ASSESSMENT OF MORPHOLOGICAL AND MOLECULAR SIMILARITY OF HUNGARIAN WHITE GRAPE VARIETIES

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The aim of this paper was to find possible link between molecular and morphological similarities of 38 Hungarian white grape varieties. Three aspects of morphological and molecular similarity were assessed in the study: comparison of the ordered variety pairs, assessment of molecular and morphological mean similarity differences and separation of varieties into similar groups by divisive cluster analysis to define (DIANA). Molecular similarity was calculated from binary data based on allele sizes obtained in DNA analysis. DNA fingerprints were determined at 9 SSR loci recommended by the European GrapeGen06 project. Morphological similarity was calculated on the basis of quantitative morphological descriptors. Morphological and molecular similarity values were ordered and categorized after pairwise comparison. Overall correlation was found to be weak but case by case assessment of the variety pairs confirmed some coincidence of molecular and morphological similarity. General similarity position of each variety was characterized by Mean Similarity Index (MSI). It was calculated as the mean of n-1 pair similarity values of the variety concerned. Varieties were ordered and compared by the difference of the index. Five varieties had low morphological and high molecular MSI meaning that they share several SSR marker alleles with the others but seems relatively distinct according to the expression of their morphological traits. Divisive cluster analysis was carried out to find similar groups. Eight and twelve cluster solutions proved to be sufficient to distinct varieties. Morphological and molecular similarity groups partly coincided according to the results. Several clusters reflected parent offspring relations but molecular clustering gave more realistic results concerning pedigree.

Keywords: Vitis vinifera – SSR (microsatellite or Simple Sequence Repeat) molecular and morphological similarity – pedigree – cluster analysis

INTRODUCTION

Early grapevine varieties of the 19th century Austro-Hungarian Monarchy were described by berry shape, leaf hair and lobes of petiole sinus [5]. Geotaxonomical classification based on plant ecology and morphological characteristics was a new approach in the 20th century. The system was elaborated by Negrul [16] and refined later by Németh [17] in Hungary and oriental, occidental and pontic origins were set

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and all existing varieties were categorized. Recently varieties have been described by several morphological and phenological characteristics in variety testing systems.

Breeding programs of the 20th century resulted in several new varieties based on crossing of old varieties. They were described by their morphological traits according to international descriptors list. Rapid development of the molecular technology such as SSR analysis resulted in high amount of genetic information. Practical application of molecular data focuses on variety identification or discrimination, as well as clarification of denominations or pedigree.

Clarification of synonyms and homonyms is important direction of molecular data applications. Denomination errors were detected by several authors [6, 14, 19]. 'Furmint' for example was proven to be identical with 'Moslavac' [14] as well as 'Blauer Portugieser' with 'Portugues Azul' [19].

Intervarietal genetic differences occurring in grapevine-growing regions made it possible to determine the geographical origin of cultivars with unknown background [21].

SSR markers were used to separate old Hungarian varieties as well. Comprehensive analysis of the old Hungarian varieties was carried out by Galbács et al. [4]. 'Kéknyelű' and 'Picolit' was differentiated by Jahnke et al. [10]. Pedigree of some old cultivars such as 'Veltliner', 'Pinot', and 'Traminer' was clarified by Kaserer and Regner [11] or Müller-Turgau by Vouillamoz and Arnold [26]. Applicability of SSR markers however, is limited in the detection of berry colour types and clones. Thirty SSR markers were not sufficient to discriminate green and grey 'Blauer Portugieser' [19], just like 24 different 'Traminer' clones remained indistinguishable by SSR markers as well [9]. Closely related 'Garnacha' berry colour mutations also resulted in the same microsatellite genotype [18] similarly to 'Pinot' varieties [20]. Berry colour differences can have several molecular backgrounds. Periclinal chimerism was reported in 'Pinot' varieties [8], and retrotransposon induced somatic mutations was described in Japanese grapevine varieties [13].

Preservation of genetic resources requires large number of morphological and molecular information. Molecular and morphological data has been collected into the GENRES-081 database in order to promote description and conservation of rare and old European grapevine cultivars [23]. Recently GrapeGen06 European project has been supporting this aim (http://www.montpellier.inra.fr/grapegen06/accueil.php), establishing a new European Vitis Database.

Comparison of both molecular and morphological similarity of grapevine varieties was studied by Cervera et al. [3] or Zulini et al. [27]. European and American 'Criolla' cultivars were successfully differentiated by using molecular and morphological data [15].

The aim of this paper was to compare and find possible link between molecular and morphological similarity of Hungarian white grape varieties. Three aspects of morphological and molecular similarity were assessed in the study.

MATERIALS AND METHODS

Plant material

Thirty-eight new and old white grape vine varieties registered in Hungary were included in the analysis. Plant material for SSR analysis was collected from national reference collections located in Helvécia and Domoszló, Hungary. Morphological descriptors were assessed at two locations. Finalized data (official variety descriptions) was provided by the national authority. Old cultivated varieties, intraspecific crossbred varieties (*Vitis vinifera* crossings) and interspecific (*Vitis amurensis* × *Vitis vinifera*) hybrids were also selected for the comparison. Varieties together with relevant pedigree are included in Table 1.

DNA analysis

DNA extraction and polymerase chain reactions were carried out as described by Halász et al. [7]. Nine SSR markers – VVMD5, VVMD7, VVMD25, VMD27, VVMD28, VVMD32, VVS2, VrZAG62, and VrZAG79 – were selected for molecular analyses according to the recommendation of the European GenRes database (http://www.genres.de/eccdb/vitis/) and GrapeGen06 project. Cy5 labelled forward primers were applied in the reactions. Primer pairs for VVMD microsatellites were published by Bowers et al. [1, 2], for VrZAG by Sefc et al. [22] and for VVS by Thomas and Scott [24]. Allele sizes were determined with ALFexpress II DNA Fragment Analyzer (Amersham Biosciences, Little Chalfont, UK). Molecular data were standardized according to the system of GrapeGen06 project using reference varieties (Table 2).

