Vitis **48** (1), 17–24 (2009)

Identification of grapevine cultivars using microsatellite-based DNA barcodes

Z. Galbács[1)], S. Molnár[1)], G. Halász[1), 2)], P. Kozma[3)], S. Hoffmann[3)], L. Kovács[4)], A. Veres[1)], Z. Galli[1)], A. Szőke[1)], L. Heszky[1)] and E. Kiss[1)]

[1)] Szent István University, Institute of Genetics and Biotechnology, Gödöllő, Hungary
[2)] Agricultural Biotechnology Centre, Gödöllő, Hungary
[3)] Research Institute of Viticulture and Enology, Pécs, Hungary
[4)] Missouri State University, Department of Agriculture, Mountain Grove, USA

Summary

Microsatellite allele sizes were determined at twelve loci in 115 grape genotypes, including 88 ancient *Vitis vinifera* L. cultivars indigenous to the Carpathian Basin. Pairwise comparison of the microsatellite data led to the identification of ambiguous nomenclature and established differences between two grapevine cultivars, Leányka and Leányszőlő, previously thought of as identical. The data also disproved erroneous assumptions about the parentage of two additional cultivars, one of which was 'Csabagyöngye' ('Pearl of Csaba'), an economically important grapevine and a renowned genetic resource for grape breeding. The results also pointed to several possible parent-progeny relationships which will be subject to further pedigree studies. The DNA typing information was used to construct a barcode system which was incorporated into the Hungarian *Vitis* Microsatellite Database for efficient and unambiguous identification of grape genotypes, thereby increasing the precision with which genetic resources are managed in Hungary.

K e y w o r d s : *Vitis vinifera*, microsatellite, SSR, genotype, DNA barcode, cultivar, Carpathian Basin.

Introduction

Microsatellite or simple sequence repeat (SSR) loci (Thomas and Scott 1993) serve as the most widely used markers in grape genomics. They facilitate the application of novel approaches, among them, the construction of marker-based genetic linkage maps (Doligez *et al.* 2006, Di Gaspero *et al.* 2007), the localization of genes of interest (Hoffmann *et al.* 2008), and marker-assisted selection (Eibach *et al.* 2007). As most are transferable among grape genotypes, SSR markers can be used to reliably identify cultivars (This *et al.* 2004), clarify nomenclature (Maletic *et al.* 2004), and trace the parentage or origin of economically important cultivars (Bowers *et al.* 1999, Tapia *et al.* 2007).

In a previous study, we used six microsatellite loci to characterize 97 ancient *Vitis vinifera* cultivars indigenous to the Carpathian Basin (Halász *et al.* 2005). As other regions where grapes have been cultivated since antiquity, the Carpathian Basin was the site of gene pool and germplasm blending, resulting from the importation of diverse genotypes and the selection of spontaneous hybrids (Hajdu 2003). Hybridizations between genotypes have only been documented since the end of the 19th century when deliberate crosses were first made. However, parentage information even for breeding-selected cultivars remains often anecdotal (Hajdu 2003). Furthermore, the spread of cultivars within and outside the Carpathian Basin gave rise to a nomenclature which is highly unreliable for genotype identification. For example, the cultivar Furmint, which produces the famed Tokaj wine of Hungary, was known by eleven synonyms in various languages (Goethe 1887).

Genotyping of indigenous grapevines of the Carpathian Basin is warranted, as many of these cultivars are economically important, while others serve as genetic resources in local or foreign breeding programs. In the present study, we extended our earlier DNA typing work by using twelve SSR loci and including an extensive and diverse assortment of reference genotypes, among them grapevines indigenous to other regions of Europe and Central Asia, as well as several new cultivars from Hungarian breeding programs. The results presented here refuted erroneous assumptions about the parentage of two important cultivars and set the direction for future pedigree studies for others.

Material and Methods

P l a n t m a t e r i a l a n d D N A e x t r a c t i o n : The 115 grapevine accessions used in this study are listed in Tabs 2 and 3. Young leaf samples of these grapevines were collected from the germplasm repository of the Research Institute for Viticulture and Enology at Pécs, Hungary. Genomic DNA extraction, polymerase chain reactions (PCRs) and allele size analysis were carried out as previously described by Halász *et al.* (2005).

M i c r o s a t e l l i t e a n a l y s i s : The twelve SSR markers applied in this study were as follows: Scu08vv, Scu10vv (Scott *et al.* 2000), VVMD21, VVMD25, VVMD28, VVMD31, VVMD36 (Bowers *et al.* 1996, 1999) ssrVrZAG47, ssrVrZAG62, ssrVrZAG79, ssrVrZAG83, ssrVrZAG112 (Sefc *et al.* 1999). Markers ssrVrZAG62, ssrVrZAG79, VVMD25, VVMD28 were from among the list of markers recommended by the European GenRes#81 and GrapeGen06 projects (http://www.montpellier.inra.fr/grapegen06/accueil.php) for the characterization of regional cultivars of Europe. Allele size determination was

Correspondence to: Dr. E. Kiss, Szent István University, Institute of Genetics and Biotechnology, Páter Károly str 1., Gödöllő, 2100, Hungary. E-mail: kiss.erzsebet@mkk.szie.hu

performed using Cy5 dye-labelled forward primers and ALFexpress II DNA Fragment Analyzer (Amersham Biosciences, Little Chalfont, England, UK) as previously described by HALÁSZ *et al.* (2005).

Statistical analysis: Allele frequencies, expected and observed heterozygosity, probable identity, and parentage were calculated using the Identity 1.0 software (WAGNER and SEFC 1999). DNA barcodes were constructed using the Microsoft Excel 2003 software.

