

Microsatellite analysis of traditional eastern grapevine varieties and wild accessions from Geisenheim collection in Germany

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Summary

The Geisenheim collection contains a number of old traditional grapevines obtained during the last century from many countries including wild grapevine accessions. Over 60 samples originating from Azerbaijan, Bulgaria, Dagestan, Egypt, Greece, Hungary, Kazakhstan, Lebanon, Moldova, Romania, Russia, Turkey, Ukraine and Uzbekistan were probed for analysis. Additionally 25 accessions of wild grapevines some acquired in Germany were included to the tested panel. Accessions were analysed on 9 microsatellite loci (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62 and VrZAG79) for standard grapevine identification done in 4 multiplex PCRs. We obtained 13.56 overall average alleles per locus (12.44 in cultivated and 7.56 in wild grapevines). Expected and observed heterozygosity in cultivated grapevines were 0.826 and 0.644, while among wild accessions it was 0.693 and 0.464 respectively. The most informative locus proved to be VVMD28 in *Vitis vinifera* L. ssp. *sativa* and VVMD7 within *V. vinifera* L. ssp. *sylvestris* GMELIN. Microsatellite profiling will enable proper identification of cultivars by obtaining groups of synonyms and homonyms through comparative analysis as well assessment future estimation of relatedness between cultivated and wild accessions.

Key words: *Vitis vinifera*; *Vitis sylvestris*; identity, microsatellites; diversity.

Introduction

The Caucasus region is considered to be a primary centre of origin of cultivated grapevine (*Vitis vinifera* L. subsp. *sativa*). From this area cultivated forms would have been spread by humans to the Near East, Middle East and Central Europe (ARROYO-GARCIA *et al.* 2006). Early studies focused on West European cultivars and included only some autochthonous cultivars from Eastern Europe. Little is known about the genetic diversity of grapevine cultivars from Eastern Europe and their relationships with other cultivars in the Mediterranean region and Western Europe. Therefore it is useful to study a large set of cultivars from less explored regions to complete the knowledge about European grapevine. Molecular characterization using microsatellites proved to be an efficient tool for proper

identification of cultivars that is needed for shedding light to the missing gaps. The goal of this work was to obtain microsatellite profiles of cultivars originating mainly from Eastern Europe and wild grapevines collected from different locations maintained at the Geisenheim collection. Established genotypes were compared to databases in order to access correct cultivar identities as well as synonyms.

Material and Methods

A total of 68 cultivated grapevine varieties (*Vitis vinifera* L. subsp. *sativa*) originating from different countries (Tab. 1) and 23 wild accessions (*V. vinifera* L. subsp. *sylvestris* GMELIN) (Tab. 2) were obtained from Geisenheim collection in Germany.

Genomic DNA was isolated from lyophilized young leaves using the Qiagen DNeasy 96 Plant Kit (Hilden, Germany). DNA quality was determined on agarose gels (1 %) and the concentration was measured by NanoDrop ND-1000 spectrophotometer (Peqlab, Erlangen, Germany). Nine microsatellite loci were studied in four independent PCRs. The multiplex PCR I included three SSR loci: VVMD5 (BOWERS *et al.* 1996), VVMD27 (BOWERS *et al.* 1999) and VVS2 (THOMAS and SCOTT 1993). The multiplex PCR II included: VrZAG62 (SEFC *et al.* 1999) and VVMD25 (BOWERS *et al.* 1996). The multiplex PCR 3 was: VrZAG79 (SEFC *et al.* 1999) and VVMD7 (BOWERS *et al.* 1996, 1999), while multiplex PCR IV was: VVMD28 (BOWERS *et al.* 1999) and VVMD32 (BOWERS *et al.* 1996; 1999). PCR were done according to IBÁÑEZ *et al.* 2009. The separation of fragments and data analysis was carried out in Beckman Coulter Genetic Analysis System GeXP (Sciex, Darmstadt, Germany).

Diversity analysis for nine microsatellites was done by estimating the average number of alleles per locus (Na), expected heterozygosity (He) and observed heterozygosity (Ho). These genetic parameters and the identity check for matching genotypes (allowing for mismatching at two loci) were carried out using the software Cervus 3.0 (KALINOWSKI *et al.* 2007).