Morphological description

Assessment of morphological descriptors was carried out according to UPOV TG050 international DUS (distinctness, uniformity, stability) test protocol. Technical details of the test protocol is available at http://www.upov.int/edocs/tgdocs/en/tg050.pdf. Five—five plants were selected at each variety as sample at both experimental sites (Domoszló and Helvécia). List of the descriptors is introduced in Table 3. Morphological data were converted into variety description matrix comprising state of expression values.

Data evaluation

Morphological distance of varieties r and q (varieties to be compared) was calculated by city block algorithm as:

Table 1
Pedigree of crossbred white grape varieties

Variety	Pedigree
Bianca	Seyve Villard 12375 × Bouvier
Budai	n.a.
Chardonnay	n.a.
Chasselas blanc	Madeleine royal × *
Csabagyöngye	Madeleine angevine × Muscat Fleur d'Oranger
Csillám	Seyve Villard 12375 × Csabagyöngye
Ezerjó	n.a.
Furmint	n.a.
Generosa	Ezerjó×Tramini
Göcseji zamatos	Seyve Villard 12375 × Medoc
Hárslevelű	n.a.
Irsai Olivér	Pozsonyi × Csabagyöngye
Jubileum 75	Ezerjó × Pinot gris
Kabar	Hárslevelű × Bouvier
Királyleányka	Leányka×*
Korona	Juhfark×Irsai Olivér
Kövidinka	n.a.
Leányka	n.a.
Odysseus	(V. amurensis × V. vinifera) × Pinot gris
Olaszrizling	n.a.
Orpheus	(<i>V. amurensis</i> × <i>V. vinifera</i>) × Irsai Olivér
Pátria	Olaszrizling × Tramini
Pelso	(Olaszrizling×Ezerjó)×Pinot gris
Pinot blanc	n.a.
Pinot gris	n.a.
Rajnai rizling	n.a.
Rizlingszilváni	Rajnai rizling×Zöldszilváni
Rozália	Olaszrizling×Tramini
Rózsakő	Kéknyelű×Budai
Sauvignon blanc	n.a.
Szirén	(Kadarka×Ottonel muskotály)×Irsai Olivér
Taurus	(V. amurensis×V. vinifera)×Afuz Ali
Tramini	n.a.
Trilla	Pozsonyi×Muscat lunel
Villard blanc (Seyve Villard 12375)	n.a.
Vulcanus	Pinot gris × Budai
Zalagyöngye	Seyve Villard 12375 × Csabagyöngye
Zeus	Ezerjó× Bouvier

^{*} Debated.

n.a. – not available.

$$d_{rq} = \sum_{k=1}^{m} \left| c_{kr} - c_{kq} \right|$$

where

m – number of descriptors,

 c_k – state of expression value at the kth characteristic.

Morphological similarity for varieties r and q was calculated as follows:

$$s_{ra}=1-d_{ra}$$

where

 d_{rq} = distance of r and q varieties.

Molecular similarity was calculated from binary matrix by using Jaccard similarity index as:

$$s_{rq} = \sum_{k=1}^{m} a_k / (a_k + b_k + c_k)$$

where

 a_k – variable that positive (1) for r and q varieties at the kth locus

 b_k – variable that positive (1) for r variety and negative (0) for q variety at the kth locus

 c_k -variable that positive (1) for q variety and negative (0) for r variety at the kth locus

m – number of loci

Mean Similarity Index (MSI) of variety q was calculated by taking the arithmetical average of the similarity values of all pair combinations (m = 37) of the variety concerned as:

$$MSI_q = 1 / m * \sum_{k=1}^{m} s_{qk}$$

where

 s_{ak} – similarity value of variety q in kth pair combination.

m – number of pair combinations

Similarity indices were grouped into high, medium and low categories. Category intervals were set to index average \pm standard deviation as follows:

high:
$$>x+s$$
, low: $< x-s$,

where x was index average, and s was index standard deviation.

Table 2
SSR allele sizes of white grape varieties

Variety									Allele :	sizes (bp)							
variety	VVN	AD5	VV.	MD7	VVM	4D25	VVM	1D27	VVN	4D28	VVN	4D32	VV	'S2	VrZA	\G62	VrZA	AG79
Bianca	228	236	247	253	244	252	186	190	220	238	255	273	134	150	196	196	242	262
Budai	228	228	251	251	244	252	190	196	236	250	273	273	144	148	204	208	252	252
Csillám	228	228	247	253	244	260	190	196	248	260	251	251	134	144	192	208	254	262
Chardonnay	236	240	243	247	242	258	182	190	220	230	241	273	138	144	192	200	246	248
Chasselas blanc	228	228	243	251	244	258	186	190	220	270	241	241	138	144	196	208	254	262
Csabagyöngye	238	238	241	241	244	244	182	182	220	270	273	273	134	156	190	208	258	262
Ezerjó	226	232	243	243	242	252	180	186	230	280	257	273	134	144	192	192	240	254
Furmint	228	242	243	253	242	244	180	190	230	250	265	273	134	154	192	208	240	252
Göcseji zamatos	228	238	241	251	244	252	182	182	240	248	241	259	134	144	184	204	258	258
Generosa	228	234	243	261	252	252	182	182	238	280	241	257	144	152	192	198	240	254
Hárslevelű	228	234	243	253	244	244	186	190	230	250	265	273	134	144	192	208	240	254
Irsai Olivér	226	238	241	241	244	258	182	182	220	270	251	273	134	156	208	208	254	258
Jubileum 75	240	240	247	253	244	252	180	190	238	238	273	273	134	152	192	198	248	262
Kabar	228	234	247	251	254	258	186	186	220	250	265	273	146	152	198	204	240	254
Királyleányka	238	242	251	251	244	254	196	196	230	262	251	265	134	134	198	208	252	252
Korona	228	228	243	253	244	258	180	190	230	250	251	273	136	148	200	200	240	246
Kövidinka	236	242	243	253	244	258	182	186	238	252	241	241	134	144	192	198	254	262
Leányka	228	238	251	255	252	258	186	196	250	262	251	253	134	134	196	198	240	254

