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Outline analysis of the grapevine (*Vitis vinifera* L.) berry shape by elliptic Fourier descriptors

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Summary

Grapevine berry morphology is one of the most important features in table grape production. In this study, berry samples of 46 grapevine accessions were investigated for 3 consecutive years with elliptic Fourier descriptors (EFD) to evaluate shape diversity. Ten reference shapes obtained from the OIV descriptor list were involved and principal component (PC) scores summarizing the EFD's were statistically evaluated with Two way ANOVA and discriminant analysis. The cummulative contribution of the five principal components was 96.83 %. Two way ANOVA revealed that berry shape had high variability within the accessions and years. Based on the linear discriminant analysis, reference shapes were compared to those of the accessions and graphic reconstruction was carried out. OIV references were considered as unknown samples and grouped into the accession classes. Overall correct classification of the accessions into their group was 13.88 %. Our results showed that EFD together with reference shapes are a powerful method to discribe berry shape and possibly give the future basis of uvometric evaluation of grapevine cultivars.

Key words: uvometry; shape description; diversity; machine vision; image processing.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the most important horticultural crops with 7.4 Mio. ha worldwide. The production is 77.8 Mio. t, with 57 % wine grapes, 36 % table grapes and 7 % dried grapes (OIV, 2019). According to the International Organisation of Vine and Wine (OIV) "The world's vine stock is composed by more than 10,000 varieties" and the morphological characteristics of these genotypes are variable, described according to multiple traits detailed in ampelographic literature (Robinson *et al*. 2012) and descriptor lists (OIV, 2009). Among the organs shoot, leaf, bunch, and berry are the most relevant for characterization and identification of the genotypes.

Berry shape is an important property in viticulture, especially for table grapes. This is one of the primary selection criteria for consumers (Ferrara *et al.* 2017). To define the phenotypic variability and idenfity the accession, descriptor lists have been developed, where different authors mention distinct grouping of berry shapes. For example Pacottet (1905) who differed 5 groups or Bioletti (1938) who described 15 groups of berry shapes. Moreover Goussard (2008) used 6 groups in his works. Nowadays, the ampelographic key given by the OIV (2009) is widely applied to describe the morphological features of the berries. In this classification 10 different shape references are offered.

There are several methods to describe morphological features, for instance simple descriptors as size, area, circularity or eccentricity. Other prevailing methods are the closed contour based techniques. We can compare shapes according to all of their points, in this case every point of the shapes are considered as feature points, this method is called correspondance-based shape matching (Zhang *et al.* 2004). Elliptic Fourier Descriptors (EFD) are also used for shape description, where only the object boundary bears information. With the help of the EFD, closed contour objects can be described regarding the shape (Kühl and Giardina 1982).

There are numerous agricultural studies evaluating the shape according to the outline of the fruits, leaves or flowers. For instance Bubeníčková (2010) determined the shape variations of the tubers of differerent potato cultivars. The figure of dissimilar grain types were evaluated based on their contours by Mebatsion *et al.* (2012). Red Haven peach cultivar's shape were described in different maturity stages by Severa (2008), who later examined the shape of coffee bean (Severa *et al.* 2010). Demir *et al.* (2018) distincted the size and shape of walnut, evaluated shape index, average diameter, surface area, sphericity and volume of the fruits. EFD's are applied in grapevine phenotyping, too. For example leaf shape of different accessions were described by Diaz *et al.* (1991) and Chitwood *et al.* (2014). Furthermore, ancient and modern grapevine seeds were analysed and distinguished by Orrú *et al.* (2012) and Pagnoux *et al.* (2015). In Chile a very promising digital image-analyses tool was developed in connection with berry attributions, such as diameters at different locations of the berries, shape and

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color (Cid et al. 2019). The objective of this recent study was to evaluate the berry shape of 46 grapevine accessions in three consecutive years, both table and wine grapes based on EFD's. Furthermore, the reference shapes of the OIV (2009) were involved to validate the berry shapes of EFD's.