Results and Discussion

Microsatellite allele sizes were determined at twelve loci in 115 grape cultivars. The numbers of different alleles at each of the twelve loci are listed in Tab. 1. The highest and lowest variability in allele size were obtained at loci VVMD36 (17 different allele sizes), and Scu08vv loci (4 different allele sizes), respectively. For 11 of the SSR loci, both the expected and the observed heterozygosity values were high, ranging from 0.696 to 0.888 and from 0.678 to 0.913, respectively (Tab. 1). An exception to this was the Scu08vv locus, which had very low heterozygosity values (0.197 to 0.182). The estimated frequency of null alleles was low, ranging from 0.005 to 0.04. The total probability of identity for the twelve loci was 1.94 x 10^{-10}. Tab. 2 presents allele size data for cultivars indigenous to the Carpathian Basin, while Tab. 3 presents allele size data for cultivars of world-wide economic importance, grapevines indigenous to other regions of Europe and Central Asia, as well as grapevines of known parentage from Hungarian breeding programs. Pairwise comparison of the combined 2,760 SSR data demonstrated that most grape genotypes could be distinguished by allele size at the twelve loci. Most importantly, the data showed that 'Leányka' and 'Leányszőlő' are different genotypes, refuting the commonly held assumption that these cultivar names were synonymous (Tab. 2). Two other cultivars, 'Betyárszőlő' and 'Fodroslevelű', on the other hand, did appear to be synonymous. This synonymy was unexpected, because these grapevines were described as two different cultivars as early as in the 13th century (CSOMA 1994-95). It will require further clarification to determine if the two names indeed denote an identical genotype, or if these results reflect an error in the records of the germplasm repository at Pécs, Hungary.

Other indistinguishable genotypes were the red/white berry colour variant pairs of 'Gohér piros'/'Gohér fehér' and 'Lisztes piros'/'Lisztes fehér' and the red/rouge variant pairs of 'Bakator piros'/'Bakator tüdőszinű' and 'Piros muskotály'/'Sárga muskotály' (Tab. 2). The identical sizes of all 12 SSR alleles within the pairs suggest that these berry skin colour variants likely arose as white-to-red bud mutations, similarly to 'Ruby Okuyama' and 'Flame Muscat' which are red-skin sports of 'Italia' and 'Muscat of Alexandria' (KOBAYASHI *et al*. 2005). These eight colour variants from the Carpathian Basin present valuable material for further genetic studies on vegetative mutations in grapevine.

The results also revealed that, in the case of cultivar 'Bakator kék', the name 'Bakator' is a homonym. It was a commonly held assumption that 'Bakator kék' was a berry colour variant of 'Bakator piros' and 'Bakator tüdőszinű'. The former, however, did not share the same size SSR alleles as the latter two (Tab. 2). The detection of these nomenclatorial errors demonstrates the usefulness of SSR-based DNA typing in improving the precision of cultivar identification and the quality of grapevine collections.

The DNA typing information also enabled us to search for possible genetic relatedness among the cultivars. Although data for twelve loci provide insufficient information to conclusively demonstrate parent-progeny relationships, we putatively identified 32 combinations that might represent such relatedness (data not shown). These possibilities will need to be confirmed with additional SSR markers in future experiments. We also included in this study three reference genotypes for which the parent-progeny relationships were known either from breeding records

Table 1

Salient features of the 12 microsatellite loci used in the analysis

Locus	Number of alleles (n)	Range of allele sizes (bp)	H_e[a]	H_o[a]	Estimated frequency of null alleles	PE[a]	PI[a]
Scu8vv	4	185-197	0.197	0.182	0.012	0.095	0.685
Scu10vv	6	202-217	0.722	0.791	0.040	0.486	0.210
VrZAG47	9	155-174	0.802	0.852	0.027	0.611	0.124
VrZAG62	8	180-207	0.764	0.773	0.005	0.551	0.162
VrZAG79	11	240-264	0.830	0.852	0.011	0.671	0.086
VrZAG83	6	191-214	0.696	0.730	0.020	0.450	0.238
VrZAG112	9	232-266	0.776	0.800	0.013	0.572	0.145
VVMD21	7	230-267	0.707	0.678	0.017	0.471	0.219
VVMD25	7	241-259	0.750	0.739	0.006	0.509	0.198
VVMD28	11	218-278	0.888	0.913	0.012	0.774	0.043
VVMD31	9	193-221	0.752	0.721	0.017	0.543	0.161
VVMD36	17	244-296	0.838	0.817	0.011	0.683	0.082
Total	104					1.90 x 10^{-4}	2.11 x 10^{-10}
Average	8.6		0.726	0.789	0.016		

Table 2

Microsatellite characterization of the varieties indigenous in the Carpathian Basin