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Odysseus	228	240	243	253	252	252	182	186	238	270	241	253	150	150	190	192	242	258
Olaszrizling	228	240	251	261	258	272	186	190	248	260	241	273	138	152	196	196	254	254
Orpheus	238	238	243	253	244	258	182	186	246	248	241	273	134	144	192	208	240	256
Pátria	228	234	247	261	244	252	180	196	238	250	241	273	138	152	192	196	248	254
Pelso	228	242	243	261	252	272	186	186	248	260	241	241	138	152	198	198	240	240
Pinot blanc	230	240	243	247	244	254	186	190	220	238	241	273	138	152	192	198	242	248
Pinot gris	230	240	243	247	244	254	186	190	220	238	241	273	138	152	192	198	242	248
Rajnai rizling	228	238	253	261	252	258	182	190	230	236	237	273	144	150	198	204	246	246
Rizlingszilváni	240	240	251	261	252	258	182	182	236	246	253	253	144	152	198	198	246	246
Rozália	240	240	261	261	254	272	190	190	238	248	241	241	138	152	196	196	248	254
Rózsakő	228	228	243	253	244	244	186	190	270	280	241	241	134	150	192	198	240	254
Sauvignon blanc	230	234	243	261	244	252	176	190	236	236	243	259	134	152	192	198	248	248
Szirén	238	240	247	253	244	258	180	190	220	270	253	253	144	156	192	204	254	258
Taurus	238	238	243	253	244	258	184	184	260	260	253	273	130	138	190	198	256	256
Tramini	228	234	247	261	252	252	190	190	238	238	241	273	152	152	192	198	248	254
Trilla	228	228	237	259	252	258	180	190	270	280	253	265	134	136	200	204	254	258
Villard blanc	234	238	241	255	244	258	180	190	236	236	241	255	134	144	184	198	258	262
Vulcanus	228	228	243	253	244	258	186	190	236	236	241	273	138	144	196	208	242	254
Zalagyöngye	230	238	241	251	244	244	182	190	220	238	273	273	134	154	190	198	262	262
Zeus	228	234	241	245	252	252	186	196	270	280	241	241	134	144	192	198	240	254

Table 3 List of morphological descriptors

Nr.	Morphological descriptors (Nr. of UPOV TG050 characteristic in brackets)
1	Young shoot: prostrate hairs (3)
2	Young shoot: anthocyanin coloration of prostrate hairs on tip (4)
3	Young leaf: prostrate hairs between main veins on lower side of blade (7)
4	Young leaf: erect hairs on main veins on lower side of blade (8)
5	Shoot: attitude before tying (9)
6	Shoot: color of <u>dorsal</u> side of internodes (10)
7	Shoot: color of <u>ventral</u> side of internodes (11)
8	Shoot: length of tendrils (15)
9	Mature leaf: size of blade (17)
10	Mature leaf: blistering of upper side of blade (18)
11	Mature leaf: number of lobes (20)
12	Mature leaf: depth of upper lateral sinuses (21)
13	Mature leaf: arrangement of lobes of upper lateral sinuses (22)
14	Mature leaf: arrangement of lobes of petiole sinus (23)
15	Mature leaf: length of teeth (24)
16	Mature leaf: ratio length/width of teeth (25)
17	Mature leaf: proportion of main veins on <u>upper</u> side of blade with anthocyanin coloration (27)
18	Mature leaf: prostrate hairs between main veins on lower side of blade (28)
19	Mature leaf: <u>erect</u> hairs on main veins on <u>lower</u> side of blade (29)
20	Mature leaf: length of petiole compared to length of middle vein (30)
21	Bunch: size (peduncle excluded) (32)
22	Bunch: density (33)
23	Bunch: length of peduncle of primary bunch (34)
24	Berry: size (35)
25	Berry: ease of detachment from pedicel (38)
26	Berry: thickness of skin (39)
27	Berry: firmness of flesh (41)

Hierarchical cluster analysis is a common multivariate statistical tool to reveal similarity groups. The main structure of the data set had priority over the direct links of the clusters therefore, divisive cluster analysis (DIANA) was applied in the study [12]. The initial number of similar groups was set to 4. The number of groups was increased up to 12 in order to see the cohesion power of the similar group concerned.

RESULTS AND DISCUSSION

Assessment of variety pairs

Molecular and morphological similarity for all variety pairs was calculated first. Molecular similarity was based on SSR allele size data, morphological similarity on the state of expression values. Calculated similarities were combined into one matrix (Table 4).

Molecular similarity (upper triangle of Table 4) varied from 0% ('Rozália/Furmint' or 'Csabagyöngye'/'Pelso') to 100% ('Pinot gris'/'Pinot blanc') according to our calculation. The 'Rózsakő'/'Zeus' pair resulted unexpectedly high (72.2%) similarity without common ancestor in their pedigree. Parent-offspring relationship was detected in the case of 'Irsai Olivér'/'Csabagyöngye' and 'Generosa'/'Tramini'. At the same time despite breeding records and 50–60% similarity the SSR data did not support the direct parentage of 'Pátria'/'Tramini' (Table 1).

'Pinot gris' and 'Pinot blanc' are berry colour variants, they cannot be differentiated by the applied 9 SSR markers similarly to earlier results [4, 8].

It was noted that 'Csabagyöngye' in pair combination with 'Generosa', 'Pelso' or 'Rozália' did not share any common SSR alleles, their molecular similarity is 0%. Variety pairs not outlined above were combined in lower category intervals (Table 5).

Morphological similarity (lower triangle of Table 4) varied from 45.5% to 89.4%. There were 48 pairs showing morphological similarity higher than 80%. The majority of the pairs (650) fell into the 50–70% category. Morphological similarity had narrower range of similarity and contrary to molecular similarity certain level of similarity always occurred even between distinct varieties.

The case of 'Pátria'/'Kabar' pair outlined that interpretation of similarity should be based on the method applied. High morphological similarity (89.4%) of this pair was in contrast with low molecular similarity (29.6%).

Pedigree was not clearly reflected at pairs with high morphological similarity. The 'Pátria'/'Rozália' pair derived from the same crossing ('Olaszrizling'בTramini') according to the ampelographic literature. 'Pátria'/'Kabar', 'Pátria'/'Pinot blanc' and 'Kabar'/'Pinot blanc' pairs were all listed in top values without having direct relationship. 'Trilla' and 'Csillám' are not related either.

