from each other and *cv.* 'Kozma' is an individual cultivar and can not be connected to any other investigated cultivar.

The 'Gohér' and 'Bajor' conculta also make an individual group from the other conculta members and from the other cultivars. But these two conculta are close to each other.

The difference between the two 'Mustos' samples are higher than in case the other concultas. It can be explained with mutation which causes the different color variations, can because other differences in the genome, also in the non-coding region.

Discussion

Nowadays old grapevine cultivars started to be in the observed of several investigations. These cultivars took important part of the vineyards in the Carpathian Basin before the *Phylloxera* crisis (*Balassa*, 1991). Some of them can be found only in cultivar collections or in old plantations. For the separation and identification of these autochthonous cultivars it is necessary to use molecular genetic method beside ampelography.

The result of the study showed there can be molecular differences inside concultas (color variations) such as: between the members of 'Gohér', 'Bajor'.

The synonyms of the most important cultivar from Tokaj were separated from each other, while the molecular background of the conculta members, subcultivars and clones of 'Furmint' were matching. However the randomly collected samples of old plantations could be identified.

Close relation between the 'Gohér' and 'Bajor' conculta was found. Our investigations confuted the morphology-based theory about the pedigree of 'Királyleánka' but the missing parent was not found.

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VIVC- Vitis International Variety Catalogue- http://www.vivc.bafz.de/index.php (Last access: 2008.07.28.)