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Morphological and molecular characterization of varieties and selected clones of 'Kadarka' grape

János Werner[A]*, Kitti Tóth-Lencsés[B]*, Anikó Veres[B], Erzsébet Kiss[B] and Pál Kozma jr.[A]

*These two authors equally contributed to the article.

[A] Research Institute for Viticulture and Enology
University of Pécs
HU-7634 Pécs, Pázmány P. u. 4.
E-Mail: werner.janos@pte.hu

[B] Institute of Genetics and Biotechnology
Szent István University
HU-2100 Gödöllő, Páter K. u. 1.
E-Mail: kiss.erzsebet@mkk.szie.hu

'Kadarka' originating from the Balkan Peninsula used to be the most commonly grown cultivar in Hungry for centuries. It played a huge role in spreading red wine production in Hungary. As a result of different ecological and technological conditions many 'Kadarka' clones and variants can be differentiated from each other in morphological and quality characteristics. We studied the variability of 'Kadarka' by examining the composition of an old plantation (established in 1898) and 'Kadarka' varieties from a gene bank and cultivars co-cultivated with 'Kadarka' (altogether 33 items). Our aim was to analyze the relationship between the numerous 'Kadarka' clones and variants based on their morphological and genetic traits and to clarify synonymy and homonymy not only among 'Kadarka' named, but also the 'co-cultivated' varieties. For ampelographic characterization we used OIV descriptors and Kruskal's Non-metric Multidimensional Scaling – NMDS and we applied SSR fingerprints for determining the genetic distances. Among the selected clones two main types, 'Kadarka' and 'Olasz kadarka' could be discriminated in the old plantation, which have different morphological traits and microsatellite profiles. Other varieties ('Lúdtalpú kadarka', 'Ménesi kadarka', 'Virághegyi kadarka') originating from gene banks could also partly be categorized into two main types. Morphological and genetic comparison support that 'Csókaszőlő' planted in the 19th century together with the 'Kadarka', shows close relationship with 'Mészi kadarka'. We confirmed the hypothesis that 'Fehér kadarka' and 'Öreg kadarka' are not in close relationship with 'Kadarka'. We demonstrated that 'Szagos kadarka' and 'Halápi szagos', as well as 'Rácfekete' and 'Cigányszőlő', considered to be distinct cultivars, are identical, that is they are synonyms and not 'Kadarka' varieties.

Keywords: 'Kadarka', variability, morphology, molecular markers, selection

Morphologische und molekulare Charakterisierung von Kadarka-Sorten und ausgewählten Klonen. *Die vom Balkan stammende Rebsorte 'Kadarka' war in den vergangenen Jahrhunderten die meist angebaute Rebsorte Ungarns und spielte dort eine bedeutende Rolle in der Verbreitung der Rotweinherstellung. Durch den Einfluss verschiedener ökologischer Umstände und Anbaubedingungen entwickelten sich viele Sorten und Klone, die morphologisch und hinsichtlich ihrer Qualitätseigenschaften unterschieden werden können. Die Variabilität der Rebsorte 'Kadarka' wurde untersucht durch die Überprüfung der Sortenzusammensetzung einer alten Anlage (gepflanzt im Jahre 1898) und von Sorten aus Genbanken und der Rebsorten, die früher mit 'Kadarka' zusammen angebaut wurden (insgesamt 33 Samples). Ziel war es, die Beziehungen von Kadarka-Sorten und deren Klone zueinander mittels morphologischer und genetischer Untersuchungen zu analysieren und Synonyme und Homonyme von als 'Kadarka' bezeichneten Sorten und der mit ihnen gemeinsam angebauten Sorten zu klären. Für die ampelographische Charakterisierung wurden OIV-Deskriptoren und Kruskal's Non-metric Multidimensional Scaling (NMDS) verwendet, die Bestimmung der genetischen Distanzen erfolgte mittels SSR Fingerprints. Unter den ausgewählten Klonen der alten*

Anlage konnten zwei Haupttypen ('Kadarka' und 'Olasz Kadarka') unterschieden werden, die unterschiedliche morphologische und genetische Merkmale aufweisen. Andere Sorten, die aus den Genbanken stammen ('Lúdtalpú kadarka', 'Ménesi kadarka', 'Virághegyi kadarka') konnten teilweise ebenso in zwei Haupttypen klassizifiert werden. Die morphologischen und genetischen Untersuchungen unterstützen die Annahme, dass die Sorte 'Csókaszőlő', die im 19. Jahrhundert gemeinsam mit 'Kadarka' ausgepflanzt wurde, eine enge Beziehung mit 'Mészi kadarka' aufweist. Die Hypothese, dass zwischen 'Fehér kadarka' ('Weisskadarka') und ' Oreg kadarka' ('Alte Kadarka') keine nähere Beziehung besteht, wurde bestätigt. Weiters konnte gezeigt werden, dass die früher als unterschiedliche Sorten beschriebenen 'Szagos kadarka' und'Halápi szagos' identisch sind ebenso wie'Rácfekete' und'Cigányszőlő', sie sind also Synonyme und keine unterschiedlichen 'Kadarka'-Sorten.
Schlagwörter: 'Kadarka', Variabilität, Morphologie, Molekularmarker, Selektion

Caractérisation morphologique et moléculaire de cépages Kadarka et de clones sélectionnés. *Au cours des siècles passés, le cépage 'Kadarka' provenant des Balkans, était le cépage le plus souvent cultivé et jouait un rôle important dans la propagation de la production de vins rouges. Une multitude de variétés et de clones se sont développés sous l'influence de différents conditions écologiques et culturales ; elles peuvent être distinguées du point de vue morphologique et en ce qui concerne leurs caractéristiques qualitatives. La variabilité du cépage 'Kadarka' a été examinée par voie de vérification de la composition des cépages d'un vieux vignoble (planté en 1898), des cépages provenant des banques de gènes et des cépages qui avaient été autrefois cultivés en commun avec 'Kadarka' (33 échantillons au total). La présente étude avait pour objectif d'analyser les relations entre les cépages Kadarka et leurs clones à l'aide d'examens morphologiques et génétiques et de clarifier les synonymes et les homonymes des cépages désignés comme 'Kadarka' et des cépages cultivés en commun avec eux. Aux fins de la caractérisation ampélographique, on a utilisé des descripteurs OIV et le Non-metric Multidimensional Scaling (NMDS) de Kruskal ; la détermination des distances génétiques s'est effectuée au moyen d'empreintes SSR (SSR Fingerprints). Deux types principaux ('Kadarka' et 'Olasz Kadarka') présentant des caractéristiques morphologiques et génétiques différentes ont pu être distingués parmi les clones sélectionnés du vieux vignoble. D'autres cépages provenant de banques de gènes ('Lúdtalpú kadarka', 'Ménesi kadarka', 'Virághegyi kadarka') ont également pu être classifiés en deux types principaux. Les examens morphologiques et génétiques confirment l'hypothèse selon laquelle le cépage 'Csókaszőlő', qui a été cultivé au 19e siècle en commun avec 'Kadarka', présente une relation étroite avec 'Mészi kadarka'. La supposition qu'il n'existe aucune relation étroite entre 'Fehér kadarka' ('Kadarka blanc') et 'Öreg kadarka' ('vieux Kadarka') a été confirmée. En outre, il a pu être démontré que les cépages 'Szagos kadarka' et 'Halápi szagos', auparavant décrits comme étant des cépages distincts, sont identiques, de même que 'Rácfekete' et 'Cigányszőlő'; il s'agit donc de synonymes et non pas de cépages 'Kadarka' distincts.*
Mots clés : 'Kadarka', variabilité, morphologie, marqueurs moléculaires, sélection

During the centuries of cultivation a lot of grape varieties have developed. Variability coming from bud mutation of asexually propagated plants is kept up by vegetative propagation, accumulating positive and negative modifications (Mullins et al., 1992).