Results and Discussion

Diversity statistics: The mean number of alleles per locus in cultivated grapevines was 12.44 and in wild accessions 7.56. Expected and observed hetero-

Table 1

Microsatellite allelic profiles of grapevine (*V. vinifera* L. subsp. *sativa*) accessions and their origins according to the *VIVC* maintained at the Geisenheim collection

Accessions	<i>VIVC</i> Origin	VrZAG62	VrZAG79	VVMD5	VVMD7	VVMD25	VVMD27	VVMD28	VVMD32	VVS2									
Adzhem Misket	Ukraine	192	194	238	257	234	236	246	246	249	255	178	182	237	245	251	270	135	156
Affenthaler	Germany	192	202	241	243	na	na	248	255	247	255	na	na	229	229	249	270	na	na
Agnol	?	194	198	249	249	224	240	248	248	245	249	182	191	237	258	249	270	135	152
Ak Lik	?	186	198	241	245	234	240	238	238	241	245	180	182	241	258	270	270	125	137
Ak Shekerek	Turkmenistan	210	220	245	245	226	240	232	251	268	282	176	191	258	272	251	270	143	145
Albourla	Ukraine	186	202	Na	na	224	240	248	248	241	262	178	188	245	258	239	255	133	152
Augster Weißer	Hungary	186	194	247	249	224	244	240	240	239	241	182	191	237	249	255	270	133	133
Aurora	France	184	186	241	249	224	230	240	248	249	255	182	182	258	258	257	270	133	135
Babeasca neagra	Moldova	198	200	255	257	236	236	248	251	249	255	180	191	231	231	255	270	145	145
Bakthior, Balgthioryi	Uzbekistan	186	202	241	245	234	240	242	248	255	255	178	186	245	258	255	270	135	143
Bastardo Magarachskii, Bastard von Majoradi	Ukraine	192	202	236	249	226	232	248	248	255	255	176	182	247	263	270	143	143	143
Batuta neagra	Moldova	186	202	na	na	230	246	240	248	239	249	182	191	237	237	251	251	133	143
Berbecel	Bulgaria	186	192	243	249	238	246	240	248	249	255	184	186	249	260	251	257	141	143
Bolgar	Lebanon	184	186	241	249	224	230	240	248	249	255	182	182	258	258	257	270	133	135
Braier weisser	Austria	192	198	238	249	238	244	248	255	241	255	182	182	237	245	249	255	143	156
Bronnertraube	Germany	192	202	243	249	234	240	246	255	255	255	182	186	219	235	239	270	143	143
Bulanyi	Russian Federation	186	202	238	249	228	240	240	240	255	255	176	180	237	258	249	261	143	145
Cabernet sauvignon	France	186	192	245	245	230	240	238	238	239	249	172	186	235	237	239	239	139	152
Chaouch Blanc, Tschautsch	Turkey	186	202	245	247	228	236	246	248	249	255	176	180	258	258	251	270	135	152
Chaouch Blanc, Tschautsch	Turkey	186	202	245	247	228	236	246	248	249	255	176	180	258	258	251	270	135	152
Chaouch de Crimee	?	186	186	249	249	226	232	238	240	239	249	178	178	237	260	249	255	135	145
Chaouch de Crimee	?	186	186	249	249	226	232	238	240	239	249	178	178	237	260	249	255	135	145
Charas	Uzbekistan	186	198	245	249	234	244	238	242	241	249	176	182	258	258	249	251	137	143
Chardonnay	France	186	194	241	243	232	236	238	242	239	255	178	186	219	229	239	270	137	143
Charka	?	186	202	245	247	228	236	246	248	249	255	176	180	258	258	251	270	135	152
Coarna alba	Moldova	186	186	249	257	238	246	240	240	239	249	178	180	237	237	263	270	133	145
Coarna neagra	Moldova	186	198	na	na	234	234	na	na	241	249	176	182	245	245	255	272	137	143
Dimyat	Bulgaria	186	202	236	259	238	244	240	248	249	255	176	178	247	247	249	263	145	145
Donskoy oblong	Russian Federation	186	198	245	249	224	240	248	248	239	249	172	182	245	258	249	261	133	145
Doroi blau	Russian Federation	186	198	247	249	224	263	242	263	237	241	188	191	237	254	259	270	141	154
Doroi blau	Russian Federation	186	198	247	249	224	263	242	263	237	241	188	191	237	254	259	270	141	154
Fayoumi	Egypt	na	na	238	253	230	230	248	251	na	na	182	191	245	254	270	270	141	143
Feteasca alba	Moldova	190	192	na	na	224	234	na	na	249	255	182	191	249	260	247	251	133	133
Feteasca neagra	Moldova	194	202	245	253	224	236	238	255	249	255	176	186	237	249	263	270	141	143
Feteasca regala	Moldova	192	202	247	249	234	240	246	248	248	239	191	191	229	260	251	263	133	133
Francuse	Moldova	192	192	247	249	226	232	248	248	248	249	182	191	247	249	251	263	141	143
Galbena de Odobesti	Moldova	186	194	247	257	224	234	240	240	239	255	182	191	229	247	270	270	135	135
Gamsa	Hungary	186	202	247	247	na	na	248	253	239	255	na	na	229	262	270	270	na	na
Gordan de Dragsani	Moldova	188	198	236	236	232	240	246	251	239	266	176	176	237	237	261	270	135	141
Grasa de Cotnari	Moldova	194	202	236	249	226	240	238	253	239	241	176	191	247	260	251	270	133	145
Haengling blauer	Germany	194	198	236	249	226	230	238	255	249	255	186	186	229	229	239	270	141	152
Hainos Kek	Hungary	186	186	238	249	230	236	238	258	186	186	176	176	237	258	261	270	135	143
Heunisch blauer	Austria	186	198	243	247	224	236	242	253	239	262	176	188	237	249	239	239	139	152

