SSR ANALYSIS OF SOME *VITIS SYLVESTRIS* (GMEL.) ACCESSIONS OF THE SZIGETKÖZ AND FERTŐ-HANSÁG NATIONAL PARK, HUNGARY

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Abstract

The evolution of cultivated plants played important role in the ascent of humanity. Research of their origin and evolution started at the beginning of the20th century, but till nowadays a lot of questions remain open. A large number of theories exist about the evolution of the European grapevine (*Vitis vinifer*a L.). The *Vitis sylvestris* GMEL. in Hungary is a protected species. The quest and reservation of its populations are significant in terms of nature conservation and reserve of biodiversity as well. Based on theoretical and practical researches, it is supposed, that this species itself, or crossing with other species could be the progenitor of the European grapevine (*Vitis vinifera* L.).

In this study the quest and the SSR analysis of the *Vitis sylvestris* GMEL. populations of the Szigetköz and Fertő-Hanság National Park of Hungary are intended. 20 different genotypes of woodland grape (*Vitis sylvestris* GMEL.), 10 cultivars of European grape (*Vitis vinifera* L.) and 10 species/genotypes of rootstocks were analysed in 16 SSR loci of different linking groups.

The results show, that the analysed *Vitis sylvestris* accessions form an distinct group, but are closer to the *Vitis vinifera* cultivars, than to the rootstocks. This raise the probability, that these woodland grapes are true-to-type *Vitis sylvestris.*

Keywords: Vitis sylvestris (GMEL.), biodiversity, progenitor, SSR analysis, woodland grape, European grape, genotypes, true-to-type

1 INTRODUCTION

A large number of theories exist about the evolution of the European grapevine (*Vitis vinifer*a L.). According to DE CANDOLLE (1894) the grape originate from the Trans-Caucasian part of Russia. Later the grape changed a lot under cultivation, and spread in larger and larger areas. After this, the wide-yielded grapes altered considerably under the effect of grown ones. In the after-glacial Eurasia, the existed woodland grape (*Vitis sylvestris* GMEL.) spread in whole Europe, and existed even in the southern part of Scandinavia. Once the *Vitis sylvestris* was taken into cultivation from the Trans-Caucasus by the peoples of the ancient Asia. Later the already *Vitis vinifera* was received by the peoples of the antique West-Asia and the people, who lived in the islands of the Aegean-see, spread it on the northern and southern bank of the Mediterranean-see (KOZMA, 1991).

According to TERPÓ (1986) the *Vitis vinifer*a L. is not uniform, but the progeny of more original grape species, the main fundaments between the *Vitis sylvestris* GMEL. could be the hermaphrodite flowered *V. hissarica* and the *V. nuristanica*. In 1988 he developed a new intraspecific system of *Vitis sylvestris* GMEL. The substance of his taxonomy was that he sorted the woodland grapes into subspecies based on the hairs of the leaves, and into varietas based on the shape of the leaves. He deduced the eco-geographical groups (convarietas) of *Vitis vinifera* L. directly or indirectly from these varieties.

According to KOZMA (1991) the natural evolution produced the *Vitis sylvestris* GMEL., and the *Vitis vinifera* L. developed from this, due to deliberate cultivation. The most of the *Vitis* species are unisexual, and dioecious.The hermaphrodite-flowered form of *Vitis sylvestris* GMEL. was developed in the male-flowered individuals by bud mutation.

The microsatellite analysis of the grape can be traced back to the early nineties. A lot of SSR loci were identified and characterised in grapes (THOMAS and SCOTT, 1993; SCOTT et al., 2000; etc.) and were mapped (ADAM-BLONDON et al., 2004.; CONSTANTINI et al., 2007.).

