

## Investigation of the stomata size and frequency of grapevine (*Vitis vinifera* L.) cultivar ‘Kékfrankos’

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**Abstract:** Grapevine (*Vitis vinifera* L.) leaves show high morphological diversity alongside the shoot. This variability has been investigated in this study to explore the change in leaf size, leaf thickness, stomata density and stomata size among the 1<sup>st</sup>, 5<sup>th</sup> and 10<sup>th</sup> leaves on the main shoots and leaves on the laterals. Results showed that leaf size altered from the basal abaxial leaves to the middle of the shoot, while the laterals had the smallest leaves. Number of stomata also varied significantly regarding the different levels of the canopy. First leaves on the shoots had the least stomata per unit leaf area while this number increased above. In contrast with this the size, i.e. length and width of the stomata did not differ. Leaf thickness was the lowest on the leaves of the lateral shoots, while the values decreased from the 1<sup>st</sup> to the 10<sup>th</sup> nodes. These results raised the question about the ontogeny and heteroblasty of the grapevine foliage.

**Keywords:** leaf morphology, canopy

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### Introduction

Grapevine (*Vitis vinifera* L.) canopy is built up of individual leaves with variable size and diverse shape attributes. The variability is remarkable along the shoot and possibly caused by heteroblasty and ontogeny. Differences in the shape of the leaves alongside the axis were already mentioned by Ravaz (1902), although detailed explanation was given only recently (Chitwood et al., 2016). In our previous study macro-morphological variability of the canopy has been investigated. We found that basal and apical leaves on the shoots are smaller than those in the middle of the shoots, besides venation pattern and serration size are also varying (Bodor et al., 2018).

Morphology of the stomata was described in the middle of the 19<sup>th</sup> century (Anonymous, 1842). In viticulture these “pores” received more attention after the appearance of the downy mildew (*Plasmopara viticola*) in Europe, since the plant is infected throughout the stomata (Gessler et al., 2011). Stomatal openings occur most frequently on the abaxial side of the leaf. According to comprehensive studies performed in the last decade stomatal density and size of *Vitis* species and cultivars (Shiraishi et al., 1996), even clones (Alonso-Villaverde et al., 2011) are already known. Stomata have primary function in plant physiology, and based on previous studies their number responds to ecological circumstances (Bálo et al., 1986). Thus altitude,

row orientation (Kok and Bahar, 2015) and climatic conditions (Gokbayrak et al., 2008) can modify the stomatal density.

Although the diversity of the stomata within genotypes is well described, we have only limited knowledge about the vertical variability inside the canopy. The aim of this study was to investigate leaf size, thickness, stomatal size and distribution of ‘Kékfrankos’ leaves alongside the shoot (on the axis and the lateral shoot as well).

### Materials and Methods

Plant material was collected during May in 2018, after berry set before *veraison*, from the experimental vineyard of the Soós István School for Oenology (Budafok, Budapest, Hungary). The experimented ‘Kékfrankos’ vines were trained on medium-height cordon, vertically shoot positioned. All plants were equally pruned and treated with the same canopy management.

Samples were collected randomly from several plants. Ten leaves were collected from the 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup> nodes and from the lateral shoot of several shoots resulted in 40 samples altogether. Samples were digitized with a Sony A58 digital camera, and each individual leaf area was calculated with the Image J (Abramoff et al., 2004).