Tab. 1 continued

Accessions	I/VC Origin	VZAG62	VZAG79	VVMD5	VVMD7	VVMD25	VVMD27	VVMD28	VVMD32	VVS2								
Heunisch Grauer	?	192	202	238	249	224	234	246	261	255	176	191	245	245	247	270	135	156
Heunisch Roter	Austria	194	198	236	249	232	236	238	255	249	176	182	231	231	239	270	133	152
Heunisch Weiß	?	212	229	249	253	224	226	251	253	295	176	191	249	260	251	263	133	143
Hudler blau	Germany	190	194	241	249	224	240	246	251	255	182	182	258	260	239	251	135	143
Keratuda, Kerutuola	Bulgaria	186	186	249	249	238	236	238	238	239	176	186	237	260	257	263	133	143
Khalili belyi	Azerbaijan	192	194	249	255	234	240	234	248	239	176	182	245	258	249	261	125	135
Khalili belyi	Azerbaijan	192	194	249	255	234	240	234	248	239	176	182	245	258	249	261	125	135
Kishmish Vir, Kischmisch von win	Russian Federation	186	200	247	247	226	232	248	248	249	176	191	247	258	249	270	149	152
Königstraube	?	186	202	238	241	232	236	242	248	249	176	182	219	229	239	270	143	152
Kuldzhinskii	Kazakhstan	194	200	<i>na</i>	<i>na</i>	226	240	<i>na</i>	<i>na</i>	249	176	178	245	245	249	270	143	152
L'enfant trouve	France	192	192	249	259	234	236	238	246	255	178	182	219	245	239	270	133	135
Miskor	?	186	198	245	245	238	244	246	248	249	176	191	237	249	255	270	135	152
NegruVirtos	Romania	192	202	236	249	226	232	248	248	255	176	182	247	247	263	270	143	143
Nimrang	Uzbekistan	186	194	249	255	228	232	242	246	249	182	191	237	245	249	270	143	152
Ofner weisser	Germany	186	202	245	257	226	232	240	246	249	178	186	237	247	251	263	135	145
Pamid	Bulgaria	186	186	243	249	226	244	240	240	249	182	186	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	137	145
Pinot noir	France	186	192	<i>na</i>	<i>na</i>	226	236	238	242	239	182	186	219	237	239	270	137	152
Rish Baba	Daghestan	<i>na</i>	<i>na</i>	249	257	222	232	248	248	<i>na</i>	178	182	237	247	249	270	139	152
Rish Baba	Daghestan	194	202	249	257	222	232	248	248	249	178	182	237	247	249	270	139	152
Sangiovese	Italy	192	194	241	257	224	234	238	261	241	176	182	235	245	251	255	133	133
Schewka	Bulgaria	186	198	238	243	236	234	248	251	239	178	182	245	245	255	270	145	147
Shafei	Azerbaijan	186	202	247	249	224	230	242	246	241	186	191	229	237	270	270	152	154
Shanii Chernyi (Sanli gionni)	Azerbaijan	192	198	<i>na</i>	<i>na</i>	232	232	<i>na</i>	<i>na</i>	241	191	191	258	258	249	270	145	156
Taiffi Rosovyi	Uzbekistan	186	194	<i>na</i>	<i>na</i>	226	232	<i>na</i>	<i>na</i>	239	176	191	245	245	249	257	137	143
Tavrída 1	Ukraine	186	186	249	257	238	246	240	240	239	178	180	237	237	263	270	133	145
Tavrída 2	Ukraine	184	186	241	249	224	230	240	248	249	182	182	258	258	257	270	133	135
Tusali Kara	?	186	202	<i>na</i>	<i>na</i>	224	240	<i>na</i>	<i>na</i>	249	182	191	229	229	251	270	135	152
Vinenka	Bulgaria	186	186	243	249	226	244	240	251	241	178	186	<i>na</i>	<i>na</i>	<i>na</i>	<i>na</i>	145	147
Weisser Aspirant	Greece	194	202	236	241	232	236	238	248	239	178	186	229	247	249	270	137	143
Yai uzyum rosovyi, Yaiizim Rozovyi	Daghestan	192	194	238	257	234	240	246	257	241	178	182	258	260	247	268	125	135