The characterisation of grapevine cultivars by microsatellite DNA markers in Europe in the framework of an international cooperation colled GENRES 081 (European Network for Grapevine Genetic Resources Conservation and Characterisation) was carried out between 1997 and 2002. In this research programme 6 microsatellite primer pairs were determined and suggested for the characterisation of cultivars. An European project The GRAPEGEN06 which can be considered as the continuation of the GENRES 081 project started in January 2007. The main objective of GrapeGen06 was to contribute to the successful long term preservation of the Vitis genetic resources for the use of future generations.

The *Vitis sylvestris* GMEL. in Hungary is a protected species. The quest and reservation of its populations are significant in terms of nature conservation and reserve of biodiversity as well.

A new project started in September 2013 in Hungary aimed in the quest and ex situ conservation of Vitis sylvetris individuals in the area of Szigetköz and Fertő-Hanság National Park as well, as the morphological description and analyses of them by molecular markers. The results of the planned analyses can go a long way into the clarification of the origin of *Vitis vinifera* L., and to the explanation of the development of the convarietases of the European grapevine.

2 MATERIALS AND METHODS

Plant material

The plant material (dormant canes) of 10 Vitis rootstocks, 10 Vitis vinifera L. varieties and 20 Vitis sylvestris accessions (listed in Table 1) for DNA extraction originated from the collection of the NAIRC RIVE in Badacsony (Hungary) and from the collection of the University of Pannonia in Cserszegtomaj (Hungary). The V. sylvestris materials from the Szigetköz and Fertő-Hanság National Park were reserved in Badacsony in 2010, and samples were collected in 2013.

No	ID/Label	Accession Name	Genetic Origin	Origin of the Accession
1	S1	Sylvestris B1	*Vitis sylvestris* GMEL.	Badacsony, Hungary (Collected form Szigetköz and Fertő-Hanság National Park)
2	S2	Sylvestris B2	*Vitis sylvestris* GMEL.	
3	S3	Sylvestris B3	*Vitis sylvestris* GMEL.	
4	S4	Sylvestris B4	*Vitis sylvestris* GMEL.	
5	S5	Sylvestris B5	*Vitis sylvestris* GMEL.	
6	S6	Sylvestris B6	*Vitis sylvestris* GMEL.	
7	S7	Sylvestris B7	*Vitis sylvestris* GMEL.	
8	S8	Sylvestris B8	*Vitis sylvestris* GMEL.	
9	S9	Sylvestris B9	*Vitis sylvestris* GMEL.	
10	S10	Sylvestris B10	*Vitis sylvestris* GMEL.	
11	S11	Sylvestris B11	*Vitis sylvestris* GMEL.	
12	S12	Sylvestris B12	*Vitis sylvestris* GMEL.	
13	S13	Sylvestris B13	*Vitis sylvestris* GMEL.	
14	S14	Sylvestris B14	*Vitis sylvestris* GMEL.	
15	S15	Sylvestris B15	*Vitis sylvestris* GMEL.	
16	S16	Sylvestris B16	*Vitis sylvestris* GMEL.	
17	S17	Sylvestris B17	*Vitis sylvestris* GMEL.	
18	S18	Sylvestris B18	*Vitis sylvestris* GMEL.	
19	ALHAROS	Alháros	*Vitis sylvestris* GMEL.	Cserszegtomaj, Hungary
20	DORGO	Dorgó	*Vitis sylvestris* GMEL.	
21	Sarga_ortlibi	Sárga ortlibi	*Vitis vinifera* L.	Badacsony, Hungary
22	Purcsin	Purcsin	*Vitis vinifera* L.	
23	Budai_goher	Budai góhér	*Vitis vinifera* L.	
24	Torok _goher	Török góhér	*Vitis vinifera* L.	
25	Balafant	Balafánt	*Vitis vinifera* L.	
26	Valtozo_Furmint	Változó Furmint	*Vitis vinifera* L.	
27	Piros_Furmint	Piros furmint	*Vitis vinifera* L.	
28	Koverszolo	Kövérszőlő	*Vitis vinifera* L.	
29	Primitivo	Primitivo	*Vitis vinifera* L.	
30	Fiano	Fiano	*Vitis vinifera* L.	
31	N._Mex.	V. Novo Mexicana	*V. riparia x V. candicans*	Cserszegtomaj, Hungary
32	Aramon_rup_G1	Aramon Ganzin N1	*V. vinifera x V. rupestris*	
33	V._vip._Ggb	Riparia Grand glabre	*V. riparia*	
34	V._rip._GdM	Gloire de Montpellier	*V. riparia*	
35	V._rup._FW1	Fort Worth N1	*V. rupestris*	
36	V._rup._FW2	Fort Worth N2	*V. rupestris*	
37	V._rup._FW3	Fort Worth N3	*V. rupestris*	
38	T5C	Teleki 5C E20	*V. berlandieri x V. riparia*	
39	SO4	Teleki-Fuhr SO4 (133)	*V. berlandieri x V. riparia*	
40	5BB	Teleki-Kober 5BB	*V. berlandieri x V. riparia*	