Growers and ampelographers discriminated and systematized the varieties based on morphological traits. In spite of this fact in many cases even nowadays the same cultivar is grown under different names (synonyms) or different cultivars have the same name (homonym). Molecular genetic analyses opened new possibilities for deciphering the identity, diversity and relationship of the different varieties. These new methods together with the ampelographic characteristics can provide precise, objective variety evaluation (Crespan and Milani, 2001; Boso et al., 2005; Halász et al., 2005; Bessis, 2007; Ibánez et al., 2007; Baneh et al., 2009; Galbács et al., 2009; Cipriani et al., 2010; Crespan et al., 2011; Martin et al., 2011; Storchi et al., 2011; Beslic et al., 2012; Lacombe et al., 2013).

'Kadarka' (syn.: 'Csetereska' – Serbia, 'Gamza' – Bulgária, 'Kadarka noir' – France, 'Negru moale' – Romania) belongs to the convarietas pontica subconvarietas balcanica provarietas mesocarpa subprovarietas dalmatica group (Németh, 1967). Hungary is the northern border of its cultivation area.

'Kadarka', deriving from the Balkan Peninsula, has been cultivated in Hungary since the 16th to 17th century (Kozma, 1963; Németh, 1967; Rácz, 1997; Andrásfalvy, 1999), its precise origin has not been clarified yet. 'Kadarka' was grown in the majority of the Hungarian wine regions, it was the most widely spread cultivar in Hungary (Kozma, 1963) both in

plains and mountains.
During the centuries different 'Kadarka' varieties have come to existence differing from each other in morphological traits, flower type, fertility, quality determinants and chemical composition in which various ecological conditions played a role as it got far from its place of origin (Kozma, 1963).
A certain part of variable stocks grown in the plain has been conserved, since *Phylloxera* could not eradicate the plantations in the plains due to the loose sandy soils of these areas.
In the previous centuries a mixed variety composition was characteristic to the Central European grape plantations where other varieties could be found beside the determinant main cultivar (mass wine production or table grape) (Rapaics, 1940; Csávossy, 2002).
Our aim was to analyze the relationship between the numerous 'Kadarka' clones and variants based on their morphological and genetic traits and to clarify synonymy and homonymy and to determine true-to-typeness not only among 'Kadarka' named, but also the 'co-cultivated' varieties.
In Hungary Kozma (1954; 1957; 1958 a, b; 1963), Németh (1958; 1967) and Hajdu (2010) studied the morphology, flower biology, fertilization (fruit setting) and selection of 'Kadarka' in detail. Drucker (1906) first separated three varieties within 'Kadarka' as follows: 'Bolond kadarka', 'Rúgós kadarka' and 'Nemes kadarka'. Based on characteristic leaf traits Kozma (1963) listed five 'Kadarka' varieties and their intermediate types (A-'Lúdtalpú', B-'Kordoványos', C-'Nemes', D-'Kereszteslevelű', F-'Fügelevelű') (Fig. 1.). These varieties differ from each other in the shape and dissectness of leaf blade, shape of the teeth and general shape of petiole sinus. According to Németh's system (1967) 'Kadarka' forms a cultivar group – similarly to 'Pinot'. He mentioned two cultivars (varieties): 'Kadarka blue' and 'Kadarka gray', however only 'Kadarka blue' has production value. Within 'Kadarka blue' Németh discriminated 9 subcultivars and in his opinion 'Fehér kadarka' and 'Öreg kadarka' are not 'Kadarka' varieties, they are only its distant relatives.

Material and methods

Plant materials

We morphologically and genetically evaluated the 'Kadarka' varieties, its selected clones and other cultivars (altogether 33 items, Table 1) in an experimental plantation established in Szekszárd (Batti field). The number of plants in the different plots was 8 to 40. The plant material was derived from the old Szekszárd plantation (Parászta field) and the gene bank of Pécs.

Phenotype analysis

Twenty-nine ampelographic characters were measured on each item in 2010 and 2011, following a list of descriptors developed by the Organisation Internationale de la Vigne et du Vin (OIV, 2009), including the preliminary minimal traits with respect to shoots, leaves, bunches and berries (Table 2, 3, 4). Bunch and berry measurements occurred at harvest, using 100 berries from 20 bunches. The number of plants per item was 5.
OIV classes of the evaluated morphological characters were interpreted as ordinal variables, except for three parameters of mature leaves: shape of blade (OIV 067), profile of blade in cross section (OIV 074) and shape of teeth (OIV 076), which were treated as nominal variables. Similarity/dissimilarity computation was carried out with the FD package (Laliberté and Legendre, 2010) of the R suite (R Development Core Team, 2011), using Gower metrics (Gower, 1971). Ordinal variables were evaluated according to

Table 1: Morphologically and molecularly analyzed 'Kadarka' varieties and clones in Szekszárd, Batti field

Origin	Accessions
Szekszárd, Parászta field	Kadarka clone: P.102; P.108; P.109; P.111; P.114; P.115; P.117; P.122; P.123; P.124; P.125; P.131; P.147; P.165; P. 166; P.167
Gene bank, University of Pécs	Kadarka clone: P.9; P.172; P. 173
	Kadarka varieties: Fehér kadarka; Kadarka (blue); Lúdtalpú kadarka; Ménesi kadarka; Mészi kadarka; Olasz kadarka; Öreg kadarka; Szagos kadarka; Szürke kadarka; Virághegyi kadarka
	Other cultivars: Cigányszőlő; Csókaszőlő; Halápi szagos; Rácfekete