Two characteristics were measured on every leaf blade between the main vein and the main lateral vein: (i) Leaf thickness was investigated with a

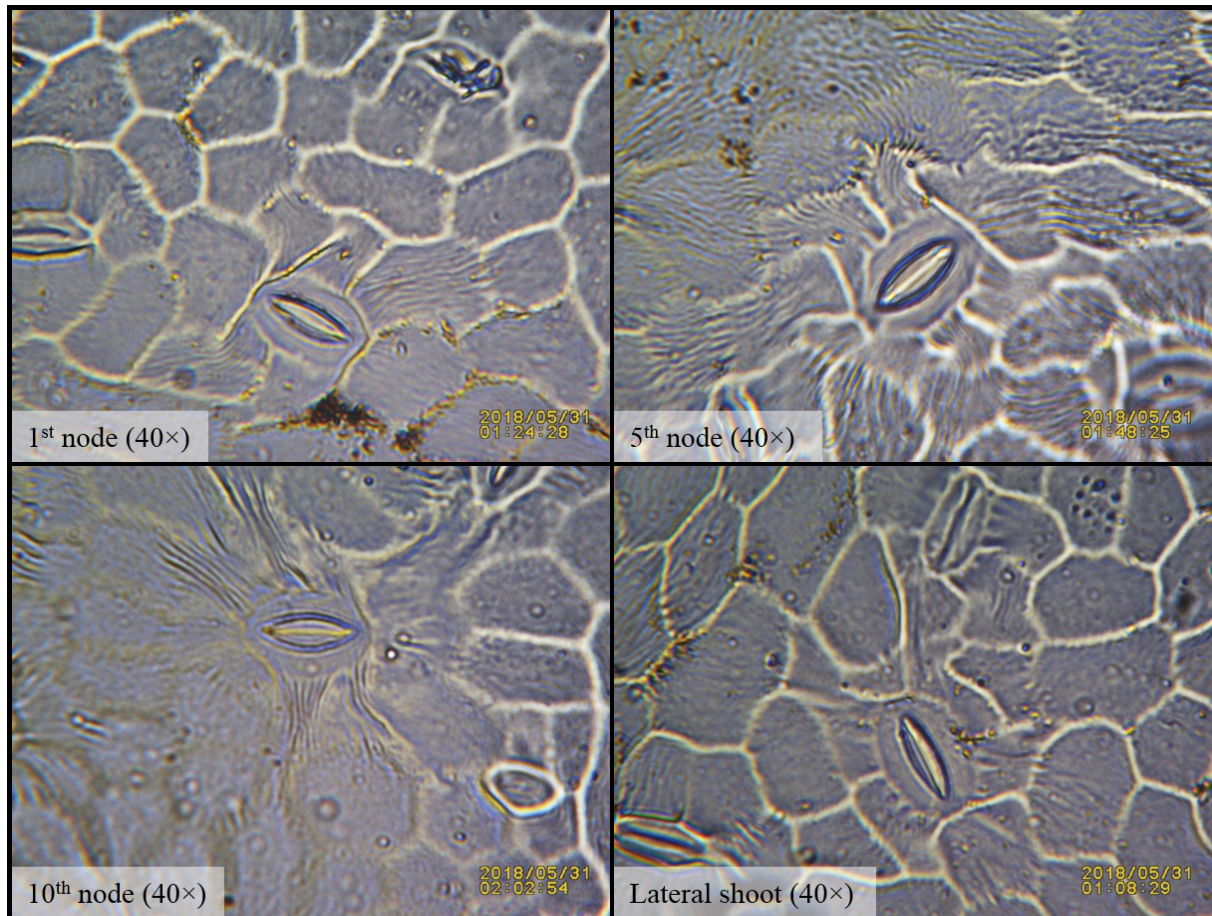


Figure 1: Stomatal imprints from the 1<sup>st</sup>, 5<sup>th</sup>, and 10<sup>th</sup> leaves of the main shoot and from the lateral shoot of the 'Kékfrankos' grapevine cultivar

digital thickness gauge (Moore and Wright Digital Thickness Gauge 053) on a 63.61 mm<sup>2</sup> surface at the same position where stomatal frequency and size were determined. (ii) Stomatal replicas were prepared with the help of a transparent nail polish collected from the lower side of all leaf samples (Figure 1). Each replica was replaced on a slide and covered with coverslip. Twenty pictures at both 10 fold and 40 fold magnification were taken from the 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup> nodes and lateral shoots. For this purpose, a Bresser Digital LCD microscope was used with 5MPx resolution. Size, e.g. width and length of the stomata, was recorded with the Image J (Abramoff et al., 2004). All of the measurements were carried out twice, and correlation was calculated to detect possible errors.