Table 1. List of the analyzed accessions

DNA extraction
DNA was extracted from the phloem of the dormant canes with DNA Plant Mini Kit (Quiagen), following the manufacture's instructions. The amount and quality of DNA was determined spectrophotometrically. The DNA was diluted to a concentration of 10 ng/ml.

SSR analysis
Microsatellite (SSR) analysis was performed in 16 loci (Table 2). The primers had been chosen from different chromosomes (COSTANTINI et al., 2007) to give well defined heterozygosis.
Polymerase chain reactions were carried out in a total volume of 25 µl containing 12,5 µl of Hot Start Master Mix (Quiagen), 0.2 µM of each primer, and 50 ng of template DNA, using the following thermal profile: (1) 94°C for 45 min; (2) 94°C for 1 min, at the annealing temperature (Table 3) for 1 min, 73°C for 1 min per 35 cycles; (3) 73°C for 7 min.
One primer of each primer pairs were fluorescently labeled with FAM (6FAM) on the 5' end of the DNA chain. PCR products were run on a PE-Applied Biosystem 3100 Automated Capillary DNA Sequencer, the length of the products were determined using GeneScan 2.0 software (Applied Biosystem).

Linkage group[a]	SSR locus name	annealing temperature	Linkage group[a]	SSR locus name	annealing temperature
1	VMC8A7	64 °C	2	VMC7G3	60 °C
3	VVMD28	62 °C	4	VrZag21	62 °C
5	VrZag79	60 °C	8	VMC1F10	57 °C
9	VMC1C10	60 °C	10	VrZag25	67 °C
13	VMC3D12	57 °C	12	VMC2H4	57 °C
15	VMC5G8	58 °C	14	VMCNG1E1	58 °C
17	Scu06vv	60 °C	18	VVIM10	57 °C
19	VMC5E9	58 °C			

[a]Linkage groups are numbered according to ADAM-BLONDON et al. (2004)

Table 2. List of the analyzed SSR loci.

Data analysis
Estimates of genetic similarity between pairs were calculated by the Jaccard index (JACCARD, 1908). For the generation of distance matrix and UPGMA dendogramm a demo version of MolMarker - a platform-independent software for the analyses of molecular marker data (under development by Gizella Jahnke and József Smidla) with fully and tested functionality in this issue - was used.

3 RESULTS AND DISCUSSION

The results of SSR analyses are shown in table 3. Based on these data a similarity matrix by Jaccard index was calculated (data not shown). Based on this matrix an UPGMA dendogram was constructed (Figure 1.).
The main groups (V. sylvestris, V. vinifera, and rootstocks) mainly forms 4 distinct groups. It is visible, that the *Vitis sylvestris* GMEL. accessions — excluding Dorgó — form two distinct group in the dendogram. The rootstock Aramon Ganzin N1 (*V. vinifera x V. rupestris)* takes place between the larger V. sylvestris group and the V. vinifera group, which is not surprising taking into accont the hybrid origin of this accession. The larger V. sylvestris group is closer to the *Vitis vinifera* cultivars, than to the rootstocks. This raise the probability, that these woodland grapes are true-to-type *Vitis sylvestris.* The smaller V. sylvestris group, and Dorgó shows similarity to rootstocks, which probably shows their hybrid (V. sylvestris x V. ripara) origin.
To clarify these questions further genetic, molecular and morphological analyses are planned.