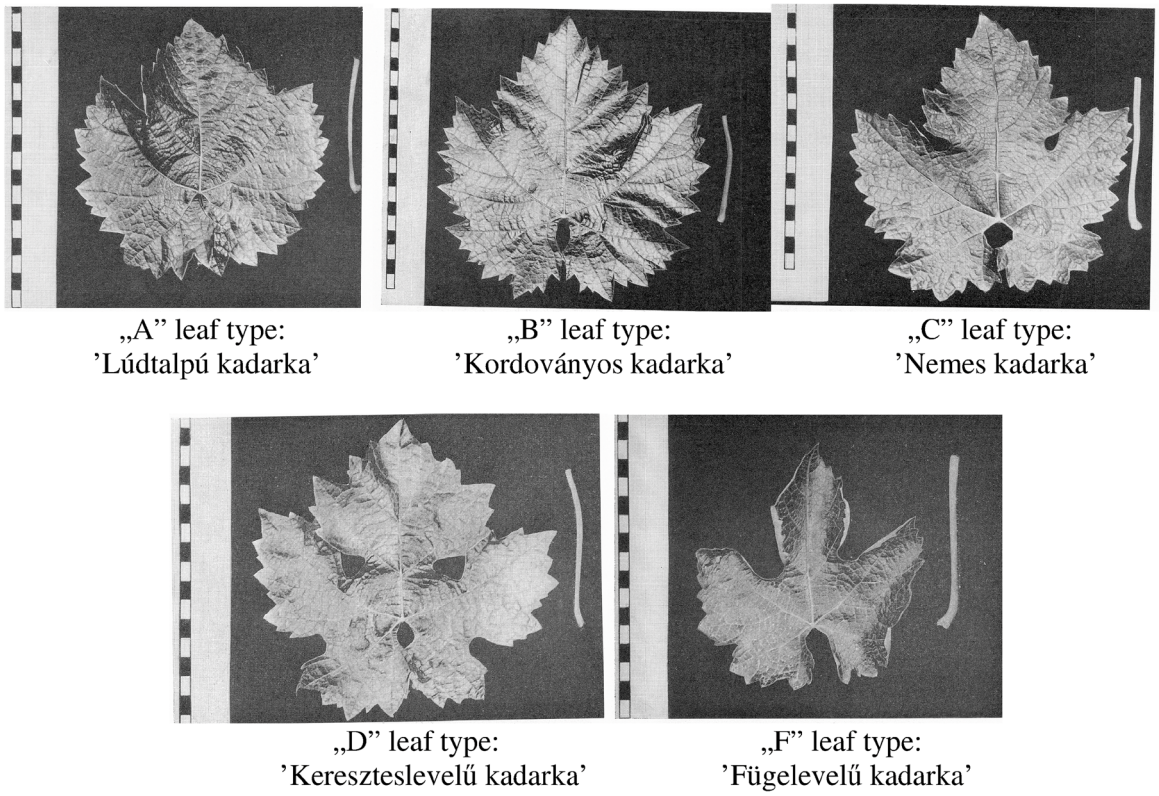

Fig. 1.: Morphological diversity in leaves of 'Kadarka' variants (Kozma, 1963)

the "metric" method described by Podani (1999). All variables were considered with equal weights. Based on Gower distances, Kruskal's Non-metric Multidimensional Scaling (NMDS, Cox and Cox, 2001) was carried out with the MASS package (Venables and Ripley, 2002) of R suit.

Genotyping with microsatellite (SSR) analysis

The total genomic DNA was extracted from freshly expanded leaves using Qiagen Plant DNeasy minikit (Halász et al., 2005). For genotyping nine microsatellite markers (VVMD5, VVMD7, VVMD27, VVMD28, VVMD32, VVS2, VrZAG62, VrZAG79) were applied according to the recommendation of GrapeGen 06 European Project. Cy-5 fluorescent dye labelled forward primers were used in the PCRs. PCRs were carried out in a 10 µl volume in a BioRad iCycler with the following profile: 1. 94 °C 2 min 2. (94 °C 30 sec, 57 °C 30 sec, 72 °C 1 min 30 sec) x 35 cylces 3. 72 °C 5 min. Reaction mixtures contained: 15 ng DNA template, 1 µM of each primer, 100 µM of each dNTP (Fermentas Biocenter, Szeged, Hungary), 1.5 mM MgCl2 (Fermentas), 1 x DNA buffer, 1 unit Taq Polymerase (WestTeam Biotech, Pécs, Hungary).
The size of the microsatellite fragments was determined by using ALF Express II. DNA Fragment Analyzer (Amersham Biosciences, Uppsala, Sweden).
The raw allele size data were coded according to This et al. (2004) involving 50 reference varieties according to the Grapegen 06 European project (http://www1.montpellier.inra.fr/grapegen06/accueil.php) as described by Katula-Debreceni et al. (2010).
SPSS 21. licenced program was applied for data analysis and dendrogram construction (using Average Linkage-, Between Groups; IBM SPSS Statistics, Version 21).

Table 2: Explanation of OIV-codes used for morphological characterization (Manual for standardization of Vitis descriptors, 2009)

Organ	Morphological trait	OIV-code	Value						
			1	2	3	4	5	7	9
Bud	Time of burst	301	very early		early		medium	late	very late
Young shoot	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	003	none or very low		low		medium	high	very high
	Density of prostrate hairs on the shoot tip	004	none or very low		low		medium	high	very high
Shoot	Color of the dorsal side of internodes	007	green	green and red	red				
	Color of the ventral side of internodes	008	green	green and red	red				
Young leaf (4th)	Color of upper side of blade	051	green	yellow	bronze	copper-reddish			
	Density of prostrate hairs between main veins on lower side of blade	053	none or very low		low		medium	high	very high
Flower	Sexual organs	151	fully developed stamens and no gynoecium	fully developed stamens and reduced gynoecium	fully developed stamens, fully developed gynoecium	reflexed stamens and fully developed gynoecium			
Mature leaf	Shape of blade	067	cordate		wedge-shaped		pentagonal	circular	kidney-shaped
	Area of anthocyanin coloration of main veins on upper side of blade	070	absent	only at the petiolar point	up to the 1st bifurcation	up to the 2nd bifurcation	beyond the 2nd bifurcation		
	Profile of blade in cross section	074	flat	V-shaped	involte	revolute	twisted		
	Blistering of upper side of blade	075	absent or very weak		weak		medium	strong	very strong
	Shape of teeth	076	both sides concave	both sides straight	both sides convex	one side concave one side convex	mixt. between both sides str. and both sides convex		
	Degree of opening / overlapping of petiole sinus	079	very wide open		open		closed	overlapped	strongly overlapped
	Teeth in the petiole sinus	081-1	none						present
	Teeth in the upper lateral sinuses	083-2	none						present
	Density of prostrate hairs between main veins on lower side of blade	084	none or very low		low		medium	high	very high
	Depth of upper lateral sinuses	094	absent or very shallow		shallow		medium	deep	very deep
Bunch	Length (peduncle excluded)	202	very short		short		medium	long	very long
	Density	204	very loose		loose		medium	dense	very dense
	Number of wings of the primary bunch	209	absent	1-2 wings	3-4 wings	5-6 wings	more than 6 wings		
Cluster	Degree of resistance to Botrytis	459			very little or little		medium		high or very high
Berry	Length	220	very short (≤8 mm)		short (about 13 mm)		medium (about 18 mm)	long	very long
	Width	221	very narrow (≤8 mm)		narrow (about 13 mm)		medium (about 18 mm	wide	very wide
	Shape	223	obloid	globose	broad ellipsoid	narrow ellipsoid	cylindric	ovoid	horn shaped
	Color of skin	225	green yellow	rose	red	grey	dark red violet		
	Intensity of flesh anthocyanin coloration	231	none or very weak		weak		medium	strong	very strong
	Particular flavor	236	none	muscat	foxy	herbaceous	other flavor		
	Begin of ripening (veraison)	303	very early		early		medium	late	very late

Results

Phenotype analysis

Comparison of the morphological data showed that there was a significant difference in the case of P. 167 clone among the 'Kadarka' clones selected from the old plantation of the Szekszárd wine region. Internodes of shoot of the P. 167 clone were green and red, while in the other 15 clones (P. 102, P. 108, P. 109, P. 111, P. 114, P. 115, P. 117, P. 122, P. 123, P. 124, P.