Cultivar	Allele size (bp) in microsatellite locus											
	Scu8vv*	Scu10vv*	Vrzag47	VrZAG62	VrZAG79*	Vrzag83	Vrzag112	VVMD21*	VVMD25	VVMD28	VVMD31	VVMD36*
Alanttermő	*185:185*	*202:208*	**174:174**	**191:207**	*254:260*	**191:197**	**237:243**	*250:259*	**253:259**	**228:236**	**209:211**	*254:276*
Aprófehér	*185:185*	*208:214*	**167:169**	**191:207**	*246:254*	**193:197**	**237:237**	*250:250*	**253:259**	**248:260**	**209:209**	*264:266*
Ágasfark	*185:192*	*202:202*	**161:174**	**191:199**	*252:262*	**191:193**	**245:245**	*244:250*	**243:259**	**236:248**	**209:209**	*254:264*
Bakarka	*185:185*	*214:214*	**165:167**	**191:207**	*254:254*	**193:197**	**237:241**	*244:250*	**243:245**	**248:260**	**207:221**	*264:266*
Bakator kék	*185:185*	*202:208*	**159:174**	**199:203**	*252:262*	**193:197**	**243:245**	*250:250*	**243:259**	**234:248**	**209:209**	*264:264*
Bakator piros	*185:185*	*202:208*	**159:165**	**191:197**	*254:254*	**191:197**	**237:241**	*244:257*	**245:245**	**234:248**	**201:209**	*266:288*
Bakator tüdőszínű	*185:185*	*202:208*	**159:165**	**191:197**	*254:254*	**191:197**	**237:241**	*244:257*	**245:245**	**234:248**	**201:209**	*266:288*
Bakszem	*185:192*	*202:208*	**161:165**	**207:207**	*240:262*	**193:203**	**232:239**	*250:250*	**243:245**	**228:278**	**207:211**	*252:264*
Balafánt	*185:192*	*202:208*	**159:174**	**207:207**	*240:254*	**191:197**	**232:243**	*244:259*	**245:253**	**228:248**	**207:211**	*276:288*
Bálint	*185:192*	*208:214*	**169:174**	**191:207**	*252:254*	**191:193**	**241:243**	*250:259*	**245:253**	**228:236**	**201:207**	*264:276*
Bánáti rizling	*185:185*	*208:211*	**159:161**	**191:207**	*254:262*	**191:203**	**237:243**	*250:257*	**245:253**	**236:236**	**209:209**	*254:288*
Beregi	*185:185*	*208:214*	**161:163**	**191:197**	*254:262*	**191:193**	**237:243**	*244:250*	**243:245**	**236:268**	**209:209**	*254:288*
Betyárszőlő	*185:185*	*202:214*	**159:161**	**197:203**	*262:262*	**191:197**	**232:237**	*250:257*	**245:259**	**246:260**	**201:207**	*264:266*
Bihari	*185:185*	*202:205*	**161:165**	**191:203**	*250:262*	**191:191**	**237:245**	*250:250*	**243:253**	**248:258**	**209:209**	*264:264*
Bogdányi dinka	*185:185*	*214:214*	**161:165**	**191:199**	*254:262*	**191:191**	**237:237**	*244:250*	**243:243**	**234:260**	**209:221**	*264:266*
Bőségszaru	*185:185*	*202:205*	**169:174**	**191:207**	*248:252*	**191:203**	**237:243**	*244:250*	**245:245**	**228:236**	**207:211**	*276:296*
Cukorszőlő	*185:185*	*202:208*	**159:161**	**191:191**	*254:262*	**191:203**	**237:241**	*257:257*	**243:253**	**228:236**	**209:209**	*254:276*
Csiri-csuri	**185:185**	**214:217**	**165:174**	**191:197**	**254:260**	**191:197**	**232:245**	**250:250**	**245:259**	**234:268**	**211:213**	**264:276**
Csókaszőlő	*185:185*	*202:208*	**159:165**	**207:207**	*240:254*	**197:197**	**237:237**	*257:257*	**245:245**	**234:260**	**207:207**	*288:288*
Csomorika	*185:185*	*208:211*	**159:161**	**191:207**	*240:262*	**191:197**	**237:243**	*257:257*	**243:243**	**234:234**	**209:211**	*288:288*
Czeiger	*185:185*	*202:208*	**165:174**	**203:207**	*254:254*	**191:197**	**237:245**	*250:250*	**245:259**	**248:268**	**207:209**	*264:288*
Demjén	*185:185*	*202:202*	**159:161**	**191:199**	*254:262*	**191:193**	**237:243**	*244:257*	**243:253**	**234:248**	**207:209**	*254:288*
Erdei	*185:185*	*202:214*	**165:174**	**197:207**	*246:254*	**191:197**	**237:243**	*244:250*	**245:259**	**248:258**	**201:211**	*264:264*
Ezerjó	*185:185*	*202:202*	**159:165**	**191:191**	*240:254*	**191:203**	**232:237**	*244:250*	**245:253**	**228:278**	**209:213**	*258:276*