ID	VMC1F10		VMC3D12		VMC7G3		VVMD28		VMC2H4		VVIM10		VMC1C10		VrZag21		VMCNG1E1		VMC5G8		Scu06vv		VMC5E9		VMC4G6		VrZag79		VVS2	
S1	204	204	226	236	128	128	-1	-1	204	204	353	353	156	168	190	190	93	101	-1	-1	168	174	-1	-1	-1	-1	-1	-1	-1	-1
S2	204	204	206	236	-1	-1	-1	-1	-1	-1	-1	-1	156	168	190	190	93	101	-1	-1	-1	-1	191	191	-1	-1	-1	-1	-1	-1
S3	204	204	226	236	-1	-1	-1	-1	204	204	353	353	-1	-1	190	190	93	101	303	303	168	174	191	191	-1	-1	251	253	-1	-1
S4	204	204	226	236	-1	-1	-1	-1	204	204	-1	-1	-1	-1	190	190	93	101	-1	-1	168	174	191	191	-1	-1	251	253	-1	-1
S5	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	190	194	93	93	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
S6	204	204	-1	-1	-1	-1	-1	-1	200	200	-1	-1	-1	-1	194	194	93	93	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	107	107
S7	190	198	228	234	128	132	-1	-1	-1	-1	-1	-1	168	168	190	190	93	93	311	311	172	174	-1	-1	108	108	255	255	-1	-1
S8	202	204	-1	-1	-1	-1	250	250	-1	-1	327	327	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	108	108	-1	-1	-1	-1
S9	192	208	202	216	116	128	238	250	204	212	353	375	168	168	190	206	101	105	303	311	164	168	-1	-1	124	128	249	251	133	151
S10	-1	-1	228	228	130	132	222	222	200	204	353	365	156	168	190	194	93	93	311	311	168	168	207	229	132	132	251	251	-1	-1
S11	-1	-1	202	202	128	132	-1	-1	-1	-1	353	365	168	168	190	190	93	93	-1	-1	168	168	205	207	106	114	-1	-1	115	115
S12	190	190	202	202	116	128	-1	-1	204	204	353	365	156	156	190	200	93	95	-1	-1	-1	-1	-1	-1	106	106	249	249	133	151
S13	-1	-1	202	226	128	128	-1	-1	200	204	353	353	156	168	190	194	93	93	311	311	-1	-1	-1	-1	132	132	251	251	147	151
S14	190	208	226	234	128	132	238	238	200	204	353	365	156	168	190	194	93	93	311	311	196	196	221	221	132	132	-1	-1	131	151
S15	190	208	226	234	128	132	238	238	200	204	353	365	156	168	190	194	93	93	311	311	196	196	-1	-1	-1	-1	251	255	131	151
S16	190	208	226	234	128	132	238	238	200	204	353	365	156	168	190	194	93	93	311	311	196	196	-1	-1	-1	-1	251	255	131	151
S17	-1	-1	224	226	-1	-1	-1	-1	-1	-1	353	353	-1	-1	190	194	93	101	-1	-1	168	174	205	207	124	132	251	255	147	151
S18	-1	-1	224	226	-1	-1	-1	-1	-1	-1	353	353	-1	-1	190	194	93	101	-1	-1	168	174	205	207	124	132	251	255	147	151
ALHAROS	190	196	200	202	124	132	250	264	214	218	-1	-1	150	156	194	198	89	91	299	311	172	174	191	205	124	124	255	265	131	135
DORGO	204	204	210	212	116	126	244	260	196	214	-1	-1	142	142	204	204	87	95	303	305	168	168	199	203	122	122	255	263	131	143
