TRADITIONAL AND LANDMARK-BASED GEOMETRIC MORPHOMETRIC ANALYSIS OF TABLE GRAPE CLONE CANDIDATES

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Leaf morphological diversity of nine 'Pannónia kincse' grapevine (*Vitis vinifera* L.) clone candidates has been evaluated according to 16 ampelometric OIV descriptors, 7 secondary characters and 17 geometric morphometric landmarks. We examined the symmetry of the morphological character values and defined the symmetric features. Based on these results missing morphological data on the right side of the leaf were replaced with the data on the left side. ANOVA was carried out for the morphological characters to find differences between the clone candidates. Results showed that the primary descriptors 602, 606 and 607 differ significantly between the samples. Based on raw data classified into categorical variables all candidates were distinguished. According to the canonical variate analysis carried out on the geometric morphometric coordinates there is no significant difference in the shape of the clone candidates. Based on the thin plate spline graphic reconstruction leaves of the candidates show diverse shape.
Keywords: *Vitis vinifera*, 'Pannonia kincse', leaf morphology, ampelometry

Traditionelle und Bezugspunkt-basierte geometrisch-morphometrische Analyse von Tafeltrauben-Klon-Kandidaten: Die Vielfältigkeit der Blattmorphologie von neun Klon-Kandidaten aus der Klonenselektion der Rebsorte 'Pannónia kincse' (*Vitis vinifera* L.) wurde nach 16 OIV-Deskriptoren, 7 sekundären Eigenschaften und 17 geometrisch-morphologischen Bezugspunkten bewertet. Auch die Symmetrie der morphologischen Merkmale wurde untersucht, und die symmetrischen Eigenschaften wurden definiert. Anhand dieser Ergebnisse konnten fehlende Werte einer Blattseite mit Werten der anderen Seite ergänzt werden. ANOVA der morphologischen Merkmale wurde durchgeführt, um Unterschiede zwischen Klon-Kandidaten festzustellen. Die OIV-Deskriptoren 602, 606 und 607 unterschieden sich deutlich zwischen den Kandidaten. Durch Transformation der Daten in Kategorien konnten alle Kandidaten unterschieden werden. Laut der kanonischen Variantenanalyse der geometrisch-morphologischen Bezugspunkte gibt es keine signifikanten Unterschiede zwischen den Blattformen der Klon-Kandidaten. Die graphische Rekonstruktion mittels "Thin-Plate Spline"-Methode zeigt hingegen unterschiedliche Blattformen der Klon-Kandidaten.
Schlagwörter: *Vitis vinifera*, 'Pannónia kincse', Blattmorphologie, Ampelometrie

INTRODUCTION

Clonal selection of the grapevine (*Vitis vinifera* L.) cultivars has high importance in viticulture to provide differences in fruit composition (Fidelibus et al., 2006; Petric et al., 2016), in vegetative performance (Miotto et al., 2014) or in resistance (Boso et al., 2006). Hungary has a long history in table grape breeding and selection (Hajdu, 2015). 'Pannónia kincse' (cross between 'Szőlőskertek királynője muskotály' × 'Cegléd szépe') is one of the most widespread cultivars in the country. Its ripening time is the end of August, density of the bunch is medium, transportability is good. Breeding of the cultivar by selection of new clone candidates has been started to improve cluster architecture and berry size (Hajdu and Borbásnè Sasköi, 2003; Hajdu, 2009). Since the clone candidates have unique, favourable characteristics there is a strong need to describe and identify them. Among other parameters leaf morphology has been in the focus of grapevine identification from the very beginnings. Ampelometry (from Greek *ampelos* “vine” and *metria* "measurement") is the study of the metric characterization of grapevines. Although ampelometry refers to all the organs (e. g. leaf, bunch, berry) it mostly means leaf description. During the last two centuries this study has been improved in many ways. Acerbi (1825) and Goethe (1887) focused on shape and lobature in classification. Ravaz (1902) defined homologous points on the leaf lamina. It served as the base of traditional morphometry which was later expanded by Galet (1956). Bourisquot et al. (1987) suggested computer supported characterization, implemented by Alessandri et al. (1996) and Soldavini et al. (2009). Most recent leaf morphological descriptions of the members of the *Vitis* taxa are based on landmark-based geometric morphometry (GMM) (Chitwood et al., 2016a, 2016b) which is mainly based on Cartesian landmark coordinates evaluated statistically (Viscosi and Cardini, 2011).