125, P. 131, P. 147, P. 165, P. 166) the colour of the internodes was green (OIV 007, OIV 008) (Table 3, 4). Colour of upper side of blade (4th leaf) is yellow-green; this is bronze-like in other clones (OIV 051). Blistering of the upper side of blade is weak or medium, in the case of the other clones it is strong or very strong (OIV 075). Overlapping of petiole sinus of mature leaf is open or closed while it is closed in the other clones (OIV 079). Density of prostrate hairs between main veins on lower side of blade of P. 167 clone is low or medium, while it is medium or high with the other clones (OIV 084). There are differences in the berry shape: broad ellipsoid in clone P. 167, contrary to the other clones having globose berries (OIV 223).

Table 3: Morphological traits of new true-to-type 'Kadarka' clones and true-to-type 'Kadarka' variants (2010, 2011)

Organ	Bud	Young shoot				Young leaf		Flower	Mature leaf										Cluster				Berry						
Variety, clone/ OIV code	301	003	004	007	008	051	053	151	067	070	074	075	076	079	081-1	083-2	084	094	202	204	209	459	220	221	223	225	231	236	303
P. 9	5	1-3	5-7	1	1	3	5-7	3	3	1	5	5	2-3	7	1	1	5	3-5	5-7	7-9	2	3	5	5	2	5	1	5	7
P. 102	5	1-3	7	1	1	3	5-7	3	3-4	1	5	5	2-3	5	1	1	7	1-3	5-7	7-9	2	7	5	5	2	5-6	1	5	7
P. 108	5	1-3	5-7	1-2	1-2	3	5-7	3	3-4	1	5	5-7	3	5	1	1	5	1-3	5-6	7	2	7	5	5	2	5-6	1	5	7
P. 109	5	3	7	1	1	2-3	7	3	3-4	1	5	5-7	2-3	5	1	1	5	1	5-7	7	2	6-7	5	5	2	5-6	1	5	7
P. 111	5	3	5	1	1	3	7	3	3	1	5	7	2-3	5	1	1	5-7	3	5-6	5-7	2	7-8	5	5	2	5-6	1	5	7
P. 114	5	1	5	1	1	3	7	3	3-4	1	2-(5)	5-7	2-3	5	1	1	7	1-(3)	5	5	1-2	9	4	3	1-3	5	1	5	7
P. 115	5	1	5-7	1	1	3	7	3	3-4	1	5	7-9	3	5	1	1	7-9	2-(3)	5-6	7-9	2	5	2	2	2	5-6	1	5	7
P. 117	5	1-3	5	1	1	3	5-7	3	3	1	5	5-7	2	5	1	1	5	1	5-6	7	2	7	5	5	2	5	1	5	7
P. 122	5	3	5	1-2	1-2	3	5-7	3	3-4	1	5	7	2	5	1	1	5-7	1	5-6	7	2	7	2	2	2	5-6	1	5	7
P. 123	5	3	5	1	1	3	5-7	3	3-4	1	5	5-7	2-3	5	1	1	5-7	(3)	6-7	7-8	2	7	5	5	2	5	1	5	7
P. 124	5	3	5	1	1	3	5-7	3	3	1	5	7	2	5-7	1	1	5-7	5	5-7	7	2	7	5	5	2	5-6	1	5	7
P. 125	5	3	5	1	1	3	5-7	3	3-4	1	2-(5)	5	2-3	5	1	1	5	1-(3)	5-6	7-8	2	7	5	5	2	5-6	1	5	7
P. 131	5	1-3	7	1	1	3	5-7	3	3	1	5	7	2-3	5	1	1	5-7	3-5	7	7	2	7	5	5	2	5	1	5	7
P. 147	5	3	7	1	1	3	7	3	3-4	1	5	5-7	2-3	7	1	1	7	1	5	5-6	2	5-7	5	5	2	5-6	1	5	7
P. 165	5	3	7	1	1	3	7	3	3-4	1	5	5-7	2-3	5	1	1	5-7	1	6-7	7-8	2	6-7	2	2	2	5-6	1	5	7
P. 166	5	3	7	1	1	3	7	3	3-4	1	5	5	3	5	1	1	5-7	3	5-6	7-8	2	5	2	2	2	5	1	5	7
P. 167	5	1	7	2	2	1-2	7	3	3-4	1	5	3-5	2	3-5	1	1	3-5	1-(3)	6-7	6	2	5-6	5-7	5-7	2-3	6	1	5	5
Kadarka blue	5	3	7	1	1	3	7	3	4	1	5	5-7	3	5	1	1	5	1-3	5-7	8-9	2	3-5	5-7	5-7	2	5	1	5	7
Lúdtalpú kadarka	5	1-3	7	1	1	3	5-7	3	3	1	5	7	2-3	5-7	1	1	5	1	5-7	7	2	5-7	5	5	2-3	5	1	5	7
Ménesi kadarka	5-6	1	5	1	1	2	5	3	3-4	1	5	5-7	2-3	5	1	1	5-7	1-3	5	5-7	2-3	5-7	5	5	2	6	1	5	7
Szürke kadarka	5	1-3	7	1	1	3	5-7	3	4	1	5	5	2-3	5	1	1	5	1-3	5-7	7	2	1-3	4-5	4-5	2	4	1	1	7

Table 4: Morphological traits of new true-to-type 'Olasz Kadarka' clones, true-to-type 'Olasz Kadarka' variants and other with 'Kadarka' co-cultivated varieties (2010, 2011)