Material and Methods

S a m p l i n g : Samples for this study were collected in three consecutive years (2018-2020) in the Institute of Viticulture and Enology, Hungarian University of Agriculture and Life Sciences (Kecskemét, Hungary; VIVC: HUN047). At the germplasm collection 5 plants per accession were maintained under the same viticultural conditions: trained on one-cane umbrella with 1.2 meter plant and 3 meter row distance with NW-SE row orientation. Plant protection, nutrient supply, and canopy management of the plants were uniform. After the visual inspection of more than 300 grapevine accessions before full ripening, those 46 were chosen for detailed morphological description, which represented large morphological variability (Tab. 1). Berry samples (n=30/accession/year) of the investigated accessions were obtained from the middle third of 5 to 10 bunches at full ripening according to the OIV (2009) (Fig. 1). Only those bunches and berries were selected for investigation which showed no symptoms or untypical phenotypic traits. Samples were stored in plastic boxes at 4 °C until digitalization.

I m a g e a c q u i s i t i o n : Pedicels and receptacles were gently removed from the berries with sharp scissors. Afterwards, berries were placed on a transilluminating LED light box in the same position and digitized individually by a Sony SLT-A58 camera (Sony Corp. Tokyo) with a Sony SAL35F18 (Sony Corp. Tokyo) lens mounted and ISO100 sensitivity (Fig. 1).

S h a p e r e f e r e n c e s : Ten shape references, namely: broad ellipsoid, cylindric, finger-shaped, globose, horn-shaped, narrow ellipsoid, obloid, obovoid, obtuse ovoid, ovoid obtained from the OIV descriptor list (OIV, 2009) have been included in the study. The reference berry figures were re-drawn in high resolution with GIMP (GIMP 2.10.28), and these figures were then included in the sample set (Fig. 1).

O u t l i n e a n a l y s i s : This step was carried out with the SHAPE software package (Iwata and Ukai 2004). Shape evaluation was performed on the images of the investigated grapevine accessions and OIV references. The file format of the images was changed from JPEG to bitmap (BMP). Altogehter 4140 images were investigated in this study. SHAPE contains 4 different softwares, namely "ChainCoder" to get the chain codes of the object's outlines. "CHC2Nef" to calculate the Elliptic Fourier Descriptors (EFDs). "PrinComp" to compute the PCs of the normalized EFDs and "PrinPrint" to draw the shape alterations by an inverse Fourier transformation. In the SHAPE there are two possibilities to determine the starting point for tracing the contour, one of them is when the starting point is the farthest point from the object's center, the other possibility is when the starting point is standardized according to the major axis. This latter method was applied in this study because the farthest point from center of the berries varied. Based on our previous examinations (data not shown) 50 harmonics were used to evaluate the Elliptic Fourier Descriptors in this study.

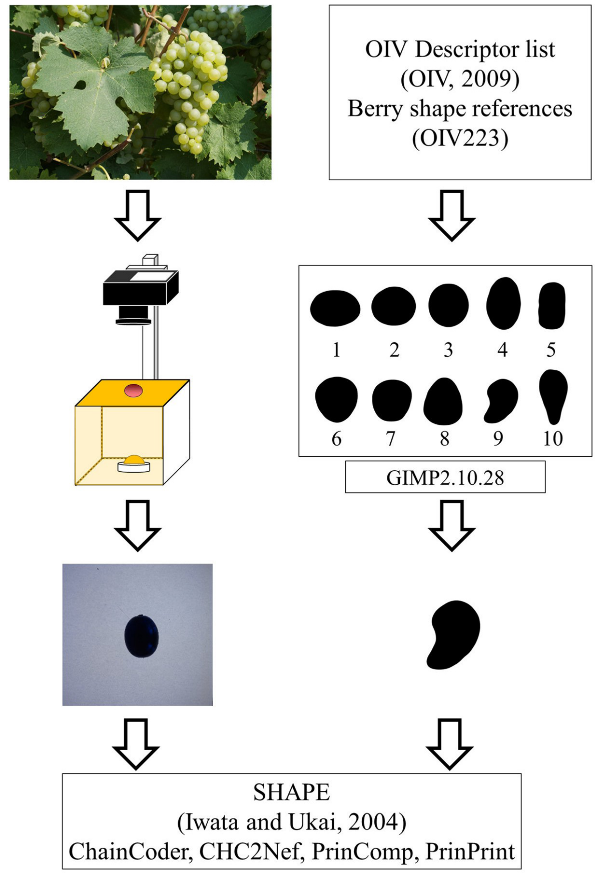


Fig. 1: Berry sample and reference image outline analysis.