The two similarity matrices were compared by Mantel test. Correlation resulted in 0.062 meaning that practically there was no correlation between the two data sets. Our data confirmed that molecular and morphological similarity did not correlate in the case of the tested grapevine varieties and only molecular similarity reflected pedigree.

Similarity values of both data sets were divided into high, medium and low categories. Variety pairs of high and low similarity were combined into four groups:

- group (1): high morphological and high molecular,
- group (2): high morphological and low molecular,
- group (3): low morphological and high molecular,
- group (4): low morphological and low molecular similarities (Table 6).

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Table 4
Molecular (upper triangle) and morphological (lower triangle) similarity matrices of white grape varieties

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	Bianca	Budai	Chardonnay	Csabagyöngye	Csillám	Ezerjó	Fehér chasselas	Furmint	Generosa	Göcseji zamatos	Hárslevelű	Irsai Olivér	Jubileum75	Kabar	Királyleányka	Korona	Kövidinka	Leányka	Odysseus	Olaszrizling	Orpheus	Pátria	Pelso	Pinot blanc	Pinot gris	Rajnai rizling	Rizlingszilváni	Rozália	Rózsakő	Sauvignon blanc	Szirén	Taurus	Tramini	Trilla	Villard blanc	Vulcanus	Zalagyöngye	Zeus
Bianca		0.25	0.18	0.16	0.19	0.19	0.28	0.13	0.15	0.10	0.22	0.14	0.29	0.21	0.07	0.19	0.10	0.18	0.10	0.19	0.17	0.27	0.11	0.31	0.31	0.22	0.07	0.16	0.15	0.23	0.10	0.03	0.25	0.10	0.18	0.29	0.29	0.14
Budai	0.50		0.11	0.17	0.20	0.15	0.25	0.28	0.12	0.20	0.24	0.25	0.16	0.23	0.22	0.20	0.07	0.19	0.03	0.16	0.14	0.19	0.08	0.10	0.10	0.29	0.12	0.08	0.21	0.15	0.11	0.04	0.17	0.11	0.15	0.32	0.21	0.15
Chardonnay	0.79	0.61		0.11	0.14	0.22	0.22	0.21	0.14	0.06	0.17	0.14	0.23	0.13	0.07	0.27	0.26	0.03	0.18	0.19	0.30	0.13	0.11	0.40	0.40	0.26	0.15	0.11	0.14	0.10	0.21	0.15	0.19	0.10	0.13	0.28	0.14	0.10
Csabagyöngye	0.71	0.64	0.76		0.16	0.19	0.32	0.19	0.00	0.26	0.15	0.53	0.22	0.19	0.17	0.12	0.15	0.11	0.16	0.08	0.24	0.07	0.00	0.07	0.07	0.11	0.13	0.00	0.12	0.07	0.25	0.13	0.04	0.16	0.20	0.12	0.47	0.07
Csillám	0.78	0.59	0.76	0.72		0.19	0.33	0.21	0.15	0.19	0.32	0.19	0.29	0.13	0.11	0.23	0.27	0.14	0.07	0.15	0.21	0.18	0.11	0.10	0.10	0.14	0.03	0.12	0.19	0.14	0.22	0.07	0.20	0.10	0.18	0.29	0.11	0.19
Ezerjó	0.56	0.80	0.69	0.71	0.62		0.23	0.31	0.48	0.10	0.43	0.10	0.15	0.17	0.15	0.28	0.18	0.22	0.14	0.15	0.21	0.27	0.15	0.21	0.21	0.18	0.07	0.07	0.48	0.19	0.14	0.11	0.25	0.19	0.14	0.19	0.07	0.45
Fehér chasselas	0.73	0.62	0.75	0.85	0.77	0.68		0.17	0.24	0.19	0.32	0.33	0.15	0.17	0.11	0.23	0.32	0.27	0.14	0.35	0.31	0.22	0.11	0.17	0.17	0.14	0.11	0.16	0.41	0.14	0.22	0.07	0.15	0.19	0.27	0.48	0.19	0.28
Furmint	0.53	0.82	0.61	0.59	0.62	0.72	0.54		0.14	0.13	0.46	0.21	0.22	0.24	0.33	0.36	0.25	0.17	0.13	0.06	0.29	0.17	0.10	0.13	0.13	0.17	0.07	0.00	0.22	0.13	0.09	0.14	0.10	0.13	0.06	0.22	0.14	0.17
Generosa	0.64	0.74	0.69	0.64	0.68	0.74	0.67	0.64		0.03	0.33	0.07	0.20	0.18	0.07	0.15	0.23	0.23	0.24	0.25	0.14	0.39	0.32	0.32	0.32	0.19	0.16	0.27	0.50	0.29	0.07	0.07	0.53	0.11	0.14	0.20	0.07	0.48
Göcseji zamatos	0.72	0.62	0.68	0.70	0.84	0.63	0.76	0.60	0.68		0.10	0.23	0.11	0.21	0.11	0.10	0.14	0.14	0.07	0.11	0.17	0.06	0.07	0.00	0.00	0.18	0.15	0.04	0.15	0.14	0.18	0.03	0.03	0.19	0.27	0.11	0.24	0.14
Hárslevelű	0.56	0.71	0.58	0.59	0.54	0.72	0.54	0.74	0.69	0.54		0.18	0.23	0.30	0.11	0.50	0.21	0.21	0.14	0.19	0.30	0.36	0.11	0.17	0.17	0.17	0.00	0.07	0.28	0.22	0.13	0.11	0.24	0.10	0.13	0.33	0.14	0.27
Irsai Olivér	0.75	0.62	0.73	0.81	0.74	0.62	0.79	0.52	0.65	0.76	0.54		0.11	0.26	0.20	0.19	0.14	0.27	0.10	0.24	0.17	0.18	0.07	0.06	0.06	0.18	0.15	0.07	0.19	0.03	0.32	0.15	0.11	0.28	0.14	0.24	0.29	0.14
Jubileum75	0.58	0.76	0.61	0.53	0.57	0.67	0.55	0.63	0.69	0.62	0.63	0.67		0.22	0.12	0.19	0.33	0.10	0.24	0.11	0.22	0.28	0.16	0.43	0.43	0.28	0.21	0.27	0.15	0.35	0.19	0.12	0.45	0.11	0.19	0.15	0.25	0.15
Kabar	0.73	0.65	0.81	0.73	0.76	0.75	0.76	0.59	0.73	0.77	0.60	0.76	0.70		0.10	0.21	0.17	0.21	0.03	0.14	0.13	0.30	0.19	0.16	0.16	0.21	0.14	0.07	0.18	0.13	0.21	0.07	0.28	0.17	0.09	0.14	0.22	0.17
Királyleányka	0.75	0.65	0.73	0.65	0.66	0.63	0.66	0.65	0.73	0.66	0.67	0.71	0.67	0.74		0.11	0.11	0.35	0.03	0.07	0.07	0.03	0.08	0.10	0.10	0.15	0.22	0.04	0.21	0.11	0.03	0.12	0.08	0.11	0.11	0.04	0.16	0.15