Organ	Bud	Young shoot				Young leaf		Flower	Mature leaf										Cluster				Berry						
Variety, clone / OIV code	301	003	004	007	008	051	053	151	067	070	074	075	076	079	081-1	083-2	084	094	202	204	209	459	220	221	223	225	231	236	303
Fehér kadarka	5	1-3	5-7	2	2	2-3	5-7	3	4	1	5	5-7	2-(4)	5-7	1	1	5	1	5-7	7	2	3-5	4-5	4-5	2	1	1	1	5-7
P. 167	5	1	7	2	2	1-2	7	3	3-4	1	5	3-5	2	3-5	1	1	3-5	1-(3)	6-7	6	2	5-6	5-7	5-7	2-3	6	1	5	5
P. 172	5	1	7	2	2	2-3	5-7	3	3-4	1	5	5	3	3-5	1	1	5-7	1	7	6-7	2	7	5-7	5-7	2-3	6	1	5	5
P. 173	5	1	7	2	2	2-3	5-7	3	3-4	1	5	3-5	2	5	1	1	5-7	1	7	6-7	2	5-7	5-7	5-7	2-3	6	1	5	5
Virághegyi kadarka	5	1	7	2	2	2-3	7	3	4	1	5	3-5	2-3	3-5	1	1	3-5	1	5-7	6-7	2	7	5-7	5-7	2-3	6	1	5	5
Olasz kadarka	5	1	7	2	2	2-3	5	3	4	1	5	3-5	2	3	1	1	3	1	5-6	6-7	2	7	5-7	5-7	2-3	6	1	5	5
Öreg kadarka	5	1-3	7	1	1	3	5-7	3	3	1	3	5	2-3	3-5	1	1	3-5	5-7	6-7	7-9	2	5-7	5	5	2	5	1	5	7
Szagos kadarka	5	7	3	1	1	4	1	3	3-4	1	2-3	3	2	3	1	1	1	5-7	5	7	2-3	3-5	5	5	2	6-7	1	2	7
Halápi szagos	5	1	3	1	1	2-3	1	3	3	1	1-3	3-5	2	3-5	1	1	1	5-7	5-6	6-7	2-3	5	3-5	3-5	2	6	1	2	5-7
Mészi kadarka	5	5-7	7	1	1	3	7	3	4	1	5	5-7	2	5	1	1	3-5	3	5	5	2	9	3	1-3	1-3	6	1	4	7
Csókaszőlő	5	5-7	7	1	1	3	5-7	3	3-4	1	4-5	5	2	5	1	1	3	3	5	3	2-3	9	3	1-3	1-3	6	1	4	3
Cigányszőlő	5	1	5	1	1	2	7	3	3-4	1	1-4	5	2-3	3	1	1	3-5	3	7-9	3	2	1-2	3	3	2	6	1	5	7
Rácfekete	5	1	7	1	1	2	5-7	3	3-4	1	5	5-7	3	3	1	1	5	1-(3)	9	5-7	2-3	3	3-5	3-5	2-3	6	1	4-5	7

Ampelograhic data of clone P. 167 selected in Szekszárd and clones P. 172, P. 173 deriving from the gene bank, as well as 'Virághegyi kadarka' and 'Olasz kadarka' are very similar to each other. In the other 15 clones of the old plantation and P. 9 (selected in 1950) the majority of the traits was identical. Ampelographic data of 'Kadarka'; 'Lúdtalpú kadarka' and 'Ménesi kadarka' also show identity with these 15 clones (P. 102, P. 108, P. 109, P. 111, P. 114, P. 115, P. 117, P. 122, P. 123, P. 124, P. 125, P. 131, P. 147, P. 165, P. 166) (Table 3, 4). Slight differences can be observed in the shape of the leaf blade (OIV 067) and Botrytis susceptibility (OIV 459). Central lobe of mature leaf of 'Lúdtalpú kadarka' is wedge-shaped (OIV 067); blistering of the upper side of blade is strong (OIV 075).

'Mészi kadarka' significantly differs from 'Kadarka' in numerous traits. Anthocyanin colouration of the shoot tip is more intensive compared to 'Kadarka' (OIV 003), while density of prostrate hairs between main veins on lower side of blade is lower (OIV 084), its berries are smaller (OIV 220, OIV 221), the berry shape is broad-ellipsoid (OIV 223), it has a particular herbaceous flavour (OIV 236) (Table 3, 4).

'Csókaszőlő' is identical with 'Mèszi kardarka`in most ampelographic values , such as anthocyanin pigmentation and density of prostrate hairs on the shoot tip (OIV 003, OIV 004), anthocyanin pigmentation of shoot (OIV 007, OIV 008), colour of upper side of blade (4th leaf) (OIV 051), shape of teeth (OIV 076), overlapping of petiole sinus (OIV 079), depth of upper lateral sinuses (OIV 094), and berry characteristics (OIV 220, OIV 221, OIV 223, OIV 225, OIV 231, OIV 236, OIV 303). It deviates only in shape of teeth (OIV 067), in blistering of upper side of blade (OIV 075), in density of prostrate hairs between main veins on lower side of blade (OIV 084), and in the bunch density (OIV 204).

'Cigányszőlő' and 'Rácfekete' varieties are different from 'Kadarka' in the majority of ampelographic traits, and they differ from each other in the characteristics of the cluster (OIV 202, OIV 204, OIV, 209) and berry (OIV 220, OIV 221, OIV 223, OIV 225, OIV 231, OIV 236, OIV 303) (Table 3, 4).

'Szagos kadarka' ('Kadarka muscat') has almost the same ampelographic values as 'Halápi szagos'; however, anthocyanin colouration of the shoot tip of 'Szagos kadarka' is intensive while this is not the case in 'Halápi szagos' (OIV 003). Both varieties have muscat flavour (OIV 236) (Table 3, 4).

Central lobe of blade of 'Öreg kadarka' is slightly protruding (OIV 067), profile of blade in cross section is involute compared to the other Kadarkas (OIV 074) and its petiole sinus is less closed (OIV 079), the density of prostrate hairs between main veins on lower side of blade is lower (OIV 084), depth of upper lateral sinuses is deeper (OIV 094).

The berry colour of 'Fehér kadarka' is green-yellow (OIV 225), it does not have a particular spicy flavour (OIV 236), the internodes of the shoots are green and red (OIV 007, OIV 008), the berries have higher resistance to Botrytis than 'Kadarka gray' (OIV 459), veraison occurs earlier (OIV 303).

Berries of 'Szürke kadarka' ('Kadarka gray') are gray (OIV 225), its clusters are very susceptible to Botrytis (OIV 459). Berries do not have a spicy flavour taste (OIV 236). The majority of its morphological traits is identical with 'Kadarka' (Table 3, 4).

Statistical analysis of the ampelographic data shows the separation of the different groups (Fig. 2.). Clones belonging to 'Kadarka' form a separate group including 'Kadarka gray' ('Szürke kadarka'), 'Fehér kadarka' and clones P. 9, P. 102, P. 108, P. 109, P. 111, P. 115, P. 117, P. 122, P.123, P. 124, P. 125, P. 147. The varieties 'Lúdtalpú kadarka' and 'Ménesi kadarka' are found on the verge of this group probably due to their distinct leaf morphology. Although clone P. 114 is graphically not included in the group, most likely because of its loose cluster structure, it does belong to 'Kadarka'. 'Olasz kadarka', 'Virághegyi kadarka', clones P. 167, P. 172 and P. 173 as a separate group can be definitely distinguished from 'Kadarka'. The old varieties, occurring mixed in the 'Kadarka' plantations such as 'Cigányszőlő', 'Csókaszőlő', 'Mészi kadarka' and 'Rácfekete' are separately displayed in Fig. 2, and among them a closer relationship of 'Csókaszőlő' and 'Mészi kadarka' is probable. 'Szagos kadarka' and 'Halápi szagos' constitute a separate group. It has been suggested that these two varieties might be the same (synonyms), since they have identical traits, referring to intravarietal variability and not two distinct cultivars.