Fodroslevelű	*185:185*	*202:214*	**159:161**	**197:203**	*262:262*	**191:197**	**232:237**	*250:257*	**245:259**	**246:260**	**201:207**	*264:266*
Furmint	*185:192*	*202:208*	**159:174**	**191:207**	*240:252*	**191:191**	**243:245**	*250:259*	**243:245**	**228:248**	**209:211**	*254:276*
Fügeszőlő	*185:192*	*208:208*	**159:174**	**199:207**	*240:252*	**191:193**	**237:243**	*244:244*	**243:243**	**234:268**	**209:211**	*264:288*
Fürjmony	*185:192*	*205:208*	**159:165**	**203:207**	*250:254*	**191:197**	**237:237**	*250:257*	**253:259**	**244:258**	**207:209**	*254:264*
Gergely	*185:185*	*208:214*	**165:174**	**191:207**	*240:254*	**197:203**	**237:243**	*244:250*	**243:259**	**248:260**	**207:211**	*266:276*
Gohér fehér	*185:192*	*202:208*	**161:174**	**191:207**	*252:262*	**193:193**	**241:243**	*244:257*	**243:245**	**234:248**	**207:211**	*254:288*
Gohér piros	*185:192*	*202:208*	**161:174**	**191:207**	*252:262*	**193:193**	**241:243**	*244:257*	**243:245**	**234:248**	**207:211**	*254:288*
Gohér változó	*185:192*	*202:208*	**161:174**	**191:207**	*252:262*	**193:193**	**241:243**	*244:257*	**243:245**	**234:248**	**207:211**	*254:288*
Gorombaszőlő	*185:185*	*208:214*	**165:174**	**191:197**	*252:252*	**191:193**	**241:245**	*250:259*	**243:245**	**248:260**	**209:209**	*254:266*
Halápi	*188:188*	*208:217*	**159:174**	**189:199**	*252:258*	**191:193**	**237:241**	*244:267*	**253:259**	**236:246**	**209:213**	*244:254*
Hamuszőlő	*185:185*	*208:208*	**169:171**	**191:191**	*248:254*	**191:193**	**237:243**	*250:250*	**253:253**	**234:258**	**201:209**	*264:276*
Izsáki	*185:185*	*208:214*	**159:169**	**191:207**	*240:246*	**197:203**	**237:245**	*244:250*	**245:259**	**236:258**	**209:211**	*254:276*
Járdovány	*185:185*	*208:214*	**159:165**	**199:207**	*240:254*	**197:197**	**245:245**	*244:250*	**243:245**	**228:260**	**209:209**	*266:276*
Juhfark	*185:185*	*208:208*	**159:174**	**199:207**	*240:252*	**191:193**	**243:245**	*250:257*	**245:259**	**248:248**	**207:209**	*264:276*
Kadarka	*185:185*	*208:214*	**165:174**	**191:207**	*252:252*	**191:193**	**237:241**	*250:250*	**243:259**	**228:260**	**207:209**	*266:276*
Kéklő piros	*185:185*	*202:208*	**161:161**	**197:197**	*252:262*	**191:191**	**232:232**	*250:257*	**243:245**	**228:246**	**201:209**	*264:270*
Királyleányka	*185:185*	*208:214*	**174:174**	**197:207**	*252:254*	**191:191**	**232:243**	*244:250*	**243:253**	**228:260**	**201:209**	*254:266*
Királyszőlő	*185:185*	*202:208*	**163:174**	**207:207**	*254:262*	**193:197**	**237:241**	*250:259*	**245:259**	**234:260**	**207:207**	*266:288*
Kolontár	*185:192*	*202:208*	**159:174**	**199:207**	*252:262*	**193:203**	**232:243**	*244:250*	**245:253**	**244:248**	**209:211**	*254:264*
Kovácskréger	*185:185*	*202:211*	**159:159**	**191:191**	*252:254*	**191:191**	**241:245**	*250:257*	**243:243**	**236:248**	**209:209**	*254:264*
Kozma	*185:185*	*202:208*	**159:161**	**191:199**	*262:262*	**193:193**	**232:243**	*257:267*	**243:253**	**236:248**	**207:209**	*254:264*
Ködös	*185:185*	*208:208*	**165:174**	**197:207**	*252:252*	**191:193**	**241:245**	*257:259*	**245:259**	**228:248**	**207:209**	*254:276*
Kövérszőlő	*185:185*	*208:208*	**159:174**	**199:207**	*240:254*	**191:197**	**237:243**	*250:259*	**243:245**	**246:260**	**207:211**	*264:266*
Kövidinka	*185:185*	*208:214*	**161:167**	**191:197**	*254:262*	**191:197**	**237:245**	*244:250*	**245:259**	**234:248**	**209:221**	*264:264*
Lányszőlő	*185:185*	*208:211*	**159:174**	**191:191**	*252:254*	**191:191**	**237:243**	*250:257*	**243:253**	**228:236**	**209:209**	*254:276*