Korona	0.77	0.67	0.71	0.74	0.75	0.67	0.78	0.58	0.76	0.76	0.61	0.76	0.66	0.75	0.73		0.18	0.22	0.07	0.15	0.26	0.18	0.11	0.10	0.10	0.27	0.07	0.04	0.15	0.14	0.14	0.15	0.11	0.19	0.18	0.29	0.15	0.10
Kövidinka	0.59	0.65	0.60	0.46	0.56	0.63	0.48	0.73	0.63	0.54	0.72	0.50	0.65	0.61	0.64	0.59		0.13	0.22	0.10	0.35	0.17	0.15	0.17	0.17	0.17	0.24	0.11	0.28	0.18	0.26	0.15	0.19	0.14	0.26	0.28	0.14	0.22
Leányka	0.76	0.61	0.77	0.69	0.78	0.59	0.78	0.59	0.67	0.72	0.51	0.73	0.64	0.70	0.76	0.77	0.59		0.10	0.28	0.13	0.21	0.15	0.09	0.09	0.21	0.24	0.11	0.39	0.10	0.13	0.15	0.15	0.22	0.17	0.14	0.14	0.32
Odysseus	0.80	0.63	0.76	0.72	0.77	0.67	0.77	0.52	0.73	0.79	0.57	0.79	0.63	0.79	0.71	0.83	0.58	0.80		0.11	0.26	0.18	0.15	0.36	0.36	0.14	0.15	0.16	0.24	0.14	0.22	0.15	0.15	0.19	0.06	0.15	0.11	0.19
Olaszrizling	0.77	0.61	0.80	0.67	0.62	0.64	0.70	0.64	0.72	0.73	0.66	0.65	0.66	0.81	0.75	0.74	0.65	0.77	0.75		0.22	0.33	0.32	0.18	0.18	0.19	0.12	0.47	0.20	0.07	0.10	0.12	0.26	0.15	0.10	0.30	0.11	0.15
Orpheus	0.74	0.53	0.69	0.61	0.62	0.56	0.65	0.51	0.69	0.60	0.53	0.63	0.59	0.63	0.72	0.72	0.60	0.66	0.72	0.66		0.13	0.19	0.16	0.16	0.17	0.14	0.11	0.22	0.10	0.17	0.23	0.07	0.10	0.17	0.32	0.18	0.17
Pátria	0.76	0.67	0.82	0.72	0.75	0.74	0.75	0.64	0.74	0.76	0.66	0.70	0.66	0.89	0.76	0.79	0.68	0.72	0.81	0.82	0.67		0.19	0.30	0.30	0.13	0.11	0.36	0.23	0.22	0.13	0.07	0.55	0.14	0.10	0.23	0.07	0.27
Pelso	0.62	0.70	0.73	0.63	0.64	0.72	0.69	0.63	0.72	0.66	0.65	0.67	0.72	0.79	0.77	0.72	0.64	0.76	0.74	0.76	0.65	0.78		0.23	0.23	0.19	0.17	0.42	0.26	0.20	0.07	0.17	0.27	0.11	0.07	0.12	0.04	0.20
Pinot blanc	0.73	0.67	0.78	0.68	0.74	0.68	0.74	0.54	0.70	0.77	0.55	0.74	0.70	0.85	0.72	0.78	0.61	0.75	0.82	0.80	0.67	0.86	0.80		1.00	0.17	0.14	0.29	0.22	0.31	0.13	0.14	0.45	0.03	0.09	0.22	0.18	0.17
Pinot gris	0.70	0.62	0.80	0.67	0.72	0.67	0.74	0.52	0.68	0.74	0.50	0.71	0.68	0.80	0.72	0.72	0.58	0.75	0.74	0.78	0.68	0.78	0.77	0.89		0.17	0.14	0.29	0.22	0.31	0.13	0.14	0.45	0.03	0.09	0.22	0.18	0.17
Rajnai rizling	0.64	.069	0.67	0.59	0.70	0.64	0.67	0.61	0.71	0.75	0.56	0.68	0.79	0.73	0.73	0.72	0.62	0.76	0.72	0.76	0.66	0.71	0.76	0.75	0.80		0.48	0.15	0.14	0.22	0.21	0.24	0.29	0.22	0.21	0.28	0.23	0.14
Rizlingszilváni	0.68	0.70	0.78	0.76	0.74	0.70	0.77	0.62	0.68	0.77	0.55	0.80	0.67	0.79	0.69	0.73	0.54	0.76	0.77	0.72	0.57	0.73	0.76	0.77	0.77	0.75		0.17	0.16	0.15	0.15	0.17	0.22	0.15	0.15	0.07	0.16	0.11
Rozália	0.72	0.75	0.75	0.67	0.72	0.78	0.69	0.65	0.78	0.79	0.65	0.72	0.73	0.82	0.72	0.78	0.66	0.72	0.84	0.78	0.67	0.83	0.79	0.84	0.77	0.81	0.80		0.12	0.21	0.07	0.04	0.42	0.12	0.07	0.17	0.04	0.12
Rózsakő	0.58	0.82	0.61	0.63	0.63	0.74	0.65	0.69	0.74	0.67	0.66	0.60	0.79	0.7	0.67	0.72	0.68	0.66	0.67	0.69	0.59	0.69	0.80	0.70	0.67	0.77	0.70	0.75		0.19	0.14	0.07	0.26	0.19	0.14	0.20	0.11	0.72
Sauvignon blanc	0.65	0.65	0.72	0.63	0.66	0.68	0.71	0.55	0.70	0.74	0.55	0.71	0.72	0.82	0.69	0.68	0.61	0.72	0.74	0.76	0.67	0.73	0.84	0.79	0.80	0.78	0.72	0.80	0.73		0.06	0.07	0.36	0.07	0.22	0.15	0.24	0.19
Szirén	0.71	0.59	0.74	0.74	0.78	0.66	0.76	0.53	0.66	0.81	0.54	0.76	0.58	0.81	0.65	0.79	0.55	0.69	0.78	0.71	0.63	0.84	0.67	0.81	0.76	0.61	0.75	0.73	0.59	0.68		0.15	0.11	0.27	0.21	0.19	0.14	0.14
Taurus	0.72	0.59	0.72	0.78	0.74	0.63	0.82	0.50	0.70	0.71	0.59	0.76	0.52	0.69	0.61	0.75	0.53	0.72	0.77	0.68	0.67	0.68	0.64	0.72	0.74	0.63	0.77	0.71	0.63	0.66	0.76		0.08	0.11	0.11	0.16	0.16	0.03
Tramini	0.66	0.63	0.67	0.56	0.87	0.66	0.62	0.59	0.59	0.63	0.54	0.62	0.72	0.75	0.68	0.59	0.63	0.64	0.67	0.67	0.64	0.71	0.76	0.72	0.75	0.79	0.70	0.76	0.69	0.80	0.58	0.54	\Box	0.11	0.15	0.21	0.12	0.30
Trilla	0.75	0.60	0.76	0.70	0.87	0.62	0.79	0.57	0.67	0.84	0.49	0.76	0.60	0.77	0.67	0.78	0.58	0.83	0.79	0.80	0.59	0.76	0.71	0.80	0.80	0.68	0.79	0.76	0.65	0.72	0.81	0.77	0.60		0.14	0.11	0.07	0.19
Villard blane	0.81	0.59	0.72	0.72	0.74	0.62	0.71	0.63	0.67	0.72	0.67	0.67	0.57	0.69	0.67	0.81	0.63	0.67	0.76	0.76	0.68	0.78	0.61	0.67	0.63	0.63	0.66	0.72	0.63	0.59	0.75	0.69	0.55	0.69	\Box	0.23	0.28	0.22
Vulcanus	0.57	0.80	0.68	0.67	0.69	0.68	0.67	0.73	0.72	0.72	0.65	0.71	0.70	0.71	0.72	0.73	0.61	0.70	0.69	0.72	0.55	0.72	0.77	0.74	0.76	0.78	0.80	0.74	0.76	0.66	0.63	0.67	0.65		0.63	\Box	0.15	0.15
Zalagyöngye	0.74	0.66	0.71	0.82	0.70	0.69	0.75	0.63	0.66	0.65	0.67	0.73	0.58	0.68	0.68	0.77	-	0.67	0.68		0.63	0.71	0.62	0.65	0.65	0.63	0.72	0.70	0.63	0.59	0.71	0.70	-	0.67	-	0.67		0.11
Zeus	0.55		0.62	0.52	0.61	0.70	0.58	0.68	0.68	-	_	0.61	0.78	0.67	0.67	0.65	0.77	_	_	-	0.60	0.67	_	_	0.71	-	0.71	0.79	0.80	0.79		0.56	-		_	0.72	0.55	$\overline{}$