Genetic diversity

N-coded microsatellite allele data are compiled in Table 5 and Table 6. New clones P. 108, P. 109, P. 111, P. 114, P. 115, P. 117, P. 122, P. 123, P. 124, P. 125, P. 131, P. 147 selected in the present project from the old plantation and clone P. 9 as well as the varieties 'Lúdtalpú kadarka', 'Ménesi kadarka', 'Kadarka gray' show identical SSR allele patterns with 'Kadarka' (Table 5.). Thus this group of clones and variants is

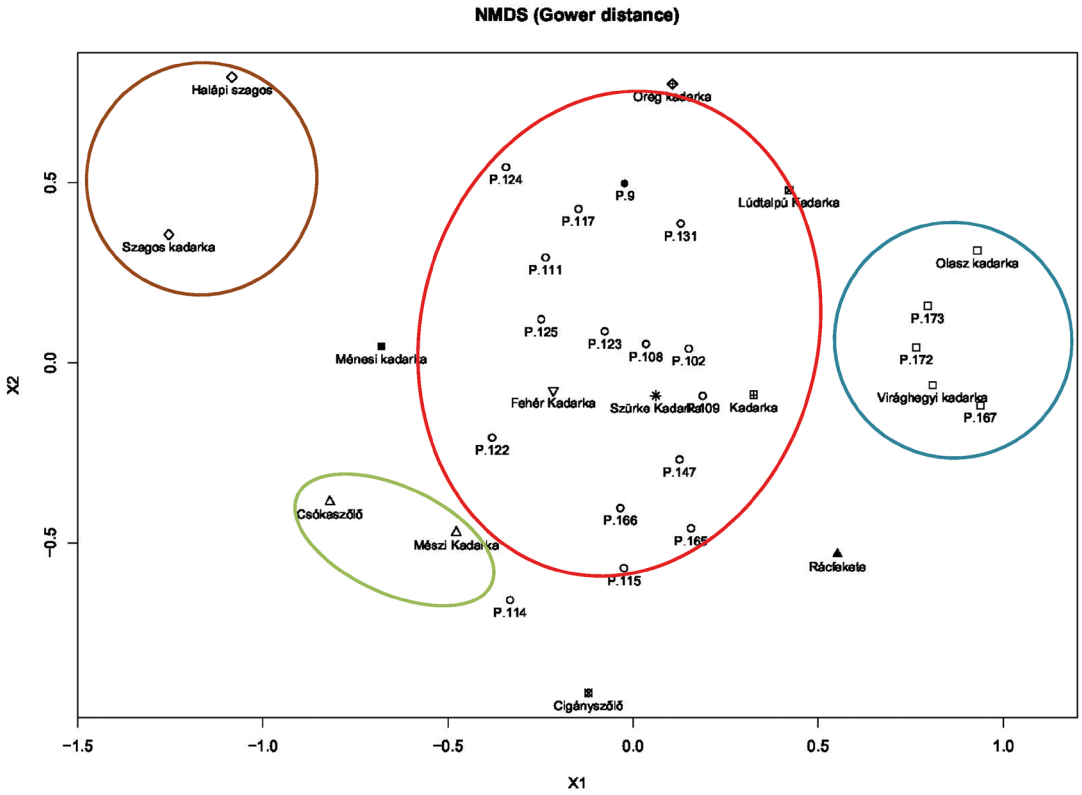

Fig. 2.: Grouping of 'Kadarka' varieties and selected clones based on the statistics of morphological traits

regarded as true-to-type 'Kadarka'. 'Fehér kadarka' (Table 6) differs from 'Kadarka' in loci VVMD5, VVMD27 and VVMD28, but these deviations do not exclude the possible parent-offspring relationship between them.

Another group with identical alleles is formed by 'Olasz kadarka' and 'Virághegyi kadarka' together with some new clones selected in the present project: P. 167, P. 172, P. 173, but differring from the 'Kadarka' (Table 6). This group of clones and variants is regarded as true-to-type 'Olasz kadarka'.

Allele profiles of 'Csókaszőlő' and 'Mészi kadarka' correspond in four loci, they have common alleles in other four loci, but the data in VrZag62 locus excludes the direct parent-progeny relationship between them.

'Cigányszőlő'-'Rácfekete' and 'Szagos kadarka'-'Halápi szagos' have the same microsatellite profiles with the selected primers, but these are different from the 'Kadarka' alleles in five-five loci.

Based on microsatellite alleles a dendrogram was constructed (Fig. 3.), showing that 'Kadarka' and 14 of the new clones (P. 108, P. 109, P. 111, P. 114, P. 115, P. 117, P. 122, P. 123, P. 124, P. 125, P. 131, P. 147, P. 165, P. 166) selected in this project from the old plantation in Szekszárd and clone P. 9 were grouped together and they are indistinguishable from each other. Only clone P. 102 was put into a separate cluster due to the allele size differences in five SSR loci (VVMD7, VVMD25, VVMD27, VVMD28, VrZag62). 'Olasz kadarka' constitutes another well distinguishable group with 'Virághegyi kadarka', and new clones P. 167) (Szek-szárd, old plantation, P. 172, P. 173 (gene bank, Pécs) having identical SSR patterns at the nine analyzed loci. This clustering and varietal distribution was confirmed by K-means analysis also (data not shown). Based on the microsatellite data 'Cigányszőlő' and 'Rácfekete' are not true-to-type 'Olasz kadarka' variants, but they stand closer to the 'Olasz kadarka' than to 'Kadarka' varieties. The same is true for 'Mészi kadarka' and 'Csókaszőlő'. 'Szagos