To find morphological differences between the 'Pannónia kincse' clone candidates for identification, leaf ampelometric characterization was carried out with three methods: primary OIV (2009) descriptors, secondary characters introduced by Galet (1956) and landmark-based geometric morphometric tools.

MATERIALS AND METHODS

PLANT MATERIAL

Samples were collected in the germplasm collection of the National Agricultural Research and Innovation Centre Research Institute for Viticulture and Enology, Kecskemét (Hungary) in 2013. Clone candidates were planted in 20 replicates derived from the original mother plants. Ten leaves were sampled from the investigated 9 clone candidates (P.K.11; P.K.13; P.K.15; P.K.18; P.K.21; P.K.23; P.K.27; P.K.28; P.K.32) between berry set and *veraison* from the middle third of several shoots. Samples were stored in plastic bags until digitalisation. Scanning was performed with an HP ScanJet 4570c (Hewlett-Packard; Palo Alto, US) with 300 dpi resolution.

MORPHOLOGICAL ANALYSIS

Characterisation of the primary descriptors and record of the geometric morphometric coordinates were carried out with the GRA.LE.D. (2.04; LAAZ Bt, Szigetszentmiklós, Hungary) semi-automatic software according to Bodor et al. (2012, 2014). Morphological description was assessed by means of 17 landmarks, 16 OIV (Oiv, 2009) and 7 secondary characters on both sides of the leaves if possible: length of vein N_1 (OIV 601), length of vein N_2 (OIV 602), length of vein N_3 (OIV 603), length of vein N_4 (OIV 604), length petiole sinus to upper leaf sinus (OIV 605), length petiole sinus to lower leaf sinus (OIV 606), angle between N_1 and N_2 (OIV 607), angle between N_2 and N_3 (OIV 608), angle between N_3 and N_4 (OIV 609), angle between N_3 and the tangent between petiole point (OIV 610), length of vein N_5 (OIV 611), length of tooth N_2 (OIV 612), width of tooth N_2 (OIV 613), length of tooth N_4 (OIV 614), width of tooth N_4 (OIV 615), length between the tooth tip of N_2 and the tooth tip of the first secondary vein of N_2 (OIV 617). Derived from the primary descriptors 7 secondary characters were evaluated on both sides of the leaves: OIV 602/OIV 601, OIV 603/OIV 601, OIV 604/OIV 601, OIV 605/OIV 602, OIV 606/OIV 603, OIV 607 + OIV 608, OIV 607 + OIV 608 + OIV 609 according to Galet (1956). Beside the traditional morphological description 31 landmark coordinates were recorded for the GMM analysis.

STATISTICAL ANALYSIS

Despite of careful sampling some lobes, serration and tips were missing, thus only morphological characters on the right side of the leaves were statistically evaluated. Outliers were removed before replacing missing data on the right side of the leaves with the data obtained from the left side. Symmetry of the leaf characters was analysed with paired sample t-test on a dataset where data of both the two sides of the leaf were present (80 to 89 data-pairs). For morphological data analysis of the complete right leaf sides (e. g. 16 ampelometric OIV descriptors, 7 secondary characters). Analysis of Variance (ANOVA), Principal Component Analysis (PCA) and Multivariate Discriminant Analysis were performed. Statistical analyses were carried out in the PAST (Hammer et al., 2001). Data were transformed into categorical variables according to Galet (1956) and Oiv (2009) and cluster analysis were carried out to visualize relations between the samples.