Variety pair	Molecular similarity (%)	Variety pair	Morphological similarity (%)
Pinot blanc/Pinot gris	100	Pátria/Kabar	89.4
Rózsakő/Zeus	72.2	Pinot blanc/Pinot gris	88.6
Pátria/Tramini	55.0	Trilla/Csillám	87.0
Irsai Olivér/Csabagyöngye	52.6	Pátria/Pinot blanc	86.1
Generosa/Tramini	52.6	Kabar/Pinot blanc	85.3
		Pátria/Rozália	83.0
	similarity interval % (nr. of pairs)		Similarity interval % (nr. of pairs)
1	50.1–100.0 (5)	1	80.1–90.0 (48)
2	40.1–50.0 (20)	2	70.1–80.0 (282)
3	30.1–40.0 (41)	3	60.1–70.0 (281)
4	20.1–30.0 (163)	4	50.1-60.0 (87)
5	10.1–20.0 (361)	5	40.1–50.0 (5)
6	0–10.0 (113)		

Table 5
Molecular and morphological similarity order of white grape variety pairs

Pair components in group (1) could be divided into 2 categories according to their progeny:

- progenies of 'Sauvignon': 'Pinot gris', 'Pinot blanc', 'Sauvignon blanc' and 'Chardonnay';

- progenies of 'Csabagyöngye': 'Irsai Olivér', 'Csabagyöngye', and 'Zalagyöngye'.

Other crossbred varieties included in this group were 'Pátria', 'Kabar', 'Pelso', and 'Rozália'.

The 'Chardonnay', 'Pinot blanc', 'Csabagyöngye', 'Irsai Olivér', 'Csabagyöngye', 'Zalagyöngye' and 'Pelso', 'Rozália' pairs have parent—offspring or grandparent-offspring relationship. The 'Pátria', 'Rozália' pair has the same parents.

The pair components of 'Chasselas blanc'/'Irsai Olivér' or 'Chasselas blanc'/'Csabagyöngye' are not in direct relationship, but one of their parents is in relation. 'Chasselas blanc' is not parent either of 'Irsai Olivér' or 'Csabagyöngye'. 'Chasselas blanc' is offspring of 'Madeleine royal', they share SSR alleles in 10 loci out of 12 [4] or 48 loci out of 57 [26]. 'Madeleine royal' and 'Madeleine angevine' are in parent-offspring relationship. Furthermore, microsatellite data proved that 'Madeleine angevine' is one of the parents of 'Csabagyöngye' [4].