Tab. 2 continued

Cultivar	Allele size (bp) in microsatellite locus											
	Scu8vv*	Scu10vv*	Vrzag47	VrZAG62	VrZAG79*	Vrzag83	Vrzag112	VVMD21*	VVMD25	VVMD28	VVMD31	VVMD36*
Lágylevelű	*185:185*	*202:214*	**174:174**	**191:197**	*252:254*	**191:197**	**237:245**	*250:250*	**243:253**	**236:236**	**201:209**	*254:254*
Leányka	*185:185*	*202:208*	**165:174**	**195:197**	*240:254*	**191:197**	**232:243**	*250:250*	**253:259**	**248:260**	**201:207**	*266:276*
Lisztes fehér	*185:185*	*208:208*	**159:161**	**207:207**	*240:262*	**193:197**	**241:243**	*250:257*	**243:245**	**234:248**	**207:211**	*276:288*
Lisztes piros	*185:185*	*208:208*	**159:161**	**207:207**	*240:262*	**193:197**	**241:243**	*250:257*	**243:245**	**234:248**	**207:211**	*276:288*
Magyarka	*185:192*	*208:208*	**163:174**	**191:207**	*248:254*	**191:191**	**232:237**	*244:250*	**243:259**	**260:278**	**207:207**	*264:288*
Mézes fehér	*185:192*	*208:214*	**161:174**	**191:207**	*254:262*	**197:197**	**237:243**	*250:257*	**245:259**	**248:260**	**207:207**	*266:276*
Mustos	*185:185*	*208:214*	**161:174**	**207:207**	*246:252*	**191:191**	**232:245**	*244:250*	**245:259**	**248:258**	**207:211**	*254:276*
Pettyesszőlő	*185:185*	*202:208*	**169:174**	**199:207**	*250:252*	**191:193**	**237:241**	*244:244*	**253:253**	**236:248**	**209:211**	*254:288*
Pécsi dinka	**185:185**	**202:208**	**163:174**	**191:199**	**252:254**	**191:193**	**237:241**	**244:244**	**245:245**	**236:236**	**209:209**	**254:288**
Pécsi szagos	**185:185**	**208:211**	**159:159**	**189:191**	**254:258**	**191:191**	**237:237**	**257:267**	**243:245**	**234:268**	**209:209**	**264:288**
Pintes	*185:185*	*202:205*	**155:169**	**191:197**	*248:250*	**191:203**	**237:237**	*250:250*	**243:253**	**236:244**	**201:207**	*254:272*
Piros gránát	*185:185*	*208:214*	**159:169**	**191:199**	*250:254*	**191:191**	**237:241**	*244:250*	**253:259**	**236:268**	**207:221**	*254:264*
Piros muskotály	*185:185*	*208:217*	**159:174**	**189:199**	*254:258*	**191:191**	**237:237**	*250:267*	**245:253**	**246:268**	**209:213**	*244:264*
Piros tökös	*185:185*	*202:214*	**163:163**	**197:207**	*252:254*	**191:191**	**237:245**	*244:250*	**243:245**	**228:268**	**211:211**	*276:288*
Polyhos	*185:185*	*202:202*	**163:174**	**191:199**	*252:262*	**191:197**	**232:243**	*244:259*	**243:245**	**234:248**	**209:209**	*254:288*
Pozsonyi	*185:192*	*202:214*	**161:174**	**207:207**	*254:254*	**191:197**	**237:245**	*244:259*	**245:259**	**268:278**	**207:211**	*264:264*
Purcsin	*185:185*	*208:214*	**159:169**	**191:197**	*250:258*	**191:197**	**237:243**	*250:250*	**253:253**	**236:258**	**201:209**	*254:276*
Rakszőlő	*185:185*	*208:214*	**159:165**	**199:199**	*254:254*	**191:197**	**237:245**	*244:244*	**243:243**	**236:260**	**209:221**	*254:266*
Rókafarkú	*185:185*	*208:214*	**159:161**	**203:207**	*240:246*	**191:193**	**237:241**	*250:250*	**243:245**	**246:248**	**207:211**	*264:276*
Rózsaszőlő	**185:185**	**208:208**	**163:169**	**197:199**	**246:254**	**191:197**	**237:237**	**244:250**	**259:259**	**248:268**	**211:221**	**264:264**
Sárfehér	*185:192*	*202:208*	**174:174**	**207:207**	*252:254*	**191:193**	**245:245**	*244:250*	**245:259**	**248:278**	**207:207**	*264:264*
Sárga muskotály	**185:185**	**208:217**	**159:174**	**189:199**	**254:258**	**191:191**	**237:237**	**250:267**	**245:253**	**246:268**	**209:213**	**244:264**
Sárpiros	*185:185*	*202:208*	**159:159**	**191:207**	*254:260*	**193:193**	**237:245**	*244:244*	**243:259**	**268:278**	**207:209**	*264:288*
Somszőlő	*185:185*	*202:214*	**161:161**	**199:203**	*252:254*	**193:197**	**232:243**	*244:250*	**243:253**	**244:258**	**193:209**	*252:256*
Szagos bajnár	*185:185*	*205:208*	**161:165**	**191:207**	*250:262*	**197:197**	**237:241**	*250:250*	**243:253**	**234:258**	**207:211**	*264:288*
Szekszárdi	**185:192**	**202:208**	**169:174**	**191:207**	**252:254**	**191:191**	**232:245**	**244:250**	**245:253**	**248:278**	**207:209**	**264:264**
Szeredi	*185:185*	*202:202*	**163:174**	**199:199**	*252:252*	**191:193**	**243:245**	*250:257*	**243:259**	**228:236**	**209:209**	*254:276*
Szerémi	*185:185*	*202:208*	**159:174**	**191:207**	*252:258*	**191:191**	**237:243**	*250:250*	**243:253**	**228:258**	**207:209**	*276:276*
Szőkeszőlő	*185:185*	*202:208*	**159:174**	**191:191**	*254:260*	**191:197**	**239:239**	*244:250*	**243:259**	**244:244**	**209:213**	*272:276*
Tótika	*185:185*	*202:214*	**165:174**	**199:207**	*252:254*	**191:193**	**237:241**	*250:257*	**245:259**	**228:236**	**207:207**	*254:276*
Tökszőlő	*185:185*	*208:214*	**159:159**	**203:207**	*240:262*	**191:193**	**232:241**	*257:257*	**245:245**	**246:248**	**207:211**	*264:276*
Tulipiros	*185:185*	*208:208*	**163:174**	**191:207**	*252:254*	**191:193**	**237:241**	*244:244*	**243:245**	**234:248**	**207:209**	*254:288*
Tükörszőlő	*185:185*	*202:214*	**161:161**	**199:207**	*246:262*	**191:193**	**243:245**	*250:250*	**243:243**	**236:246**	**209:211**	*254:264*
Ürömi dinka	*185:185*	*214:214*	**159:165**	**191:207**	*246:254*	**193:203**	**237:237**	*250:250*	**243:259**	**258:260**	**207:209**	*266:276*
Vékonyhéjú	*185:185*	*202:208*	**161:161**	**191:207**	*246:262*	**191:191**	**243:245**	*250:250*	**243:243**	**228:248**	**209:211**	*264:276*
Vörösdinka	*185:185*	*208:214*	**159:161**	**191:199**	*254:262*	**191:193**	**237:245**	*244:250*	**243:245**	**236:236**	**209:221**	*254:264*
Zöld dinka	*185:185*	*202:208*	**159:174**	**191:207**	*254:254*	**191:191**	**237:245**	*244:257*	**243:243**	**234:234**	**209:211**	*264:264*