S t a t i s t i c a l e v a l u a t i o n : Effective principal component (PC) scores of the single berry's data (n=30/accession/year) were statistically analysed. Two-way Anova (Two-Way Analysis of Variance) and Kruskal-Wallis test was performed as non-parametric comparison to evaluate the berry shape differences among the accessions influenced by the year. Dunn post-hoc test was carried out, where the level of the significance was set to $p < 0.05$. Linear Discriminant Analysis (LDA) was applied to determine the successful recognition rate of the accessions. For this analysis reference shape samples obtained from the OIV descriptor list were considered as 'unknown' samples with the aim to group them into the investigated accession groups. Jackknife technique was used for that which means that the method works by removing a small amount of data at each time and reclassifies the shortened data set, continuously until all the data are classified. An UPGMA dendrogram was constructed based on the Euclidian distance matrix of the reference shape PC values and mean PC values (PC1-PC5) of each accession. All the statistical evaluations were carried out in PAST (Hammer et al. 2001).

S h a p e r e c o n s t r u c t i o n : Shape reconstruction of those accessions ('Pölöskei muskotály', 'Rajnai rizling', 'KM95', '13/2', 'Pannónia kincse', 'Moldova', 'KM249', 'R80') were performed by inverse Fourier transformation, where OIV shape references were grouped in based on the LDA. This step was carried out based on the 90 berries collected in the 3 years (Tab. 4).

Table 1

The list of the 46 investigated grapevine accessions and classification accuracy according to the LDA

No.	Accession	VIVC Number	Color	Country of origin	Utilization	Correct classification based on the LDA
1	Admirable de Courtiller	68	B	Fr	W	9 %
2	Agata	93	B	Fr	W/Ro	14 %
3	Boglárka	1510	B	Hu	T	20 %
4	Bouvier	1625	B	Slo	W/T	5 %
5	Cardinal	2091	R	USA	W/T	8 %
6	Chardonnay	2455	B	Fr	W	8 %
7	Chasselas	2473	B	Fr	W/T	0 %
8	Cirfandli	13443	R	A	W	11%
9	Cabernet sauvignon	1929	N	Fr	W	8 %
10	Csokonai	3280	B	Hu	T	6 %
11	Erzsébet királyné emléke	3950	B	Hu	T	1 %
12	Eszter	20341	N	Hu	T	1 %
13	Irsai Olivér	5557	B	Hu	W/T	14 %
14	Italia	5582	B	Ita	W/T	9 %
15	Karola	5557	B	Hu	W/T	19 %
16	Kékfrankos	1459	N	Slo	W/T	25 %
17	Kismis moldavszkij	14053	R	Md	T/Ra	4 %
18	Malaga kék	2672	N	Fr	W/T	1 %
19	Mátrai muskotály	15928	B	Hu	W	21 %
20	Mathiász Ernőné	7497	B	Hu	T	21 %
21	Merlot	7657	N	Fr	W	9 %
22	Mikszáth	7714	N	Hu	T	7 %
23	Moldova	7896	N	Md	T	35 %
24	Muscat Bouschet	8194	N	Fr	W	3 %
25	Olasz rizling	13217	B	Ita	W	15 %
26	Palatina	14012	B	Hu	T	9 %
27	Pannónia kincse	8915	B	Hu	T	34 %
28	Perlette	9168	B	USA	T/Ra	2 %
29	Pölöskei muskotály	8207	B	Hu	W/T	18 %
30	Rajnai rizling	10077	B	D	W	5 %
31	Ruszbol	16995	B	Rus	T	4 %
32	Sztrasenszkij	15631	N	Md	T	1 %
33	Trollingi kék	10823	N	Ita	W/T	2 %
34	Urozsajnüj	12790	B	Md	T	17 %
35	Usztojcsivüj gyikij	17783	B	Ua	-	5 %
36	Viktória gyöngye	14318	B	Hu	T	12 %
37	Vitis typ Weiss	-	-	-	-	25 %
38	KM249	-	B	Hu	T	27 %
39	KM144	-	B	Hu	T	20 %
40	KM238	-	B	Hu	T	4 %
41	KM95	-	-	Hu	-	6 %
42	R12	-	B	Hu	T	13 %
43	R24	-	B	Hu	T	4 %
44	R80	-	B	Hu	T	50 %
45	13/2	-	-	-	-	49 %
46	13/5	-	-	-	-	2 %