Seventeen landmarks were recorded for the GMM analysis on the right side (Fig. 1) of the leaves. In the case of samples with missing landmarks on the right side coordinates were inverted from the other side of the leaf. If a landmark was missing on both sides (3 out of the 90 leaves) samples were omitted. Landmark superimposition was carried out. Outliers were removed, and covariance matrix was generated followed by PCA. Canonical variate analysis was performed to distinguish leaf samples of the clone candidates. Statistical analysis and GMM investigations were carried out with the PAST (Hammer et al., 2001) based on Viscosi and Cardini (2011).

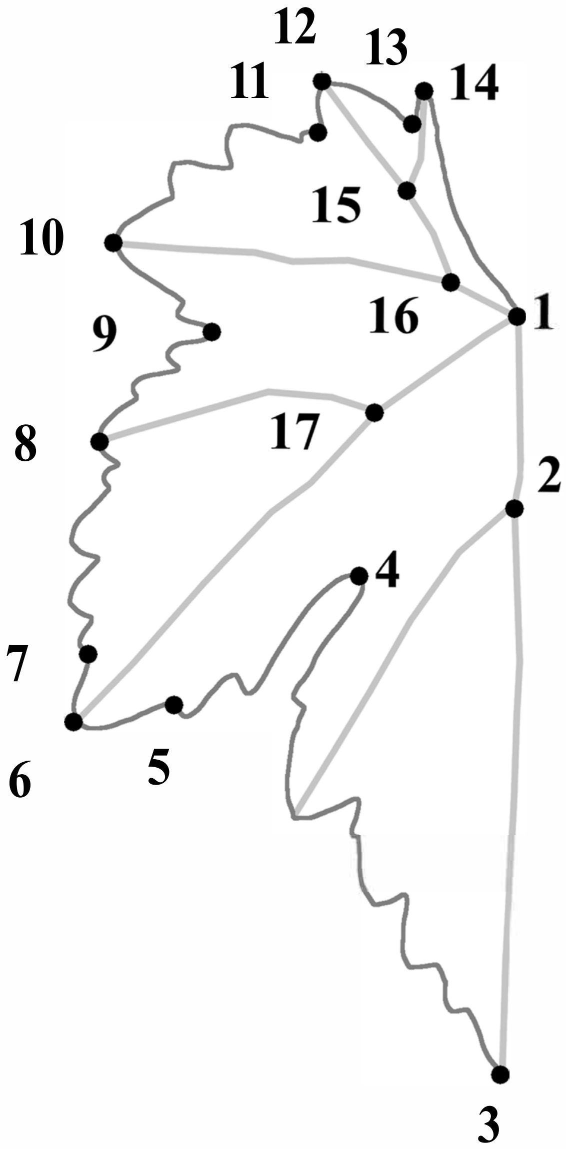

Fig. 1: Position of the 17 landmarks studied in the geometric morphometric analysis

RESULTS

TRADITIONAL MORPHOLOGICAL ANALYSIS

Altogether 90 samples were described based on 16 primary OIV and 7 secondary descriptors calculated by the positions of 17 geometric morphometric landmarks. Symmetry of the bilateral data was evaluated after removing outliers. According to the paired sample t-test all descriptors are symmetric along the leaf, but correlation is not significant in all cases. For example in the case of 608 correlation was weak, (corr.: 0.08 sig = 0.47) so this character was not included in the further evaluation. Beside primary descriptors secondary characters were also evaluated and symmetry of them was assumed (Table 1).