*Allele size data for these markers in italics were published previously by HALÁSZ et al. (2005) and are available in the Hungarian Microsatellite Database (http://mkk.szie.hu/dep/gent/).

Table 3

Microsatellite characterization of reference grape cultivars

Cultivar	Allele size (bp) in microsatellite locus											
	Scu8vv*	Scu10vv*	VrZAG47	VrZAG62	VrZAG79*	VrZAG83	VrZAG112	VVMD21*	VVMD25	VVMD28	VVMD31	VVMD36*
Cultivars of worldwide significance												
Cardinal	185:192	208:214	**159:165**	**189:189**	254:258	**191:197**	**237:245**	250:267	**259:259**	**248:268**	**211:215**	252:262
Chardonnay	185:192	205:214	**161:169**	**191:199**	246:248	**191:203**	**243:243**	250:250	**243:259**	**218:228**	**211:213**	254:276
Chasselas rouge	185:185	205:214	**165:169**	**197:207**	254:262	**193:203**	**243:245**	250:267	**245:259**	**218:268**	**209:213**	264:296
Grenache	185:192	205:205	**159:174**	**189:189**	258:258	**191:208**	**232:232**	267:267	**245:259**	**244:260**	**211:218**	262:268
Merlot	185:185	202:217	**165:167**	**197:197**	260:260	**197:203**	**232:245**	244:248	**243:253**	**228:234**	**209:213**	252:252
Muscat of Alexandria	185:185	208:208	**159:174**	**189:207**	250:258	**191:191**	**237:251**	257:267	**253:253**	**244:268**	**213:221**	254:264
Muscat Ottonel	*185:185*	*208:214*	**159:169**	**191:197**	*258:262*	**193:203**	**237:245**	*267:267*	**253:259**	**258:268**	**209:213**	*264:276*
Oportó	185:185	202:208	**161:174**	**191:207**	252:262	**193:197**	**232:243**	250:259	**253:253**	**228:260**	**201:207**	264:276
Pinot noir	*185:192*	*205:217*	**165:169**	**191:197**	*242:248*	**191:203**	**243:245**	*250:250*	**243:253**	**218:236**	**213:213**	*254:254*
Superior Seedless	185:185	208:214	**159:161**	**189:191**	258:262	**191:197**	**237:237**	244:250	**159:259**	**234:248**	**209:209**	264:276
Regional European cultivars												
Aubin	185:185	202:202	**169:174**	**197:207**	252:254	**203:214**	**232:241**	244:257	**241:247**	**258:258**	**213:213**	248:262
Bronnerstraube	185:185	202:214	**165:169**	**197:207**	248:254	**191:193**	**243:245**	250:250	**259:259**	**218:234**	**209:213**	244:296
Heunisch weiss	*185:185*	*208:214*	**159:161**	**199:207**	*240:246*	**191:197**	**243:245**	*250:250*	**243:259**	**228:246**	**209:211**	*264:276*
Madeleine Angevine	185:185	202:205	**161:174**	**197:207**	252:262	**193:203**	**234:243**	244:250	**245:259**	**218:244**	**209:221**	264:296
Muscat Lierval	185:185	205:211	**159:169**	**191:207**	258:260	**193:193**	**237:243**	257:267	**245:245**	**218:258**	**209:213**	276:288
Central Asian cultivars												
Afuz Ali	185:192	208:214	**165:165**	**180:189**	246:254	**191:197**	**245:245**	257:257	**253:259**	**234:258**	**201:209**	270:270
Dzsandzsal kara**	185:185	202:208	**159:174**	**191:207**	250:264	**193:193**	**237:266**	250:257	**243:243**	**218:234**	**209:209**	250:250
Icskimar	185:185	208:208	**165:174**	**197:199**	252:262	**197:208**	**237:266**	244:250	**253:259**	**234:244**	**207:207**	270:270
Iszpiszár	185:185	205:208	**174:174**	**191:191**	254:260	**197:208**	**237:237**	250:257	**249:259**	**244:244**	**209:209**	270:270
Kishmish vatkana	185:192	202:208	**159:174**	**191:207**	250:262	**191:208**	**237:266**	250:257	**243:243**	**218:234**	**209:209**	250:250
Nimrang	185:185	202:208	**165:174**	**191:199**	254:258	**191:197**	**232:237**	250:257	**253:259**	**234:244**	**207:207**	268:276
Cultivars from Hungarian breeding programs												
Bianka	185:185	205:208	**165:169**	**197:197**	242:264	**191:193**	**232:243**	230:250	**245:253**	**218:236**	**211:213**	254:270
Csabagyöngye	*185:185*	*205:214*	**159:161**	**189:207**	*258:262*	**191:203**	**243:243**	*244:267*	**245:245**	**218:268**	**213:221**	*264:296*
Ezerfürtű	185:185	205:208	**159:169**	**191:197**	240:248	**191:191**	**232:237**	244.250	**245:253**	**234:248**	**211:213**	264:264
Favorit	185:185	205:208	**159:165**	**189:197**	242:254	**191:193**	**243:243**	267:267	**259:259**	**218:234**	**209:213**	276:296
Irsai Olivér	185:185	205:214	**159:161**	**207:207**	254:258	**197:203**	**243:245**	244:244	**245:259**	**218:268**	**211:221**	264:296
Kossuth	185:185	205:208	**161:161**	**207:207**	262:262	**191:203**	**234:237**	244:257	**259:259**	**244:246**	**209:209**	264:288
Mátrai muskotály	185:185	214:214	**159:159**	**191:191**	240:258	**197:203**	**237:237**	244:267	**259:259**	**258:268**	**209:209**	264:276
Szőlőskertek királynője	185:185	208:214	**159:165**	**189:207**	254:258	**191:197**	**243:245**	257:267	**245:259**	**234:268**	**209:213**	264:276

* Allele size data for these markers in italics were published previously by HALÁSZ *et al.* (2005) and are available in the Hungarian Microsatellite Database (http://mkk.szie.hu/dep/gent/).

** Allele size discrepancy exists between this (Pécs, Hungary) and other Dzsandzsal kara accessions.

('Irsai Olivér', 'Mátrai muskotály') or from previous SSR-based pedigree studies ('Chardonnay'). Our results provided unequivocal confirmation of the 'Muscat Ottonel' x 'Izsáki' parentage of 'Mátrai muskotály' (Hajdu 2003, Kiss *et al.* 2005), the 'Pozsonyi fehér' x 'Csabagyöngye' parentage of 'Irsai Olivér' (Hajdu 2003, Kiss *et al.* 2005), and the 'Gouais blanc' x 'Pinot noir' parentage of 'Chardonnay' (Bowers *et al.*1999) (Tab. 2).

While allele size data at twelve SSR loci are insufficient to conclusively demonstrate parent-progeny relationships, they are adequate to disprove such relationships. Thus, the results presented here enabled us to reject the hypothesis that 'Királyleánka' resulted from a spontaneous cross between 'Kövérszőlő' and 'Leánka'. This corroborated earlier findings by Bisztray *et al.* (2005) and Jahnke *et al.* (2007), who also excluded the possibility of the 'Kövérszőlő' x 'Leánka' parentage for 'Királyleánka'. Further, the data unequivocally disproved the long-held assumption that 'Csabagyöngye' (known in the English-language literature as 'Pearl of Csaba') derives from a 'Bronnerstraube' x 'Muscat Ottonel' cross (Hajdu 2003). Instead, our results are consistent with the assumption that 'Madeleine Angevine' is one of the parents of 'Csabagyöngye'. This is in agreement with suggestions by Bauer (2002) and Hillebrand (1972) who proposed for 'Csabagyöngye' a 'Madeleine Angevine' x 'Muscat Courtillier' and a 'Madeleine Angevine' x 'Muscat Précoce de Saumur' pedigree, respectively.