Color: B - white, N - blue, R - rouge. Utilization: T - table grape, W - wine grape, Ra - raisin, Ro - rootstock.
Origin: A - Austria, D - Germany, Fr - France, Hu - Hungary, Ita - Italy, Md - Moldova, Rus - Russia,
Slo - Slovenia, Ua - Ukraine, Usa - United States of America.

Results

P r i n c i p a l c o m p o n e n t a n a l y s e s : Among the principal components (PC) explained, the shape variblity of those investigated 30 berries of each grapevine accession in three consecutive years (altogether 4140 berries), 5 proved to be effective, from which the PC1 and PC2 explained 89.53 % and 4.01 % respectively. Though the other 3 effective principal components explained 3.27 % of the berry shape features (PC3: 1.38%; PC4: 1.35%; PC5: 0.54 %). The 5 effective components explained altogether 96.83 % of the shape variablity (Tab. 2). The PC1 was associated with the roundness of the berries (Fig. 2). The higher PC1 values explained the globoid berries and the lower values those of more ovoid ones. The PC2 was associated with the wideness of the berry, for instance on which part the berry was wider. If the PC2 value has been higher, the berry shape was obtuse ovoid, so it was wider on the top. If the PC2 has been lower, the berry was obovoid, it was wider at the bottom. The PC3 and PC4 were in relation with the symmetry of the berries. The PC5 showed the squareness of the berries. When the PC5 was higher, the berry was more oval and the lower PC5 value meant more blockish shape.

T a b l e 2

The 5 effective principal components (PC) corresponding the berry shape variability of the 46 grapevine accessions

PC	Eigenvalue	Proportion (%)	Cumulative (%)
PC1	$7.55*10^{-03}$	89.54	89.54
PC2	$3.38*10^{-04}$	4.01	93.55
PC3	$1.17*10^{-04}$	1.39	94.93
PC4	$1.14*10^{-04}$	1.35	96.29
PC5	$4.58*10^{-05}$	0.54	96.83

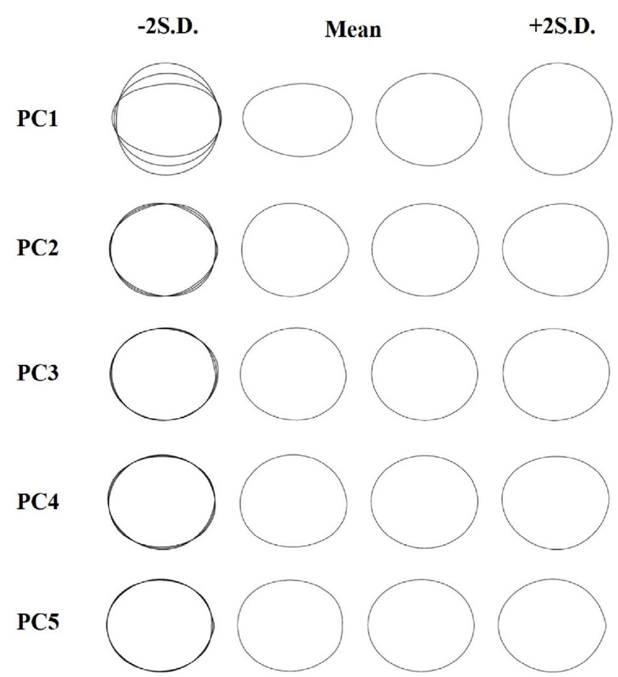


Fig. 2: Reconstruction of the mean berry shapes ± 2 S.D. of the 46 grapevine accessions corresponding to the 5 effective principal components.