Table 1: Paired sample t-test performed on the 22 bilateral morphological characters

No.	Character OIV-Descriptor	Paired differences			t	df	Sig. (2-tailed)	Correlation	Sig.
		Mean	Std. dev.	Std. error mean					
1	602	0.025	6.950	0.754	0.033	84	0.974	0.771	0.000
2	603	0.354	6.009	0.648	0.546	85	0.586	0.746	0.000
3	604	-0.288	4.852	0.517	-0.557	87	0.579	0.617	0.000
4	605	0.089	6.248	0.674	0.133	85	0.895	0.609	0.000
5	606	0.545	6.940	0.736	0.741	88	0.461	0.668	0.000
6	611	-0.059	3.450	0.376	-0.158	83	0.875	0.616	0.000
7	612	0.112	2.252	0.244	0.459	84	0.647	0.707	0.000
8	613	0.395	2.851	0.306	1.291	86	0.200	0.505	0.000
9	614	-0.242	2.116	0.231	-1.050	83	0.297	0.438	0.000
10	615	0.075	2.096	0.229	0.328	83	0.744	0.461	0.000
11	617	0.889	7.739	0.830	1.071	86	0.287	0.612	0.000
12	607	-1.267	10.810	1.159	-1.093	86	0.277	-0.335	0.002
13	608	0.951	7.052	0.747	1.272	88	0.207	0.077	0.474
14	609	0.811	6.983	0.762	1.065	83	0.290	0.280	0.010
15	610	-0.150	9.952	1.113	-0.135	79	0.893	0.277	0.013
16	602/601	-0.002	0.056	0.006	-0.308	81	0.759	0.597	0.000
17	603/601	0.005	0.056	0.006	0.742	83	0.460	0.410	0.000
18	604/601	-0.002	0.044	0.005	-0.353	87	0.725	0.388	0.000
19	605/602	0.001	0.075	0.008	0.063	79	0.950	0.459	0.000
20	606/603	0.000	0.100	0.011	-0.036	84	0.971	0.632	0.000
21	607+608	0.373	12.808	1.397	0.267	83	0.790	0.415	0.000
22	607+608+609	0.170	16.827	1.836	0.092	83	0.927	0.508	0.000

Since symmetry was assumed missing data on the right side of the leaf were completed with the data of the left side and ANOVA was performed. According to the results 3 (602, 606 and 607) out of the 22 primary characters (608 was excluded) significantly differed among the clone candidates.

Principal component analysis was done based on the morphological characters. In the PCA two axes explain 67.25 % (PC1: 43.47 %, PC2: 23.78 %) of the variance. PC1 is linked to the length of the veins while PC2 explained more with the sum of the angles between the veins (607 + 608, 607 + 608 + 609). Multivariate discriminant analysis was carried out. Confusion matrix of the primary data showed 8.88 % correct classification (jackknifed data).

Primary and secondary morphological data were classified into categories which also reduce information but assist grouping of the samples (Table 2). Cluster analysis was carried out where all clone candidates could be differentiated (Fig. 2). Although some characters were constant in the dataset: OIV 601, OIV 604, OIV 611, high variability within the candidates (intraclonal variability) was observed in the case of the secondary data (607 + 608, 607 + 608 + 609) with bi-, or multimodal distribution of the data.

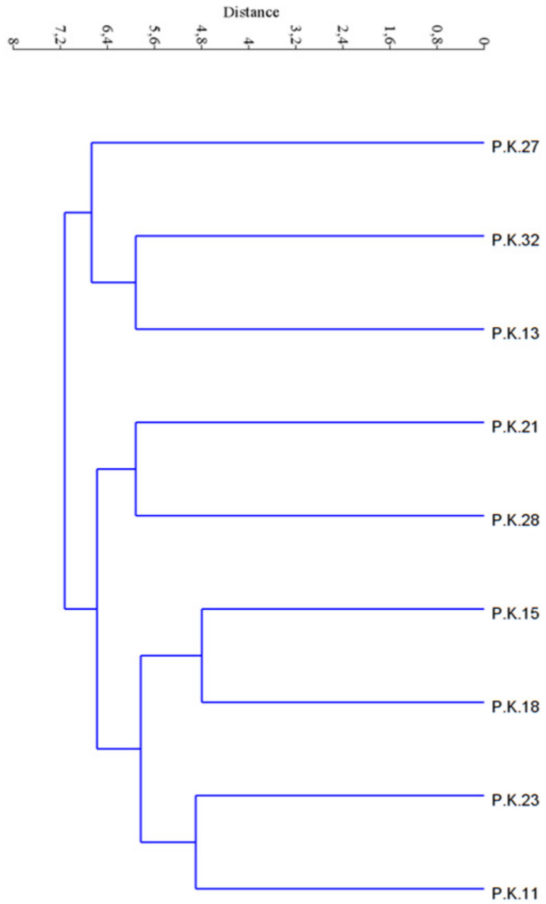

Fig. 2: Dendrogram showing similarities between the clone candidates based on the ampelometric features of the samples, constructed by the Euclidian distance matrix of the classified dataset