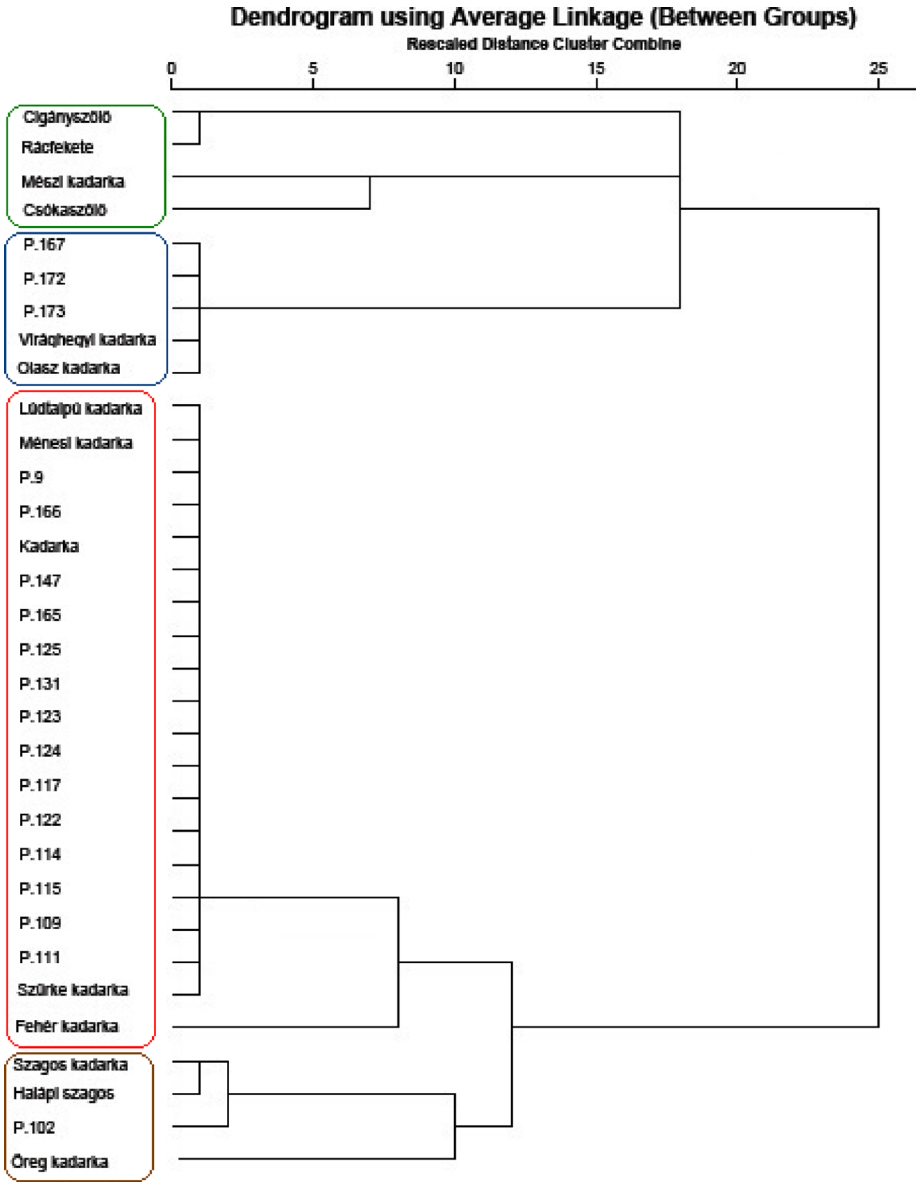

Fig. 3.: Dendrogram of 'Kadarka' varieties, variants and selected clones. Colour frames show the characteristic groups: 'Kadarka', 'Olasz kadarka', 'Mészi kadarka' and 'Szagos kadarka'.

kadarka' and 'Halápi szagos' cannot be differentiated from each other based on the microsatellite allele composition and they are both distinct from the new clone P. 102. Variety 'Öreg kadarka' stands apart from 'Fehér kadarka', 'Szagos kadarka' subcluster also from the most populous 'Kadarka' group (that includes 'Lúdtalpú kadarka', 'Ménesi kadarka', 'Szürke kadarka', clone P. 9, and new clones: P. 108, P. 109, P. 111, P. 114, P. 115, P. 117, P. 122, P. 123, P. 124, P. 125, P. 131, P. 147, P. 165, P. 166, which have identical SSR profiles at the 9 loci.

Discussion

Results of morphological and molecular analyses that confirmed the variability of 'Kadarka' varieties had been previously reported in literature (Drucker, 1906; Kozma, 1967; Németh, 1967). We stated that the old plantation in Szekszárd (Parászta) is rich in different forms of 'Kadarka', well representing the intravarietal genetic diversity of the previous centuries. Statistical analysis of the morphological traits and the SSR data showed the clones of old plantation (Szeks-

Table 5: N-coded microsatellite allele data of new true-to-type ‘Kadarka’ clones and true-to-type ‘Kadarka’ variants

SSR loci	VVMD5		VVMD7		VVMD25		VVMD27		VVMD28		VVMD32		VVS2		VrZag62		VrZag79	
Variety	Coded allele		Coded allele		Coded allele		Coded allele		Coded allele		Coded allele		Coded allele		Coded allele		Coded allele	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
P. 9	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 102	N+4	N+4	N+8	N+18	N+14	N+20	N+6	N+20	N+20	N+30	N+29	N+37	N+10	N+10	N+14	N+22	N+12	N+12
P. 108	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 109	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 111	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 114	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 115	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 117	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 122	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 123	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 124	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 125	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 131	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 147	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 165	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
P. 166	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
Kadarka blue	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
Lúdtalpú kadarka	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
Ménesi kadarka	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12
Szürke kadarka	N+4	N+4	N+16	N+24	N+4	N+20	N+10	N+20	N+12	N+44	N+37	N+37	N+10	N+10	N+14	N+30	N+12	N+12

Table 6: N-coded microsatellite allele data of new true-to-type ‘Olasz Kadarka’ clones, true-to-type ‘Olasz kadarka’ variants and other with 'Kadarka' co-cultivated varieties

SSR loci	VVMD5		VVMD7		VVMD25		VVMD27		VVMD28		VVMD32		VVS2		VrZag62		VrZag79	
Variety	Coded allele		Coded allele		Coded allele		Coded allele		Coded allele		Coded allele		Coded allele		Coded allele		Coded allele	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Feher kadarkar	N+4	N+18	N+16	N+24	N+4	N+4	N+20	N+20	N+12	N+32	N+15	N+37	N+10	N+20	N+14	N+30	N+12	N+12
P. 167	N+4	N+16	N+18	N+24	N+4	N+4	N+6	N+10	N+12	N+32	N+15	N+37	N+10	N+30	N+30	N+30	N+12	N+22
P. 172	N+4	N+16	N+18	N+24	N+4	N+4	N+6	N+10	N+12	N+32	N+15	N+37	N+10	N+30	N+30	N+30	N+12	N+22
P. 173	N+4	N+16	N+18	N+24	N+4	N+4	N+6	N+10	N+12	N+32	N+15	N+37	N+10	N+30	N+30	N+30	N+12	N+22
Viraghegyi kadarka	N+4	N+16	N+18	N+24	N+4	N+4	N+6	N+10	N+12	N+32	N+15	N+37	N+10	N+30	N+30	N+30	N+12	N+22
Olasz kadakar	N+4	N+16	N+18	N+24	N+4	N+4	N+6	N+10	N+12	N+32	N+15	N+37	N+10	N+30	N+30	N+30	N+12	N+22
Öreg kadarka	N+4	N+10	N+16	N+16	N+14	N+20	N+10	N+20	N+28	N+44	N+17	N+37	N+12	N+20	N+14	N+30	N+12	N+12
Szagos kadarka	N+4	N+4	N+6	N+16	N+14	N+20	N+6	N+20	N+20	N+30	N+25	N+37	N+10	N+10	N+12	N+22	N+12	N+12
Halapi szagos	N+4	N+4	N+6	N+16	N+14	N+20	N+6	N+20	N+20	N+30	N+25	N+37	N+10	N+10	N+12	N+22	N+12	N+12
Meszi kadarka	N+4	N+10	N+16	N+16	N+4	N+20	N+6	N+100	N+20	N+44	N+17	N+37	N+10	N+22	N+12	N+22	N	N
Csokaszolo	N+10	N+10	N+16	N+16	N+6	N+20	N+6	N+10	N+20	N+44	N+17	N+23	N+10	N+22	N+30	N+30	N	N+14
Ciganyszölö	N+4	N+18	N+18	N+24	N+4	N+4	N+6	N+20	N+30	N+30	N+25	N+31	N+10	N+10	N+30	N+30	N	N+14
Racfekete	N+4	N+18	N+18	N+24	N+4	N+4	N+6	N+20	N+30	N+30	N+25	N+31	N+10	N+10	N+30	N+30	N	N+14

zárd) and gene bank origin (Pécs) to be distinguishable. It can be concluded that for characterization, differentiation and exploration of genetic relationships of 'Kadarka' varieties a combination of morphological and molecular methods similarly to the investigation of other cultivars can successfully be applied (Ortiz et al., 2004; Boso et al., 2005; Zulini et al., 2005; Martin et al., 2011; Storchi 2011).