S t a t i s t i c a l a n a l y s i s o f t h e P C s c o r e s : Kruskal-Wallis test was used to determine the significant difference of the PC values among the accessions. The further examination using the Dunn's second order test proved that the samples were significantly different. The Two-way ANOVA analysis of the PC values have been carried out. One of the two factors was the vintage, the other was the accession. Values of the PC1, PC2, PC3 and PC4 were significantly influenced by both of the vintage and the accession, and furthermore by the interaction of the two factors. Values of PC5 were significantly influenced by the accessions to which the berries belonged to and by the interaction of the two factors (Tab. 3). Considering the average data of the three consecutive years, the lowest PC1 value was belonging to '13/2', so the '13/2' accession's berries were the most ovoid ones, while the highest value was observed for 'Mátrai muskotály', which had the most globoid berries. In case of PC2, the 'Matthiász Ernőné' had the lowest value, the accession had the most obovoid shape and 'R80' accession the highest, so the most obtuse ovoid shaped. Along PC3, 'Moldova' had the lowest value, thus this accession had the vertically most symmetrical berry shape, while the highest value belonged to '13/2', which means that '13/2' had the most asymmetric shape and it had the highest value along the PC4 also, so the most horizontally asymmetric as well. In the case of this principal component 'Pannónia kincse' had the lowest values, the horizontally most symmetric shaped and in the meantime it was the highest of PC5, so the berries were the blockiest of all. Lastly, the PC5's lowest value belonged to 'Kékfrankos', that means this accession had the most oval shape.

D i s c r i m i n a n t a n a l y s i s : A discriminant analysis was carried out to reveal the ratio of the succesfully recognised accessions based on the principal components of the berries. First discriminant functions explained 89.22 % of the variance, while the functions 2, 3 and 4 explained 6.86 %, 2.31 % and 0.88 % respectively. In this study, 13.88 % of the samples have been classified correctly (Jackknifed data). The 49.00 % of berries of the '13/2' accession was classified correctly. 'R80's' berries were characteristical in 60.00 % and other 2 accession's berries ('Moldova', 'Pannónia kincse') were refered in 35.00-34.00 %. There were 7 accessions ('KM249', 'Kékfrankos', 'Mátrai muskotály', 'Matthiász Ernőné', 'Boglárka', 'KM144', '*Vitis* typ Weiss') which berries' 20.00-27.00 % were listed into their original group. The next 30 accession's berries were listed in between 18.89 % and 2.00 %. While the 'Erzsébet királyné emléke', 'Eszter', 'Malaga kék', 'Straszenszkij' (1.11-1.12 %) and 'Chasselas' (0.00 %) cultivar samples fitted least into their genuine classes.

C o m p a r i s o n o f t h e s h a p e r e f e r e n c e t o t h e a c c e s s i o n s : Furthermore the LDA classified the OIV's reference berry shapes depending on their similarity to the examined cultivars. After the classification, contour reconstruction of the 90 berries of each accession were carried out with the help of the Shape (Iwata and Ukai 2002) software package. The obtained average contours were placed into a table (Tab. 4) also representing the reference shapes. Based on the LDA the *cylindric, narrow* and *finger-shaped* reference shapes were grouped into the '13/2'