One of the advantages of the SSR allele size data is that they lend themselves well to digitalization. The resulting numerical data can be then subjected to pair-wise comparison to detect genotypic differences. The SSR data can also be converted to real fingerprints by the construction of barcodes (Jeffrey *et al.* 1985). We converted the SSR results to DNA barcodes by uncoupling the allele size and the corresponding SSR locus information and then sorting the allele size data from lowest to highest. The Figure shows the allele size bars drawn to a linear scale for 31 of the cultivars included in this study. The resulting barcode system is a visual representation of the data, allowing easy detection of genotypic differences. If an overlap occurs in the allele sizes of two or more loci, the bar can be marked by an index showing the number of data points represented by the bar. In our data alleles produced by for example VrZag62-VrZag83 markers are of the same size and the barcodes produced from them overlap in several cases. To indicate that this particular barcode stands for two markers, the number 2 is inserted under the bars in the Figure. Microsatellite allele size values generated in different laboratories are known to differ by 1 to 4 base pairs due to different analytical and rounding methods (This *et al.* 2004). As such laboratory-specific deviations tend to be systematic, they will cause a minor shift in the position of the size bars, but leave the overall structure of the barcode unchanged. The integration of such DNA barcodes into internationally coordinated databases, such as the "European Vitis Database" or the "International Vitis Variety Catalogue" could provide useful tools for cultivar identification, intellectual property protection, or resolution of commercial disputes.

Acknowledgements

This project was supported by grants from the Hungarian Scientific Research Fund (OTKA K62535, M36630 and M45633) and the GrapeGen06 European project. Additional support was provided by the Institute of Genetics and Biotechnology, Szent István University at Gödöllő, the Institute of Viticulture and Enology at Pécs, and the Department of Agriculture at Missouri State University at Mountain Grove. The authors thank Z. Tóth for help in editing the manuscript.

References

Bauer, K.; 2002: Weinbau. Österreichischer Agrarverlag. Druck und Verlagsgesellschaft mbH, Wien.

Bisztray, Gy.; Deák, T.; Eisenheld, C.; Pedryc, A.; Balogh, I.; Regner, F.; 2005: Microsatellite based identification of grapevine cultivars traditional in Hungary and in the Carpathian Basin. Int. J. Hortic. Sci. **11**, 71-73.

Bowers, J. E.; Boursiquot, J. M.; This, P.; Chu K.; Johanssen, H.; Meredith, C.; 1999: Historical Genetics: The parentage of Chardonnay, Gamay and other wine grapes of Northeastern France. Science **285,** 1562-1565.

Bowers, J. E.; Dangl, G. S.; Vignani, R.; Meredith, C. P.; 1996: Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome **39**, 628-633.

Csoma, Z.; 1994-95: Szőlészeti, borászati hagyományok, a megújulás és a közösség kötelékében. (Viticultural and Enological Traditions, in Bond of Reform and Community). Debrecen-Budapest. Debreceni KLT, Centrál-Európa Alapítvány.

Di Gaspero, G.; Cipriani, E.; Adam-Blondon, A. F.; Testolin R.; 2007: Linkage maps of grapevine displaying the chromosomal locations of 420 microsatellite markers and 82 markers from the R-gene candidates. Theor. Appl. Genet. **114,** 1249-1263.

Doligez, A.; Adam-Blondon, A. F.; Cipriani, G.; Di Gaspero, G.; Laucou, V.; Merdinoglu, D.; Meredith, C. P.; Riaz, S.; Roux, C.; This, P.; 2006: An integrated SSR map of grapevine based on five mapping populations. Theor. Appl. Genet. **113,** 369-382.

Eibach, R.; Zyprian, E.; Welter, L.; Töpfer, R.; 2007: The use of molecular markers for pyramiding resistance genes in grapevine breeding. Vitis **46**, 120-124.

Goethe, H.; 1887: Handbuch der Ampelographie (Rebenkunde). Beschreibung und Klassification der bis jetzt kultivierten Rebenarten und Traubenvarietäten mit Angabe ihrer Synonyme, Kulturverhältnisse und Verwendungsart. Verlag von Paul Parey, Berlin.

Hajdu, E.; 2003: Magyar szőlőfajták (Varieties of Hungarian Grapes). Mezőgazda Kiadó, Budapest.

Halász, G.; Veres, A.; Kozma, P.; Kiss, E.; Balogh, A.; Galli, Zs.; Szőke, A.; Hoffmann, S.; Heszky, L; 2005: Microsatellite fingerprinting of grapevine (*Vitis vinifera* L.) varieties of the Carpathian Basin. Vitis **44,** 173-180.

Hillebrand, W.; 1972: Taschenbuch der Rebsorten. Dr. Bilz & Dr. Fraund KG, Wiesbaden.

Hoffmann, S.; Di Gaspero, G.; Kovács, L.; Howard, S.; Kiss, E.; Galbács, Zs.; Testolin, R.; Kozma, P.; 2008: Resistance to *Erisyphe necator* in the grapevine 'Kishmish vatkana' is controlled by a single locus through restriction of hyphal growth. Theor. Appl. Genet. **116,** 427-438.

Jahnke, G.; Korbuly, J.; Májer, J.; Lakatos, A.; Györffyné Molnár, J.; 2007: Analysis of grapevine varieties by molecular markers. XXXth OIV World Congress. 10-16 June 2007. Budapest. CD\documents\viticulture\161_analysis_of_grapevine__vitis_vinifera_l_1___varieties_by_molecular_markers_pdf

Jeffrey, A. J.; Wilson, V.; Thein, S. L.; 1985: Hypervariable 'minisatellite' regions in human DNA. Nature **314,** 67-73.

