Landmark-based morphometry reveal phyllometric diversity along the shoot axis of the grapevine (*Vitis vinifera* L.)

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Leaf morphology of the grapevine (*Vitis vinifera* L.) cultivar Kövdinka was evaluated based on 32 landmarks. The aim of this study was to reveal phyllometric diversity along the shoot axis. For this purpose 10 shoots were collected with 26 to 35 leaves. Altogether 304 samples were digitized and analysed with the GRA.LE.D 2.04. raster graphic software. Based on the results length of the veins, angles between the veins and further features such as size of the serrations show high diversity along the shoot axis. Lowest variability (CV= 0,126) of the investigated 54 morphological characteristics were observed among the leaves on the 11th nodes of the shoots, which is in accordance with the literature. Leaf damage was estimated based on the missing landmarks on the lamina. Our results showed that the leaves on the 11th and 13th nodes are the most intact, without missing landmarks. These results underline the careful sampling during the phyllometry-based cultivar comparison.

Introduction

Grapevine (*Vitis vinifera* L.) leaf morphology shows high variability among the cultivars but certain traits are homologue. Venation is palmate, built up by five main veins which arise from a single point at the petiolar junction. These main veins end in lobes, between the lobes there are sinuses which depth is typical to each cultivar, also as the angle between the veins which determines the general leaf shape (Mullins et al. 2004). Leaf morphological characteristics have high importance in grapevine description and identification (OIV, 2009). Since cultivars show variability in size, shape, lobature of the leaf, this organ is in the focus of the ampelographic literature from the very beginning. Ravaz (1902) has been introducing the leaf venation patterning, general leaf shapes, asymmetry, and giving the base of modern *ampelometry*, which study is based on the metric characterization of homologous features: length of veins, angles between the veins opening of the petiole sinus and size of teethes. In the middle of the XXth Century Galet (1956) and Németh (1967) have been carried out comprehensive characterization of cultivars according to ampelometry. Beside the manual metric characterization of the leaf, computer software environment are also frequent in ampelometry (Alessandri et al., 1996; Soldavini et al., 2009). Above mentioned studies were mainly based on traditional and landmark based morphometric measurements with description of the lengths, angles, ratios and outlines, than Chitwood et al. (2016a, 2016b) have been introducing geometric morphometry (GMM) in ampelography. Grapevine leaves show diversity along the shoot axis. This phenomenon is explained with heteroblasty and ontogeny (Chitwood et al., 2016b) and present not only on *Vitis vinifera* L. but also on other *Vitis* species (Cousin and Prins, 2008). Leaf morphological variability has already been mentioned by Ravaz (1902) who suggested using the leaves at the 9 to 12 nodes for comparison. Many ampelographers did the same recommendation (Németh, 1967), while others nominate the middle third of the shoots for sampling (OIV, 2009). The aim of this paper is to explore morphological diversity of the Kövidinka grapevine (*Vitis vinifera* L.) cultivar leaves along the shoot axis and get reliable data which would be used for landmark-based geometric morphometric purpose in the future.

Materials and Methods

Plant material: Study was carried out at the experimental field of the Sóos István Secondary School in Szigetcsép (Hungary) on Kövidinka grapevine (*Vitis vinifera* L.) cultivar grafted onto Berlandieri × Riparia T. 5C. Vineyard was planted in 1992 with 0,8×2 m vine and row spacing on head training system with vertical shoot positioning. Vines were pruned with equal bud load, with short spurs. Ten shoots were collected from 10 plants in July 2016. To evaluate the present/absent of the landmarks, shoot samples were collected randomly. Leaves were removed, numbered from the base to the top position and stored in plastic bag until scanning.

Digitalization: Digitalization of the 304 leaves was carried out individually with a HP Scanjet 4570c Scanner on 300 dpi in the Department of Viticulture, Faculty of Horticultural Science, Szent István University.

Data acquisition and graphic reconstruction: Thirty-two biometric landmarks (Figure 1a) were recorded with the GRA.LE.D. 2.04. raster graphic software according to Bodor et al. (2012, 2014). Origin of the Cartesian coordinate system in the software is considered the connection point of the petiole where leaf veins arise from. After the landmark record coordinates were rotated with the R (R Core Team, 2014) around the petiole junction until the first branching point of the midvein (Lm4) fit to the y axis. Missing landmarks were explored at all leaf layers at all landmark positions. Plotting of the average leaves at the 1st, 5th, 10th, 15th, 20th and 25th nodes were carried out based on the average and standard deviations of the coordinates with the PAST 2.13 (Hammer et al., 2001). Figures were depicted and completed manually.