na = not amplified.

Table 2

Microsatellite allelic profiles of wild grapevines (*V. vinifera* L. subsp. *sylvestris* GMELIN) accessions maintained at the Geisenheim collection

Accession	VrZAG62	VrZAG79	VVMD5	VVMD7	VVMD25	VVMD27	VVMD28	VVMD32	VVS2									
unknown origin	192	192	241	243	224	232	255	255	249	255	178	186	235	235	251	270	143	152
Afghanistan	186	198	na	na	224	224	na	na	239	239	178	186	233	237	249	257	135	145
Alba Z 5943	192	194	249	249	226	226	261	261	239	247	186	188	237	237	249	251	139	152
Dirnstein 2	192	192	249	249	226	230	261	261	249	255	186	186	235	264	255	259	152	152
FR 5420 J 24	192	192	249	249	226	236	255	259	239	255	186	191	237	264	239	255	149	152
Fr 5481	198	198	249	249	226	230	246	248	241	241	186	186	237	237	239	255	133	133
Fr 5481 J 39	192	198	245	249	226	240	261	263	255	255	186	188	237	245	249	251	143	156
Fr 5481 J 49	198	198	249	249	226	230	246	248	241	241	186	186	237	237	239	255	133	133
Ketsch 7 Nr.2	192	192	249	249	226	230	261	261	247	266	186	186	237	264	239	239	141	152
Ketsch 7 Nr.2	192	192	249	249	226	230	261	261	249	266	186	186	237	264	239	239	141	152
Ketsch Nr.10	192	192	249	249	226	230	261	261	249	255	186	186	235	264	255	259	152	152
Ketsch Nr.17	186	186	243	243	230	236	240	242	249	249	186	186	219	219	270	270	149	152
Ketsch Nr.32	192	192	249	249	224	230	238	263	266	266	186	186	229	264	239	255	149	152
Ketsch Nr.34	192	192	309	309	230	240	308	321	239	255	186	186	237	264	239	255	149	152
Ketsch Nr.6	192	202	247	249	224	226	246	261	249	266	186	191	237	247	270	270	149	152
Ketsch Nr.8	192	192	249	249	na	na	238	263	266	266	na	na	229	264	239	255	na	na
La 2/3	192	192	249	249	226	230	261	261	249	255	186	186	237	237	239	239	149	152
Mannheim Nr.2	192	192	249	249	226	230	238	261	239	266	186	186	235	237	239	239	152	152
Nigra galjejevo	192	202	245	247	224	226	253	255	239	239	186	186	260	260	270	270	133	133
Otterstadt	192	192	249	249	226	226	240	240	239	255	186	186	237	264	255	255	149	152
S 30664	192	192	249	249	226	236	255	259	239	255	186	191	237	264	239	255	149	152
Turkovic	186	200	245	249	230	240	248	251	241	255	186	186	237	237	239	270	133	156
Violaca Homolj.	192	202	249	249	226	230	253	261	255	255	186	186	260	264	239	270	152	152

na = not amplified.

zygosity in cultivated grapevines were 0.826 and 0.644; while among wild accessions it was 0.693 and 0.464 respectively.