Table 2: Ampelometric characterization of the 'Pannonia kincse' clone candidates based on the most frequent values of each morphological character categorized according to GALET (1956) and OIV (2009)

	OIV-Descriptor																				
Clone c.	601	602	603	604	605	606	607	609	610	611	612	613	614	615	617	602/601	603/601	604/601	605/602	606/603	607+ 608	607+ 608+ 609
P.K.11	3	3	3	5	1	3	7	7	7	1	5	5	3	3-5	3	1	4	6-7	6	4	4	7-8
P.K.13	3	5	5	5	1	1	7	5	7	1	7	7	3	5	5	1	4	6	6	2-4	5-6	7-8-9
P.K.15	3	3	3	5	1	1	5	7	7	1	5	5-7	3	5	5	1-2	4	6	6	5	4-5	8
P.K.18	3	3	3-5	5	1	1	7	7	7	1	5	5	3	5	7	1-2	4	6	5	5	5	7
P.K.21	3	3	3	5	1	1	7	7	5	1	5	5-7	3	3	5	2	4	6	6	4	3	6
P.K.23	3	3	3	5	1	1	7	7	7	1	5	5	3	3	3	1-3	3- 4	6	6	4	5	9
P.K.27	3	3-5	5	5	3	3	7	7	7	1	5	7	3	3-5	5	2	4	6	5	2	4-5	6-8
P.K.28	3	3	3	5	1	3	7	5-7	7	1	5	7	3	3	7	1	4	6	6	2-3	4-5	7
P.K.32	3	5	3-5	5	1	3	7	7	7	1	7	5	3	5	5	1-2	4	6	5	4	5-6	7

LANDMARK-BASED GEOMETRIC MORPHOMETRY

Classification of the leaf samples was carried out with canonical variate analysis. The first two CV explained 51.08 % of the variance with 30.92 % and 20.16 % on the CV1 and CV2, respectively. Results of the CVA did not indicate significance (Wilks lambda = 0.0068; F = 1,14; p = 0.1399). Confusion matrix of the classification showed 0 to 20 % percent correct classification (jackknifed data). Based on the scatterplot there is no clear difference between the samples (Fig. 3). Shape deformations were depicted based on thin plate spline (TPS) of the extreme values of each canonical variate axes. Main deformations are related to the depth of the upper sinus and the lobe next to the petiole sinus (Fig. 4).

DISCUSSION

'Pannónia kincse' is one of the most important table grape cultivars in Hungary. In order to improve the morphological characteristics of the cultivar new clone candidates have been selected to modify cluster architecture increasing the size of the bunch and berry. Since in the case of 'Pannónia kincse' the breeding targets were the cluster and the berry, leaf shape and size were not necessarily differing between the samples, although earlier investigations showed that leaf morphology sometimes varies between grapevine clones and candidates (NIEDDU et al., 2006; WERNER et al., 2013).

Analysis of the leaf ampelographic features can be performed on multiple levels. Traditional ampelography is focusing more on the shape of the lamina, serrations, and petiole sinuses. Characterization of these features need experience and the plant material of the reference cultivars. These references are listed for example in the OIV (2009) descriptor list. In contrast with these, metric characteristics such as length of the veins or angles between the veins do not require reference samples. Although caused by the large numbers of descriptors definition of the discriminant characters is required (PREINER et al., 2014). Ampelometric data can be evaluated in many ways. Raw data give the possibility to describe for example the size while the secondary data such as ratios would give more information about the shape. Both data are continuous which usually show high intra-varietal variability. RAVAZ (1902) already suggested ten categories for the angles between the veins (α + β) from ≤ 70° with 10° graduations to 151° - 160°. Later GALET (1956) also classified his secondary data and the OIV (2009) descriptor list also provides categories from 1 to 9 for the ampelometric data.