Statistical analysis: Fifty-four leaf morphological characteristics (see Bodor et al. 2014) were evaluated: length of the veins, angles between the veins, depth of sinuses, distance between lobe tips, size of the serrations in the top of the lobes. ANOVA analysis was carried out to explore the difference among the leaf layers from the 1st to the 25th nodes. Mean, standard deviation and coefficient of variation ($c_v = \frac{\sigma}{\mu}$) were calculated for each morphological characteristic at each node according to the 10 collected leaves. In the next step we calculated the average and the standard deviation these c_v values of the 54 morphological characters. These data represent the overall morphological variability at each node. Deviation of the landmark coordinates were analysed at each leaf layer one-by-one along the x and y axis and results were statistically evaluated by ANOVA analysis with the PAST 2.13 (Hammer et al., 2001). Since scanning was carried out with the same resolution at 300 dpi, deviations of the coordinates at the same landmark were possible to represent in distance (1 dpi is 0,084666667 mm on a 300 dpi picture).

Results and Discussion

Data acquisition: Altogether 304 leaf samples were collected and digitalized, however during the landmark record it was discovered that leaf samples collected from the positions above the 25th node are difficult to characterize due to the small size and undifferentiated lobes and sinuses. In this way stability of the landmarks was defined based on 250 samples collected from the 1st to the 25th leaf layer (Figure 1b). Most reliable landmark was the connection point of the petiole Lm1 (100%), while the least consistent was Lm11 where in 18 out of the 250 cases (92,8 %) this landmark i.e. tip of the vein was missing. Landmark absence was possibly caused by senescence of the leaves, hail, pests, or other damage. Most intact leaves were collected from the 11th and 13th nodes of the shoots where all landmarks were present, while highest rate of damage was observed at the 7th node with 90,93 % presence (29 out of the 320 landmarks were missing).

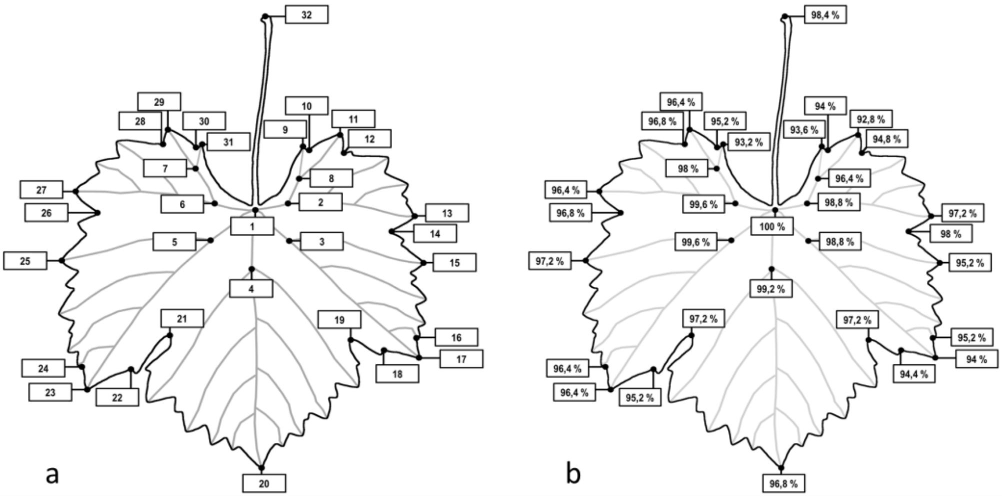

Figure 1: Location (a) and the reliability (b) of the 32 landmarks on the collected 250 grapevine (*Vitis vinifera* L. cv. 'Kövidinka') leaves

Graphic reconstruction: Only samples without missing landmarks were used in this part of the study. To represent morphological differences among the leaf samples coordinates were rotated. Average and standard deviation of the x and y coordinates of each leaf layer was calculated and graphic reconstruction was carried out based on these values for the 1st, 5th, 10th, 15th, 20th, and 25th leaf layers (Figure 2). Landmark coordinates show relative high deviation on the 1st, 5th and 25th leaf layers, while on the 10th, 15th, and 20th the coordinates are closer to each other.