Identity analysis: The set of cultivated grapevines represented by 68 accessions (Tab. 1), including 5 replicates ('Chaouch blanc', 'Chaouch de Crimee', 'Doroi blau', 'Khalili belyi', 'Rish Baba') and 3 references ('Cabernet Sauvignon', 'Pinot Noir', 'Sangiovese') were further compared to the 138 genotyped cultivars collected from the Balkans in order to establish a unique set of samples as well as synonyms (<http://vitis.atcglabs.com/> and ŠTAJNER *et al.* 2014). By identity analysis, we obtained 62 unique genotypes. The analysis revealed 1 pair of cultivars having the same name but different allelic profiles at 8 out of 9 loci analyzed: 'Tavridal' ≠ 'Tavrida 2'. 'Tavrida' is described in *VIVC* as a Ukrainian black berry wine cultivar. Some new synonyms among accessions have been revealed. 'Bastardo Magarachskii' (Ukraine; Noir) and 'Negru Virtos' (Romania; Noir) from Geisenheim collection showed to have identical genotype and with this marker set could not be distinguished from 'Ružica' (Montenegro; Noir). One could speculate that this is one cultivar which was introduced to Montenegro where it was given the name 'Ružica' due to its pink berry or wine colour ('Ružica' is small rose in the local language). 'Chaouch Blanc' is described as a white berry table grape cultivar from Turkey having an identical genotype with both 'Charka' from Geisenheim collection and with 'Elezovka' from the Balkans (collected in Bosnia and Herzegovina). At the same time, 'Chaouch Blanc' had a different genotype from 'Chaush Bel' ('Chaush') from Macedonia (Bel is white in the local language) and 'Chaouch de crime' from Geisenheim. It seems that 'Chaouch' was a very popular table cultivar and thus introduced to different

countries often in conjunction with local names. This leads to some confusion on what is the original 'Chaouch' cultivar. 'Tavrida 1' showed to be 'Coarna Alba' white berry cultivar from 'Moldova' also having identical genotype to 'Bakator Beli' = 'Drenak Beli' = 'Bele Kozije Sise' = 'Begljerka Bela' from the Balkans. Here, some equivalency in naming could be noticed as 'Beli' = 'Alba' and is referring to the berry colour. Another pair of synonymic cultivars: 'Drenak Crni' = 'Coarna Neagra' have been discovered. 'Coarna Neagra' is a Moldovan black berry cultivar used for wine and table grape production (*VIVC*) while 'Drenak Crni' is a table grape cultivar from Serbia. 'Neagra' or 'Crni' means black and is referring to the berry colour. 'Drenak' is a common name in the Balkans for table grape cultivars. 'Bolgar', 'Tavrida 2' and 'Aurora' from Geisenheim collection had an identical genotype to 'Radovača IX' from the Balkans, all of them being the cultivar 'Afus Ali'. 'Afus Ali' is stated as a prime name for cultivar 'Bolgar' in the *VIVC* database while accessions 'Tavrida 2' and 'Radovača IX' showed to be 'Afus Ali'. The correct identity of the accession 'Aurora' could not be clearly determined from this study.

Among 23 wild accessions (Tab. 2), 20 unique genotypes have been found. Three groups of identical genotypes were observed: 'Ketsch Nr. 10' = 'Dirnstein 2' and 'FR 5420 J 24' = 'S 30664'. 'Fr 5481' was identical to 'Fr 5481 J 49' at all analyzed loci but different to 'Fr 5481 J 39'.

Conclusions

Within the accessions studied from the Geisenheim collection a high allelic richness (12.44) could be found. This might be due to the presence of diverse cultivars

originating from different gene pools. Low genetic diversity observed within wild grapevine accessions could be explained by low number of individuals that probably have been exposed to the severe selection pressure of the habitat itself. Many of them were collected on the island Ketsch in the river Rhine.

Comparison between genotyped cultivars from Geisenheim collection and the Balkans discovered several new synonyms: e.g. 'Drenak Beli' from Balkans is 'Coarna Alba' originating from Moldova and 'Drenak Crni' is 'Coarna Neagra'. This is in accordance with the assumption that many table grape cultivars were introduced to the Balkans from Eastern countries. Identity analyses are also raising new questions of the true identity and origin of some cultivars e.g. 'Chaush' or 'Tavrida'?

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