Kiss, E.; Kozma, P.; Halász, G.; Veres, A.; Galli, Zs.; Szőke, A.; Hoffmann, S.; Molnár, S.; Balogh, A.; Heszky, L.; 2005: Microsatellite

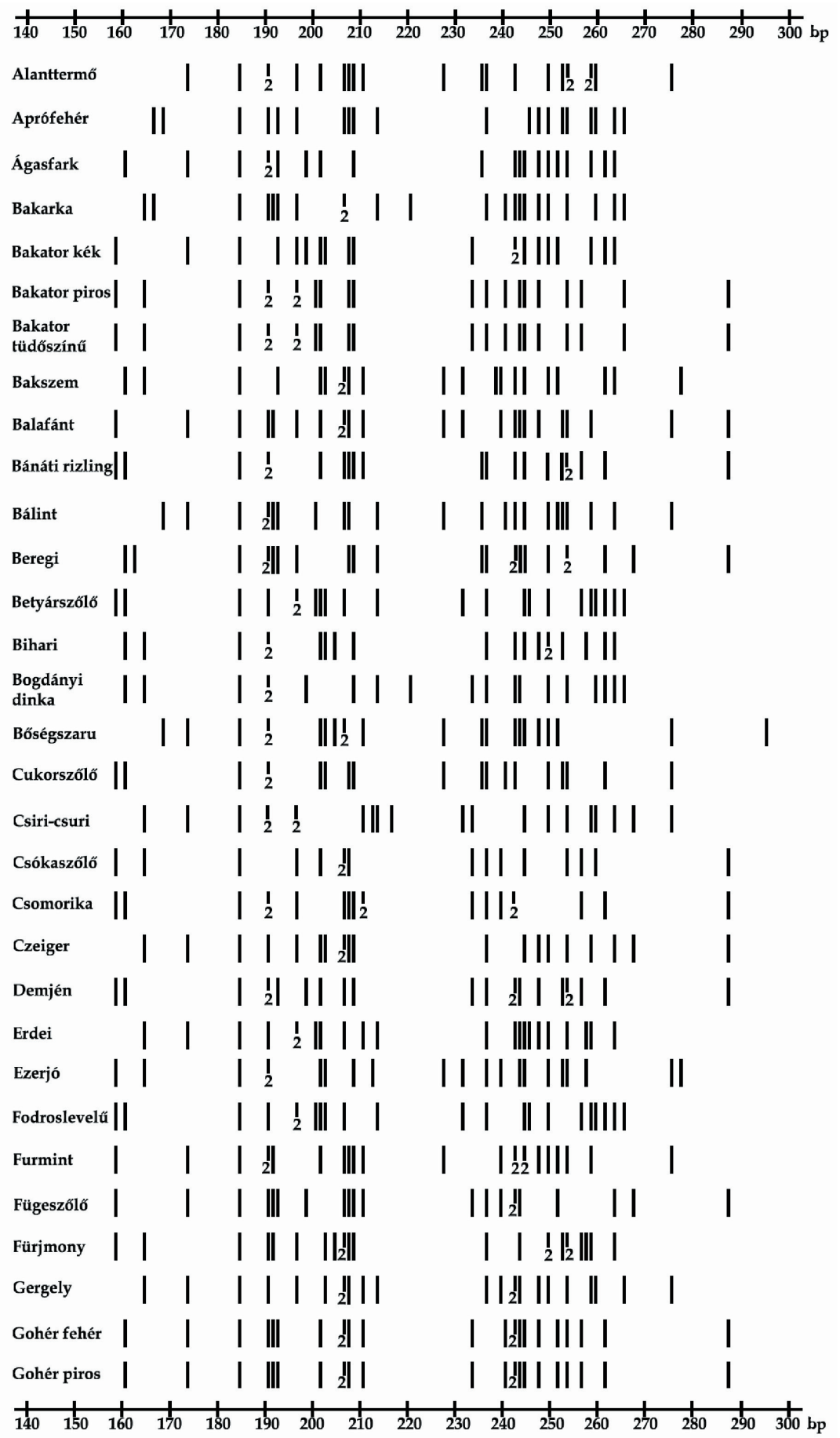

Figure: DNA barcodes of 31 grape cultivars from the Carpathian Basin. Bars marked by the number 2 represent two different markers of identical allele size.

based fingerprints and pedigree analysis of grapevine cultivars of Carpathian Basin origin, 79-87. Proc. Int. Grape Genomics Symp., St. Louis, Missouri, USA.

KOBAYASHI, S.; GOTO-YAMAMOTO, N.; HIROCHIKA, H.; 2005: Association of *VvMybA1* gene expression with anthocyanin production in grape *(Vitis vinifera)* skin-colour mutants. J. Japan. Soc. Hort. Sci. **74,** 196-203.

MALETIC, E.; PEJIC, I.; KAROGLAN KONTIC, J.; PILJAC, J.; DANGL, G. S.; VOLURKA, A.; LACOMBE, T.; MIROSEVIC, N.; MEREDITH, C. P.; 2004: The genetic relationship among three cultivars of the Dalmatian coast of Croatia. Am. J. Enol. Vitic. **55,** 174-180.

SCOTT, K. D.; EGGLER, P.; SEATON, G.; ROSSETTO, M.; ABLETT, E. M.; LEE, L. S.; HENRY, R. J.; 2000: Analysis of SSRs derived from grape ESTs. Theor. Appl. Genet. **100,** 723-726.

SEFC, K. M.; REGNER, F.; TURETSCHEK, E.; GLÖSSL, J.; STEINKELLNER, H.; 1999: Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome **42,** 367-373.

TAPIA, A. M.; CABEZAS, J. A.; CABELLO, F.; LACOMBE, T.; MARTINEZ-ZAPATER, J. M.; HINRICHSEN, P.; CERVERA, M. T.; 2007: Determining of Spanish origin of representative American varieties. Am. J. Enol. Vitic. **54,** 318-321.

THOMAS, M. R.; SCOTT, N. S.; 1993: Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites. Theor. Appl. Genet. **86,** 985-999.

THIS, P.; JUNG, A.; BOCCACCI, P.; BORREGO, J.; BOTTA, R.; CONSTANTINI, L.; CRESPAN, M.; DANGL, G. S.; EISENHELD, C.; FERREIRA-MONTEIRO, F.; GRANDO, S.; IBANEZ, J.; LACOMBE, T.; LAUCOU, V.; MAGALHAES, R.; MEREDITH, C. P.; MILANI, N.; PETERLUNGER, E.; REGNER, F.; ZULINI, L.; MAUL, E.; 2004: Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theor. Appl. Genet. **109**, 1448-1458.

WAGNER, H.; SEFC, K.; 1999: Identity 1.0 Centre for Applied Genetics. Univ. Agric. Sci., Vienna (http://www.boku.ac.at/zag/forsch/identity.htm).

Received February 27, 2008