Table 3

Two-Way ANOVA of the 5 effective principal components

PC1	Sum of squares	df	Mean square	F	p
Vintage:	3.52×10^{-01}	2	1.76×10^{-01}	183.80	4.66×10^{-77}
Accession:	23.0	45	5.12×10^{-01}	534.30	0.00
Interaction:	2.85	90	3.17×10^{-02}	33.11	0.00
Within:	3.81	3980	9.58×10^{-04}		
Total:	30.1	4117			
PC2	**Sum of sqrs**	**df**	**Mean square**	**F**	**p**
Vintage:	7.85×10^{-03}	2	3.92×10^{-03}	30.24	9.27×10^{-14}
Accession:	2.00×10^{-01}	45	4.46×10^{-03}	34.33	1.98×10^{-245}
Interaction:	3.57×10^{-01}	90	3.96×10^{-03}	30.54	0.00
Within:	5.17×10^{-01}	3980	1.30×10^{-04}		
Total:	1.08	4117			
PC3	**Sum of sqrs**	**df**	**Mean square**	**F**	**p**
Vintage:	8.63×10^{-03}	2	4.32×10^{-03}	52.98	1.96×10^{-23}
Accession:	2.14×10^{-02}	45	4.76×10^{-04}	5.84	2.59×10^{-31}
Interaction:	7.05×10^{-02}	90	7.83×10^{-04}	9.62	1.10×10^{-112}
Within:	3.24×10^{-01}	3980	8.15×10^{-05}		
Total:	4.25×10^{-01}	4117			
PC4	**Sum of sqrs**	**df**	**Mean square**	**F**	**p**
Vintage:	4.48×10^{-03}	2	2.24×10^{-03}	27.77	1.06×10^{-12}
Accession:	2.07×10^{-02}	45	4.59×10^{-04}	5.70	3.32×10^{-30}
Interaction:	6.75×10^{-02}	90	7.50×10^{-04}	9.30	4.02×10^{-108}
Within:	3.21×10^{-01}	3980	8.06×10^{-05}		
Total:	4.13×10^{-01}	4117			
PC5	**Sum of sqrs**	**df**	**Mean square**	**F**	**p**
Vintage:	9.18×10^{-05}	2	4.59×10^{-05}	1.14	3.20×10^{-01}
Accession:	1.31×10^{-02}	45	2.91×10^{-04}	7.22	5.17×10^{-42}
Interaction:	3.48×10^{-02}	90	3.86×10^{-04}	9.59	2.48×10^{-112}
Within:	1.60×10^{-01}	3980	4.03×10^{-05}		
Total:	2.08×10^{-01}	4117			

accession's berries, while the *obovoid* category resembled to the 'KM249' accession. *Broad ellipsoid* showed the main features of the 'KM95' accession and *ovoid* referred the shape of 'Moldova' accession's berries. In addition, *horn-shaped* berries were characteristical of 'R80'accession, berries of 'Rajnai rizling' were *globose.* The *obtuse ovoid* shape was typical of 'Pannónia kincse' berries and *obloid* shape to 'Pölöskei muskotály'. A dendrogram construced by the Euclidian distance matrix of the mean PC (PC1-PC5) values of the accessions and the reference shapes showed large variablity (Fig. 3). *Finger-shaped* reference showed difference from all samples and located on a seprated cluster. Beside this 3 main clusters were identified where *cylindric*, *narrow ellipsoid* and *horn-shaped* reference are together with the accession '13/2'; and *obovoid* reference shape with 'KM238', 'R24', 'Urozsajnüj', 'R80', '13/15' and '*Vitis* typ. Weiss'. The largest cluster with two sub-clusters consists most of the samples. Members of one sub-cluster are similar to the *globose* shape reference, while the others more to the *obtuse*, *ovoid*, *obloid* and *broad ellipsoid*.