In group (2) some new crossings like 'Korona', 'Kabar', 'Trilla', or 'Odysseus' took part in pair combinations. These pairs were very similar morphologically, and

Table 6
Positioning of white grape variety pairs into high and low categories on the basis of molecular and morphological similarity

			MORPHOLOGICA	AL	
		hi	gh	low	
		GROU	JP (1)	GROUP	(3)
		Chardonnay	Pinot blanc	Jubileum 75	Bianca
		Chardonnay	Pinot gris	Jubileum 75	Csillám
		Chasselas blanc	Irsai Olivér	Furmint	Korona
		Csabagyöngye	Chasselas blanc	Furmint	Orpheus
		Csabagyöngye	Irsai Olivér	Generosa	Tramini
		Csabagyöngye	Zalagyöngye	Hárslevelű	Kabar
		Kabar	Pátria	Hárslevelű	Korona
	high	Odysseus	Pinot blanc	Hárslevelű	Orpheus
		Pátria	Pinot blanc	Hárslevelű	Csillám
		Pátria	Rozália	Orpheus	Kövidinka
		Pelso	Rozália	Orpheus	Vulcanus
MOLECULAR		Pinot blanc	Pinot gris	Chasselas blanc	Kövidinka
ECL		Pinot blanc	Rozália		
40L		Pinot blanc	Sauvignon blanc		
~		Pinot gris	Sauvignon blanc		
		Rajnai rizling	Tramini		
		Sauvignon blanc	Tramini		
		GRO	JP (2)	GROUP	
		Chasselas blanc	Taurus	Csabagyöngye	Tramini
		Ezerjó	Rozália	Csabagyöngye	Zeus
		Göcseji zamatos	Rozália	Taurus	Budai
	low	Kabar	Odysseus	Taurus	Tramini
	10	Kabar	Rozália	Taurus	Zeus
		Korona	Odysseus		
		Korona	Rozália		
		Pinot blanc	Trilla		
		Pinot gris	Trilla		

very different according to SSR marker results. 'Rozália' ('Olaszrizling'×'Tramini') is genetically not related with 'Korona', 'Kabar' and 'Göcseji zamatos' despite high morphological similarity.

In low morphological and high molecular similarity group (3) 'Csillám', 'Orpheus', 'Furmint', 'Hárslevelű', and 'Jubileum 75' formed pairs. Parent-offspring relation was found in this group in the case of 'Generosa'/'Tramini'. 'Generosa' received SSR

 ${\it Table~7}$ Molecular and morphological ranking and rank differences of Mean Similarity Indices (MSI)

Variety	Molecular MSI rank	Morphological MSI rank	Rank difference
Rozália	27	2	25
Odysseus	26	4	22
Rizlingszilváni	28	6	22
Trilla	29	9	20
Göcseji zamatos	30	13	17
Pelso	25	8	17
Kabar	18	3	15
Királyleányka	31	16	15
Chardonnay	19	7	12
Korona	17	5	12
Olaszrizling	17	5	12
Taurus	32	21	11
Csillám	21	12	9
Pátria	9	1	8
Szirén	24	16	8
Sauvignon blanc	20	13	7
Csabagyöngye	26	22	4
Leányka	15	11	4
Pinot blanc	7	3	4
Rajnai rizling	11	10	1
Villard blanc	24	25	-1
Zalagyöngye	23	24	-1
Irsai Olivér	15	17	-2
Bianca	15	19	-4
Budai	22	26	-4
Vulcanus	8	14	-6
Pinot gris	7	15	-8
Ezerjó	10	23	-13
Generosa	6	19	-13
Furmint	16	30	-14
Zeus	12	26	-14
Orpheus	14	29	-15
Chasselas blanc	2	18	-16
Kövidinka	13	32	-19
Rózsakő	1	20	-19
Jubileum 75	5	28	-23
Tramini	3	27	-24
Hárslevelű	4	31	-27

Table 8
Result of divisive cluster analysis of molecular and morphological similarity of white grape varieties

N	Iolecular clu	sters		Mor	phological cl	lusters					
	numb	er of the clu	ster in	number of the cluster in							
Varieties	12 cluster	8 cluster	4 cluster	Varieties	12 cluster	8 cluster	4 cluster				
varieties	solutions	solutions	solutions	varieties	solutions	1	solutions				
	Large grou	^ <u> </u>			Large group						
Bianca	1	1	1	Chardonnay	3	3	1				
Chardonnay	1	1	1	Csillám	3	3	1				
Jubileum 75	1	1	1	Göcseji zamatos	3	3	1				
Pátria	1	1	1	Kabar	3	3	1				
Pinot blanc	1	1	1	Leányka	3	3	1				
Pinot gris	1	1	1	Odysseus	3	3	1				
Sauvignon blanc	1	1	1	Pátria	3	3	1				
Tramini	1	1	1	Olaszrizling	3	3	1				
Csillám	4	4	1	Pinot blanc	3	3	1				
Chasselas blanc	4	4	1	Pinot gris	3	3	1				
Furmint	4	4	1	Rozália	3	3	1				
Hárslevelű	4	4	1	Szirén	3	3	1				
Korona	4	4	1	Trilla	3	3	1				
Kövidinka	4	4	1	Jubileum 75	8	6	4				
Orpheus	4	4	1	Pelso	8	6	4				
Vulcanus	4	4	1	Rajnai rizling	8	6	4				
- Caroninas	Small grou	ıns	-	Sauvignon blanc	8	6	4				
Csabagyöngye	3	3	2	Tramini	8	6	4				
Irsai Olivér	3	3	2	Zeus	8	6	4				
Zalagyöngye	3	3	2		Small group	_					
Ezerjó	5	4	1	Bianca	1	1	1				
Generosa	5	4	1	Korona	1	1	1				
Rózsakő	5	4	1	Villard blanc	1	1	1				
Zeus	5	4	1	Zalagyöngye	1	1	1				
Olaszrizling	9	1	1	Csabagyöngye	4	1	1				
Pelso	9	1	1	Chasselas blanc	4	1	1				
Rozália	9	1	1	Irsai Olivér	4	1	1				
Budai	2	2	2	Taurus	4	1	1				
Kabar	2	2	2	Budai	2	2	2				
Göcseji zamatos	6	3	2	Ezerjó	2	2	2				
Villard blanc	6	3	2	Rózsakő	2	2	2				
	11	7	2		12		1				
Szirén				Rizlingszilváni		3	_				
Trilla	11	7	2	Vulcanus	12	3	1				
Királyleányka	7	5	3	F	Individuals						
Leányka			3	Furmint	5	2	2				
Rajnai rizling	10	6	3	Hárslevelű	7	5	2				
Rizlingszilváni	10	6	3	Kövidinka	10	7	2				
	Individua			Generosa	6	4	3				
Taurus	12	8	4	Királyleányka	9	4	3				
Odysseus	8	1	1	Orpheus	11	8	3				

alleles from 'Tramini'. In group (4) 'Taurus', and 'Csabagyöngye' had 5 pairs combinations with 'Budai', 'Tramini', and 'Zeus', where both molecular and morphological similarity are low.