Statistical analysis: Morphological variability of the samples was evaluated from the 1st to the 25th leaf samples. Among the 54 leaf morphological characteristics all significantly differed along the shoot axis except 5 angular characters: 1-9θ1-13; 1-11θ1-13; 1-2θ1-3; 1-5θ1-6.

Coefficient of variability was calculated for the morphological characters, based on the average and standard deviation of the 54 characters. The c_v values were the lowest on the 11th node while the highest on the 25th node with 12,68% and 40,9% respectively. This is in accordance with the literatures that leaf morphology shows uniformity between the 9th and 12th leaves on the shoot axis (Figure 3).

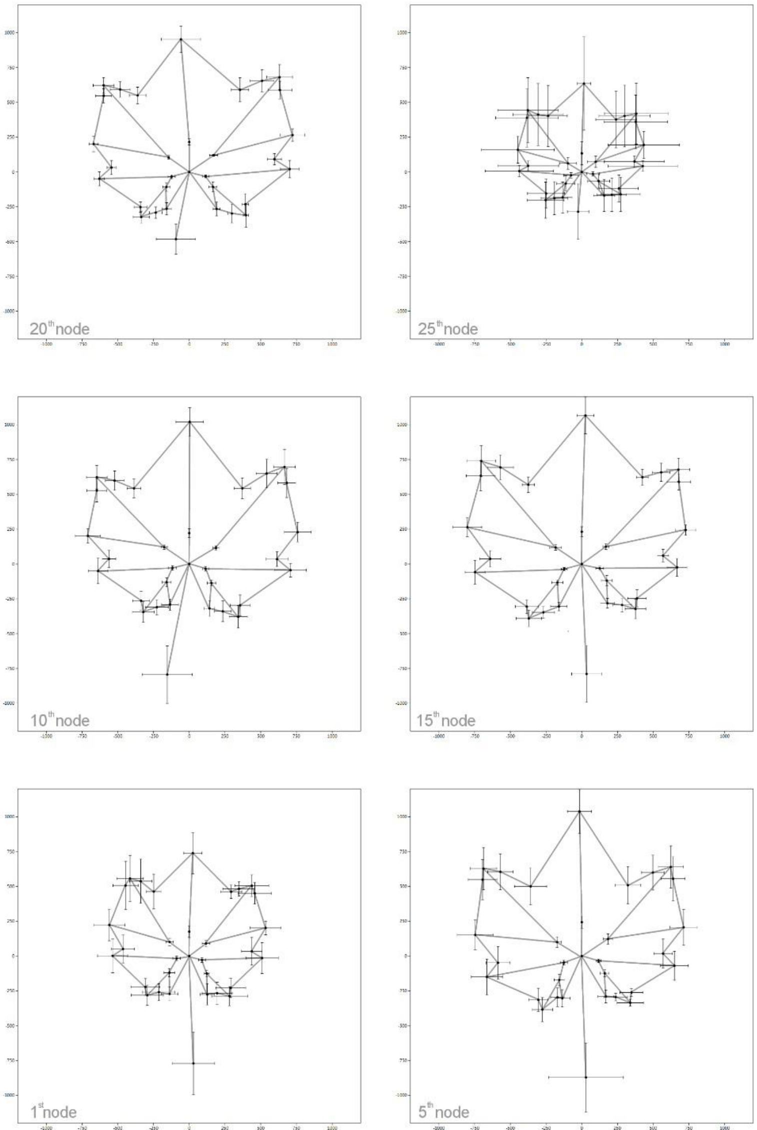

Figure 2: Graphic reconstruction of the typical leaf shapes at 1st, 5th, 10th, 15th, 20th, and 25th nodes based on the average and standard deviation of the Procrustes coordinates