In our present study primary and secondary leaf morphological characters show low variability per se, since only few characters showed significant difference between the clone candidates. Categorization of the data into classes and use of the most frequent value among the data per each morphological character show efficiency in differentiation of the candidates. This type of leaf analysis has already been applied in clone candidate investigations (NIEDDU et al., 2006). Our results showed that all candidates can be differentiated based on the classified descriptors.

Traditional ampelometric analysis focuses on metric description while geometric morphometry is more describing the shape of the objects based on outlines or

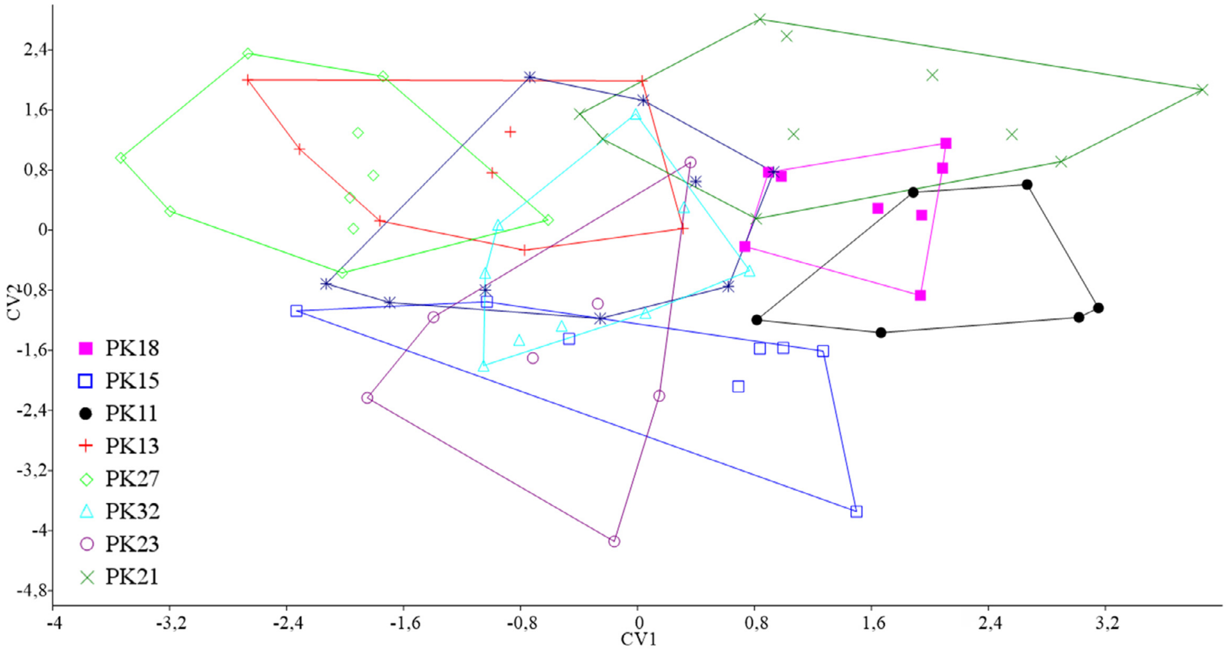

Fig. 3: Canonical variate analysis of the clone candidates

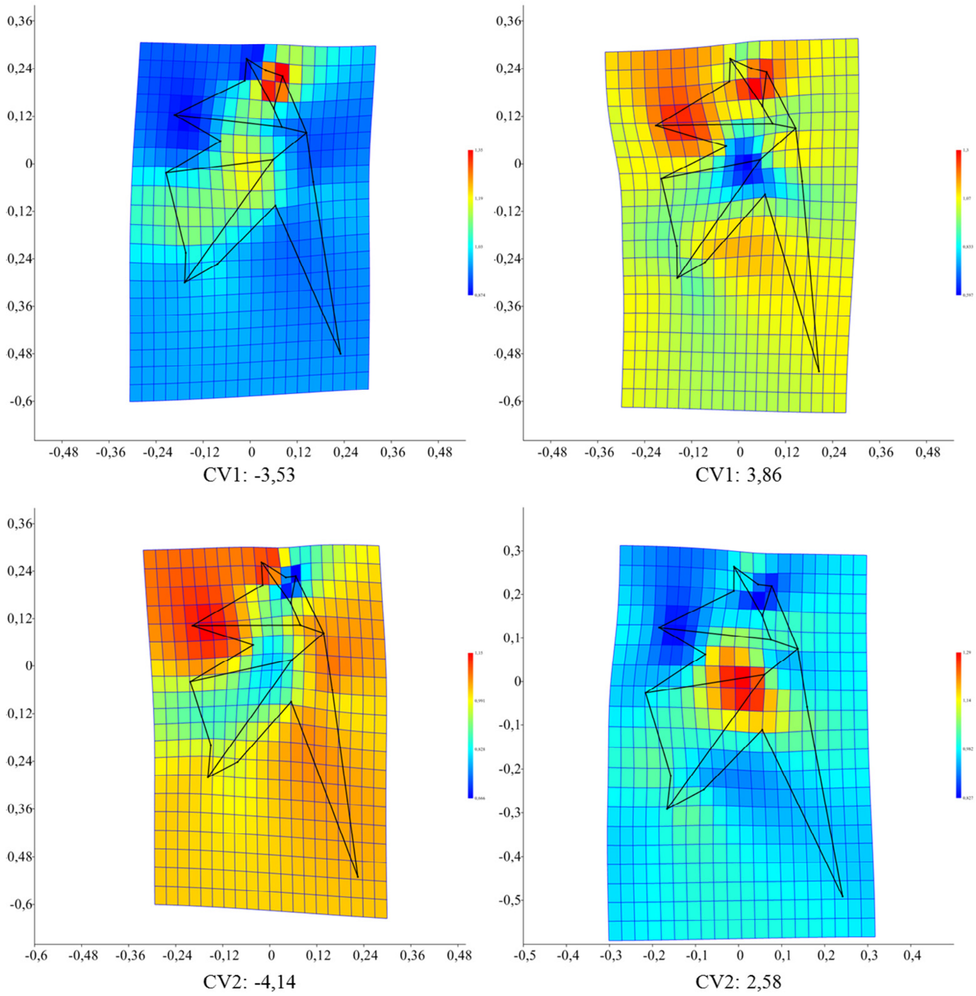

Fig. 4: CVA deformations from the mean shape on the CV1 and CV2 with representing extreme values on each axis

Procrustes-based geometric morphometrics. In leaf ampelography among others Diaz et al. (1991), Mancuso (1999) and Mancuso et al. (2001) are the pioneers in the application of GMM using Elliptic Fourier Analysis (EFA) for distinguishing cultivars and clones. Nowadays both EFA and Procrustes analysis are applied (Chitwood et al., 2014) opening new perspectives in ampelometry. In this study 17 landmark points were used to analyse shape of the leaves. We observed high shape variability among the clone candidates which mainly related to the depth of the sinuses and size of teeth next to the petiole sinus. Canonical variate analysis showed high misclassification of the samples, which is caused by the close relation of the clone candidates.

CONCLUSION

Three methods of leaf morphological analysis were applied in this study to reveal foliometric diversity among 'Pannónia kincse' table grape clone candidates. Our results showed that traditional ampelometric analysis was powerful for distinguishing samples from each other while landmark-based geometric morphometry reveals the morphological differences based on the possible graphic reconstruction of the extreme values of the samples.

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