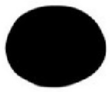
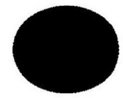
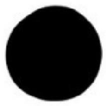
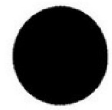
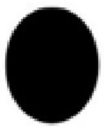
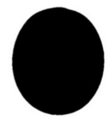
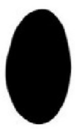
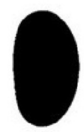
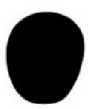
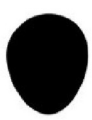
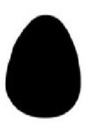
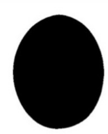
Discussion

This experiment showed that digital outline analysis is an accurate method for grapevine berry phenotyping. Our earlier results verified that berry shape is a variable trait and those descriptions based on image analysis revealed intravarietal diversity and the effect of different canopy management practices on the uvometric traits (Bodor *et al.* 2020, Somogyi *et al.* 2019). Recently Kupe *et al.* (2021a, 2021b), analysed the morphological traits on horizontally and vertically oriented berry samples of Turkish grapevine cultivars. Their findings showed that both the horizontal and vertical properties are significantly different among the cultivars. Investigation of the vertical section of the berries would provide valuable information about the shape diversity in this way. We consider to include this into our future experiments.

Bioletti (1938) claimed that there are several factors affecting berry shape. One factor can be the condition of the vine, well nourished plants have more characteristical and more uniform berry shapes. Other influencing modulus is the compactness of the cluster, berries touching each other can change each other's forms while they grow, so they become distorted. On the other hand the shape and number of the included seeds can have an effect on the berry shape (Facsar 1971, 1972, Bioletti 1938). Our earlier findings are in agreement with these and showed that seed number of the 'Italia' grapevine cultivar has significant effect on the berry morphological attributes (Somogyi *et al.* 2021). Furthermore the rate of the morphological variability is

Table 4

The classification of the 10 grapevine reference berry shapes (OIV223) obtained from the OIV (2009) descriptor list

Nr.	Reference shape*	Berry shape	Example variety of OIV	Classification**	Accessions' shape***
1.		obloid	Chasselas Michel Tompa	Pölöskei muskotály	
2.		globose	Chasselas	Rajnai rizling	
3.		broad ellipsoid	Barbera	KM95	
4.		narrow ellipsoid	Olivette noir	13/2	
5.		cylindric	Kahlili Belyi	13/2	
6.		obtuse ovoid	Ahmeur Bou	Pannónia kincse	
7.		ovoid	Bicane	Moldova	
8.		obovoid	Mouscat d'Alexandrie	KM249	
9.		horn-shaped	Santa Paula	R80	
10.		finger-shaped	-	13/2	

*obtained from the OIV (2009) desriptor list: OIV223
** the name of the accession where OIV reference figure was classified in based on the LDA
*** shape reconstruction of the accession based on the 90 berries collected in the 3 years