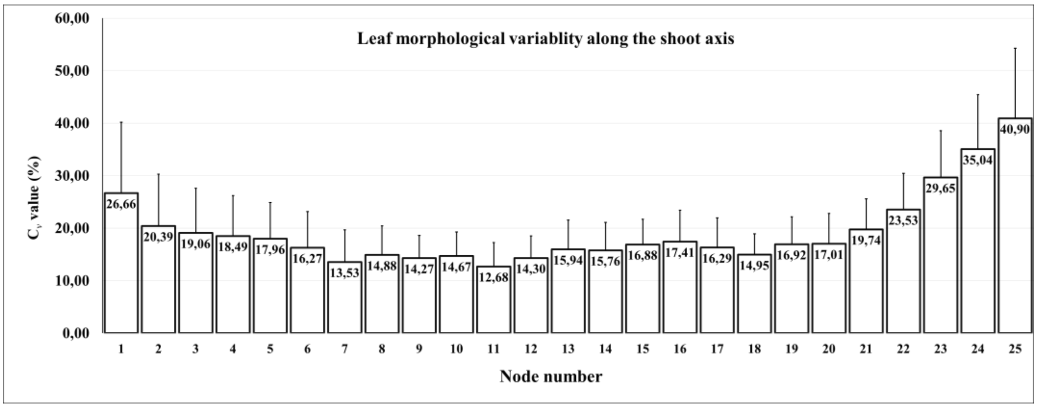

Figure 3: Coefficient of variability of the 54 characteristics along the shoot axis

Position of the same landmarks shows differences along the leaves. Since the origin of the Cartesian coordinate system was the Lm1 in this way its variability was 0 because it was located to the same position at all samples. As distance of the coordinates increases from the base (Lm1) variability of the coordinate location is also increasing. Since position of the petiole is relative, depends on scanning procedure, its location is the most divers (Figure 4). Among the leaves along the shoot axis 24th node showed the highest variability in the position of the same landmark coordinates while the lowest was observed at the 20th node (data not shown).

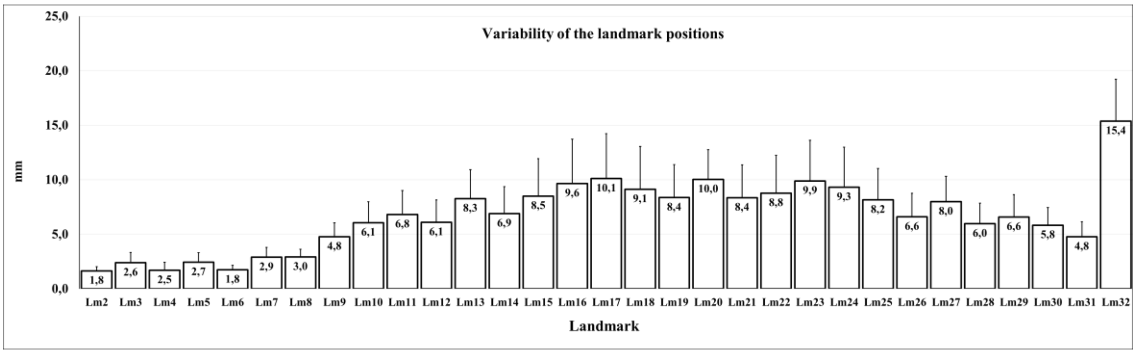

Figure 4: Variability of the 31 landmark positions on the leaf based on the standard deviations of the coordinates at each node

Discussion

Leaf morphology has high importance in the identification of the genus *Vitis*. Ampelometry has already been applied in description of species (Chitwood, 2016b), cultivars (Preiner et al., 2014), and clones (Nieddu et al. 2006). Since the plants show ampelometric variability along the shoot axis it is important to define the sampling position for the proper comparison of the genotypes and explain the difference among the leaf layers. Cousin and Prins (2008) have been reported that *V. piasezkii* and its hybrids showed leaf morphological change within three nodes from palmate (lobed) to entire and palmate again from the 8th to the 10th nodes. These results underline the morphological variability along the shoot axis in spite of that they report is in contrast with our report but opposite results are possible caused by the difference of the investigated species. In our report *V. vinifera* shows relative stable morphology in the middle third of the shoot. Chitwood et al. (2016b) have also been reported about this phenomenon on samples collected from 12 *Vitis* species and 4 *V. vinifera* hybrids and 3 species from the genus *Ampelopsis*. Their results suggest that variability is caused by multiple reasons: age of the leaves and the position of each leaf along the shoot.

Conclusion

Leaf morphology of the grapevine cultivar 'Kövidinka' was evaluated based on 54 characters derived from 32 biometric landmarks. Our results showed that samples collected from the 11th and 13th nodes were the most intact without missing landmarks. Data suggest that leaf morphological characters change significantly along the shoot except a few angular characteristics. Variabilities of these characters are decreasing from the base of the shoot to the 7th and 11th nodes and increase again from the 20th node. Since landmark based morphometric evaluations are more and more frequent, presence and diversity of the landmarks on the different leaves along the shoot axis are important to be explored.

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