Sarga_ortlibi	190	196	222	238	-1	-1	230	238	200	224	353	353	-1	-1	200	206	95	123	311	313	164	172	191	221	122	138	237	245	141	141
Purcsin	196	204	216	216	-1	-1	-1	-1	198	220	353	365	-1	-1	202	206	97	97	-1	-1	164	176	191	211	-1	-1	-1	-1	-1	-1
Budai_goher	196	202	194	208	116	116	-1	-1	194	212	365	375	168	168	-1	-1	95	105	317	317	176	176	193	217	108	134	-1	-1	-1	-1
Torok _goher	-1	-1	216	222	116	116	-1	-1	194	212	365	373	-1	-1	-1	-1	105	105	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Balafant	190	192	202	222	116	116	-1	-1	224	234	365	373	142	168	190	190	-1	-1	303	317	164	176	193	217	128	138	237	249	-1	-1
Valtozo_Furmint	192	204	216	222	116	116	230	250	206	236	365	365	142	168	200	206	95	95	303	317	164	176	193	217	106	106	-1	-1	-1	-1
Piros_Furmint	192	204	216	222	116	116	230	230	206	236	365	365	142	168	200	206	95	95	303	317	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Koverszolo	192	204	216	222	116	116	230	230	206	236	365	365	142	168	200	206	95	95	-1	-1	164	176	-1	-1	-1	-1	-1	-1	-1	-1
Primitivo	202	204	224	226	-1	-1	250	260	200	206	365	365	-1	-1	200	206	95	105	303	317	172	176	193	193	108	108	237	259	131	141
Fiano	204	204	-1	-1	-1	-1	-1	-1	-1	-1	365	367	-1	-1	202	206	93	95	-1	-1	172	172	-1	-1	120	122	249	252	131	141
Novo_Mex.	194	200	198	198	122	130	248	248	194	202	365	365	142	150	204	210	83	91	301	305	180	188	203	203	-1	-1	253	263	-1	-1
Aramon_G1	192	198	198	214	118	118	238	262	212	236	363	363	156	168	200	214	79	93	301	313	172	178	193	195	138	138	243	255	135	141
G_g	194	196	196	196	122	126	234	250	200	202	363	363	142	142	206	212	79	89	293	301	170	188	203	203	-1	-1	255	255	135	151
G_d_M	196	196	196	206	-1	-1	-1	-1	204	212	363	363	142	142	204	210	83	89	285	305	170	170	203	203	106	106	255	259	139	143
FW_1	194	196	198	198	118	134	238	238	196	230	365	365	142	142	204	208	83	91	305	305	184	192	193	215	-1	-1	257	263	-1	-1
FW_2	194	202	198	224	122	134	238	248	-1	-1	363	363	142	142	204	208	79	91	301	305	184	184	197	213	-1	-1	255	263	-1	-1
FW_3	194	196	198	222	118	118	-1	-1	204	230	365	365	142	142	204	208	91	91	301	305	184	184	197	215	126	128	255	257	-1	-1
T5C	194	196	204	208	114	126	216	252	208	212	365	365	142	150	196	210	83	83	295	305	170	180	203	211	124	124	255	255	143	147
SO4	196	196	208	212	114	126	216	236	208	212	365	365	142	150	196	210	83	83	285	295	170	170	203	211	124	124	263	263	143	145
5BB	196	196	208	212	120	126	216	218	-1	-1	363	363	142	150	202	210	83	83	-1	-1	170	170	201	203	124	124	259	259	139	147