Similarity differences of individual varieties

Each variety had n-1 combinations in pairwise comparison meaning that 37 similarity values were assigned to each variety. The calculated mean of these values was considered as Mean Similarity Index characterizing the similarity position of the variety concerned. The two sets of MSI were arranged in descending order and the appropriate rank number was assigned to each variety. Identical rank numbers were assigned to varieties of identical MSI. Rank differences were calculated as $MSI_{mol}-MSI_{morf}$ for each variety. Results are introduced in Table 7.

'Trilla', 'Odysseus', 'Rizlingszilváni', and 'Rozália' had high positive position difference [(+)24–(+)31]. Such positive difference refers to high morphological and low molecular similarity. SSR markers revealed some rare alleles in these varieties.

'Hárslevelű', 'Jubileum 75', 'Rózsakő', 'Kövidinka', and 'Tramini' on the other hand had high negative position difference [(–)19–(–)27] meaning that molecular similarity was found to be high. Their SSR alleles occur frequently in other varieties. Morphologically they had significant distance from the others (Table 7).

Distance calculation is suitable to position varieties within the sortiment and molecular and morphological similarity can be compared on variety level. Mean similarity however, is not suitable to draw conclusion on pedigree relations.

Divisive cluster analysis

Divisive cluster analysis was carried out on molecular and morphological similarity matrices in order to find similar groups. Initial cluster number was set to four. Clusters were relatively large for both morphological and molecular data therefore, cluster number was increased to eight. The eight cluster solutions already divided varieties more properly. The number of clusters was further increased up to twelve. This solution did not result in further significant change in cluster numbers. Varieties grouping together at the twelve cluster solutions were nevertheless considered as really similar groups. This clustering solution was evaluated in detail. Complete coincidence between molecular and morphological clusters was not found however, some partial overlaps were identified. Varieties were separated into large and small groups and into individuals (Table 8).

There were two large groups (clusters 1 and 4) identified concerning molecular data. Cluster 1 comprised some old varieties like 'Pinot blanc', 'Sauvignon blanc' or 'Tramini'. Cluster 4 included some traditional Hungarian varieties like 'Furmint', 'Hárslevelű' or 'Kövidinka'.

Varieties in the small groups (clusters 2, 3, 5, 6, 7, 9, 10, and 11) were linked properly concerning pedigree. 'Zalagyöngye', and 'Irsai Olivér' are offsprings of 'Csabagyöngye' in cluster 3, 'Zeus', and 'Generosa' are offsprings of 'Ezerjó' in cluster 5.

'Leányka', and 'Királyleányka' in cluster 7 have parent-offspring relationship. 'Pelso', 'Rozália' in cluster 9 are offsprings of 'Olaszrizling'.

Similarity of 'Szirén' and 'Trilla' in cluster 11 as well as 'Budai' and 'Kabar' in cluster 2 cannot be explained by pedigree.

Despite the putative parent-offspring relation of 'Bianca', 'Csillám', 'Zalagyöngye' and 'Villard blanc' they did not group together in cluster 6. Similarly, cluster 3 does not include 'Csillám', 'Korona', 'Szirén' and 'Orpheus' however, they are offsprings of 'Csabagyöngye' or 'Irsai Olivér'. The reason can be that average linkage clustering algorithm always link the most similar pairs together first and than their average is compared to the next variety or to cluster average. 'Taurus' and 'Odysseus' remained individuals as interspecific hybrids.

Clustering morphological data resulted in two large groups (clusters 3, and 8), four small groups (clusters 1, 2, 4, and 12) and six individuals. Varieties in cluster 3 had various pedigree. There were old French varieties like 'Pinot gris', or 'Chardonnay' and old Hungarian varieties like 'Leányka' grouping together in cluster 3. Cluster 8 was also a relatively heterogeneous group concerning pedigree including 'Tramini', 'Sauvignon blanc' or 'Zeus'.

'Bianca', and 'Zalagyöngye' are offsprings of 'Villard blanc', they were found in cluster 1. 'Csabagyöngye' and 'Irsai Olivér' 'Chasselas blanc' and 'Zeus' were together in cluster 4. These cases were in line with the pedigree. 'Rizlingszilváni' and 'Vulcanus' in cluster 12 are not in relationship however, they were linked together.

'Furmint', 'Hárslevelű', and 'Kövidinka' formed cluster 2, 'Generosa', 'Királyleányka', and 'Orpheus' formed cluster 3 in the column of four cluster solutions but they all became individuals in the column of 12 cluster solution. Comparing the two data sets it can be concluded, that clustering based on molecular data reflects pedigree more properly.

CONCLUSIONS

Comparison of morphological and molecular similarity of grapevine varieties was based on similarity values calculated by pairwise comparison. Comparison of the ordered similarity values confirmed that there is a low correlation between the two data sets. Morphological characteristics are expressed by coding gene sequences, while SSR markers are located mostly in the non-coding regions of the DNA. Location of coding and non-coding regions may result coinciding high or low molecular and morphological similarity. Both methods can be used for variety discrimination, but SSR markers reflected parent-offspring relations more reliably. Morphological traits are currently used for numeric description. It is noteworthy that high morpho-

logical similarity may be linked with low similarity of microsatellite fingerprints. Combined evaluation of molecular and morphological similarity can improve variety testing systems.

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