Based on morphological and molecular results four groups could be differentiated within the recently selected 'Kadarka' clones: 'Kadarka', 'Olasz kadarka', 'Szagos kadarka' and 'Mészi kadarka' types (Fig. 2, 3). The morphological and molecular classifications perfectly coincide in the case of 'Olasz kadarka' group. The largest morphological and molecular group is 'Kadarka', the consensus of the two categories is high, with the exception of 'Öreg kadarka' and clone P. 102. Inspite of the morphological similarity based on SSR data they got into the 'Szagos kadarka' group. To the only two-membered 'Mészi kadarka' morphological group the molecular analysis added two more cultivars: 'Cigányszőlő' and 'Rácfekete'.

'Virághegyi kadarka' and the clones P. 167, P. 172, P. 173 have different morphological traits from 'Kadarka' and belong to 'Olasz kadarka': the colour of young leaf (OIV 051) is green, the mature leaf is lighter green, the depth of lateral sinuses of mature leaf (OIV 094) is shallow, the overlapping of petiole sinus of mature leaf (OIV 079) is open, the berry shape (OIV 223) is broad ellipsoid, the time of bud burst (OIV 301) is late, the time of beginning of berry ripening (OIV 303) is late (Table 3, 4; Fig. 4.). Their genetic relatedness is reflected in the microsatellite profiles as well and is illustrated by the dendrogram constructed from the SSR data (Table 5, 6; Fig.3.). Based on morphological and SSR data 'Virághegyi kadarka' and clones P. 167, P. 172, P. 173 are identical to 'Olasz kadarka' ('Italian kadarka').

Their characteristic morphological traits are as follows: the colour of young leaf (OIV 051) is bronze,

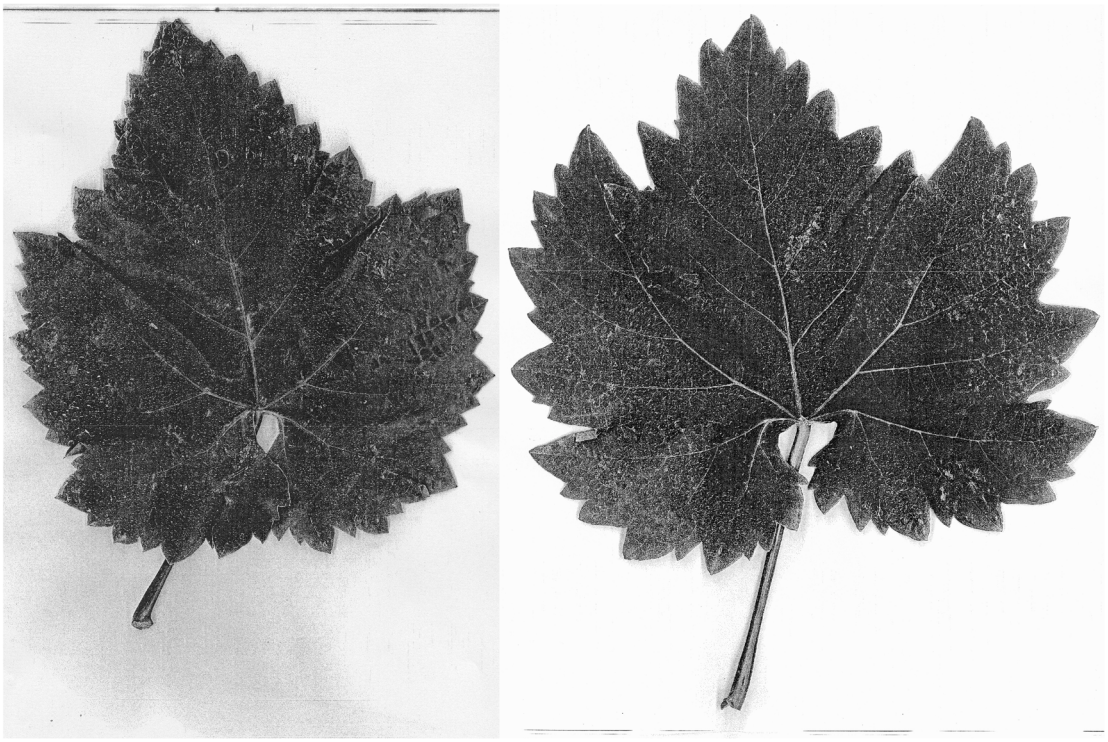

Fig. 4.: The mature leaf of 'Kadarka' and 'Olasz kadarka'

the leaf is dark green, the overlapping of petiole sinus of mature leaf (OIV 079) is closed and overlapped, the berry shape (OIV 223) is globose, the time of bud burst (OIV 301) is late, the time of beginning of berry ripening (OIV 303) is late (Table 3, 4; Fig. 6). Their genetic relatedness is reflected in the microsatellite profiles as well, the same allele sizes were obtained in all the nine selected loci (Table 5, 6; Fig. 3).

'Olasz kadarka' (Németh, 1967) along with the clones of P. 167, P. 172, P. 173 and 'Virághegyi kadarka' shows closer relationship with 'Mészi kadarka' – 'Csókaszőlő' and 'Halápi szagos' – 'Szagos kadarka' both at morphological and molecular level compared to Kadarka.

The P. 102 clone selected from the old plantation at Szekszárd were grouped together with 'Kadarka' based on its morphologic ampelographic traits, however, the molecular marker analysis characterized it as a different variety and separated it from the 'Kadarka' group. The allele size differences in five SSR loci (VVMD7, VVMD25, VVMD27, VVMD28, VrZag62) do not support the assumption that the P. 102 clone can be regarded as a 'Kadarka' seedling.

Based on morphological traits and NMDS analysis (Fig. 2) we can assume close genetic relationship between 'Csókaszőlő' and 'Mészi kadarka'. According to the SSR analysis they have the same allele composition at four loci, they share common alleles in other four loci, but the data in VrZag62 locus excludes the parent-progeny relationship, which can be explained by the assumption that they might have derived from intercrossings between each other and their seedlings. The OIV descriptor pointed out that the majority of the morphological traits correspond to the characteristics described by Németh (1966). The gene bank of the Institute of Viticulture and Oenology Pécs provided the possibility of clarifying the relationships supposed by Németh (1966, 1970).

Our morphological and molecular genetic results prove that 'Szagos kadarka' and 'Halápi szagos' are the same cultivars and they stand closer to 'Kadarka' than 'Olasz kadarka'.

The morphological and microsatellite results confirm the hypothesis of Németh (1967) that 'Fehér kadarka', 'Öreg kadarka' and 'Szagos kadarka' differ from the 'Kadarka', and they are distinct varieties.

Németh (1967) considered 'Kadarka gray' ('Szürke kadarka') as a berry colour variant of 'Kadarka', their identical SSR profile at nine loci supports this assumption. In order to provide a full proof further investigation is necessary: discrimination of berry colour variations of 'Kadarka gray' from 'Kadarka' by other genetic markers, e. g by retrotransposon-based markers.

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