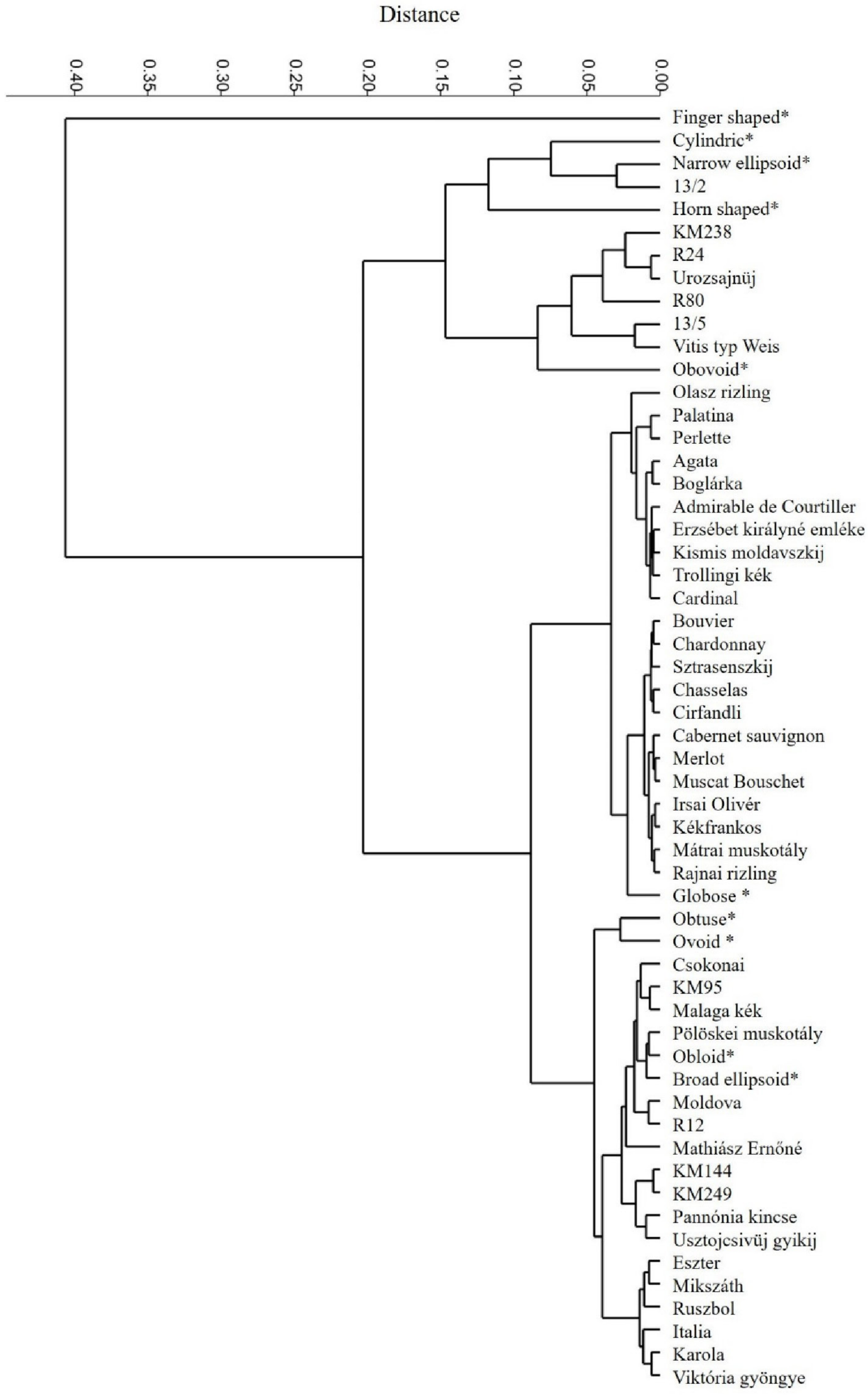


Fig. 3: UPGMA dendrogram constructed based on the Euclidian distance matrix of the reference shape PC values and mean PC values (PC1-PC5) of each accession (*indicates the OIV reference shapes).

different not only within the accessions, but within each of the bunches too. This was verified in Bodor *et al.* (2020) where samples from the different position of the bunches were compared and berries collected from the top, middle and bottom of the bunch had different morphology. To exclude any external factor, in the present study samples were collected from uniform cultivation circumstances from the middle of several bunches. We found that wihin each accession the berry shape is not uniform and there is a noticable difference among the samples belonging to the same genotype, even those being collected in the same year and same position of the bunch. The intravarietal variability is a known phenomenon and already mentioned by Kozma (1968). This inhomogeneity would cause that LDA showed low efficiency in the correct grouping of the samples in this study. Highest correct classification (50.00 %) was observed in the case of "R80", while the overal correct classification was 14.04 %. This result confirms that berry shape is a divers trait not only among, but within the accessions. Beside the above mentioned factors on the berry shape diversity, year to year effect is also noticable. Duchêne and Schneider (2005) showed that phenological stages and berry development is highly influenced by the climatic conditions. We found that there are certain accessions where berry shape was affected by the year as a factor. PC values for example belong to '13/2', '13/5', 'Boglárka', and 'Italia' showed significant differences among the investigated years, assuming that berry shape is influenced by the vintage.

We conclude that digital image outline analysis is a powerful, time saving and accurate tool to evaluate grapevine berryshape diversity, where included reference berry figures would improve the accuracy of the phenotyping.

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