Table 3. Results of microsatellite (SSR) analysis in 15 loci. (-1 indicates missing data or null alleles)

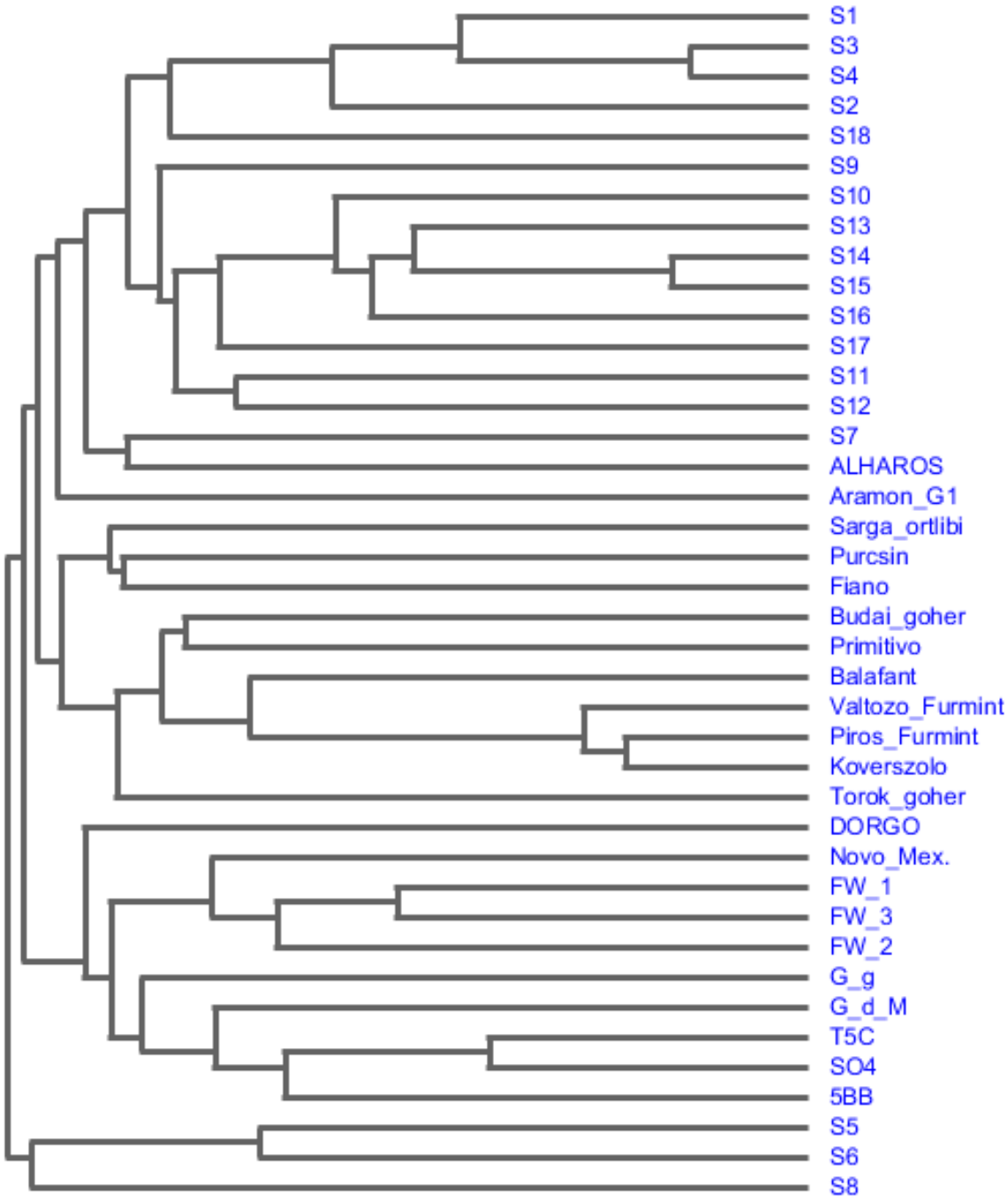

Figure 1. Dendogramm based on microsatellite results (Labels as in Table 1.).

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