Statistical analysis (mean, st. dev., rel. st. dev., correl., ANOVA) for the values of leaf area, leaf thickness, numbers of stomata, as well as stomatal width, length and shape index (width/length) was carried out with the PAST software (Hammer et al., 2002).

## Results

Results are summarized in Table 1. Leaf area showed significant difference among the leaves arising from the different nodes ( $p < 0.001$ ). Smallest leaves were collected from the lateral shoots while the largest ones originated from the 5<sup>th</sup> nodes. Leaf thickness also showed significant ( $p < 0.01$ ) difference. Samples collected from the lateral shoots were the thinnest, while those originated from the nodes of the main shoot were the thickest. Numbers of stomata also proved to be significantly different ( $p < 0.001$ ). Lowest amount was observed on the leaves collected from the 1<sup>st</sup> nodes, while the highest was detected on the 10<sup>th</sup> node (Figure 2). Stomatal size was measured twice, and replications were statistically analysed. Linear correlation was: 0.9919 ( $p < 0.001$ ) which proved the accuracy of the readings. Neither width, nor length, nor stomatal shape index showed significant alteration among the different levels of the canopy.

Table 1: Morphological characteristics of the leaf samples collected from the 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup> nodes and from the lateral shoots. Superscripts indicate the significant difference (p<0.001 and p<0.01) among the samples.

Character	Position	Difference	Mean	St. dev.	Rel. st. dev.
Leaf area (cm <sup>2</sup> )	Lateral shoot		54.04 <sup>a</sup>	20.89	38.67
	1 <sup>st</sup> node	*	57.11 <sup>a</sup>	18.38	32.19
	5 <sup>th</sup> node		299.47 <sup>c</sup>	72.75	24.29
	10 <sup>th</sup> node		199.05 <sup>b</sup>	85.85	43.13
Leaf thickness (mm)	Lateral shoot		0.26 <sup>a</sup>	0.02	7.26
	1 <sup>st</sup> node	**	0.40 <sup>b</sup>	0.10	24.44
	5 <sup>th</sup> node		0.37 <sup>b</sup>	0.04	10.45
	10 <sup>th</sup> node		0.34 <sup>b</sup>	0.06	18.22
Numbers of stomata/mm <sup>2</sup>	Lateral shoot		117.03 <sup>b</sup>	26.94	32.01
	1 <sup>st</sup> node	*	94.75 <sup>a</sup>	21.67	22.87
	5 <sup>th</sup> node		128.82 <sup>b</sup>	13.99	10.85
	10 <sup>th</sup> node		156.98 <sup>c</sup>	15.46	9.85
Stomatal width (µm)	Lateral shoot		20.69	2.65	12.80
	1 <sup>st</sup> node	n.s.	21.05	2.80	13.28
	5 <sup>th</sup> node		20.98	2.77	13.20
	10 <sup>th</sup> node		19.52	2.02	10.37
Stomatal length (µm)	Lateral shoot		31.15	3.27	10.51
	1 <sup>st</sup> node	n.s.	32.39	3.12	9.62
	5 <sup>th</sup> node		32.05	3.93	12.25
	10 <sup>th</sup> node		30.42	2.70	8.87
Stomatal shape index (W/L)	Lateral shoot		0.66	0.06	8.89
	1 <sup>st</sup> node	n.s.	0.65	0.05	8.08
	5 <sup>th</sup> node		0.66	0.07	9.90
	10 <sup>th</sup> node		0.64	0.06	9.50

\* significant at p<0.001, \*\* significant at p<0.01, n.s.: not significant

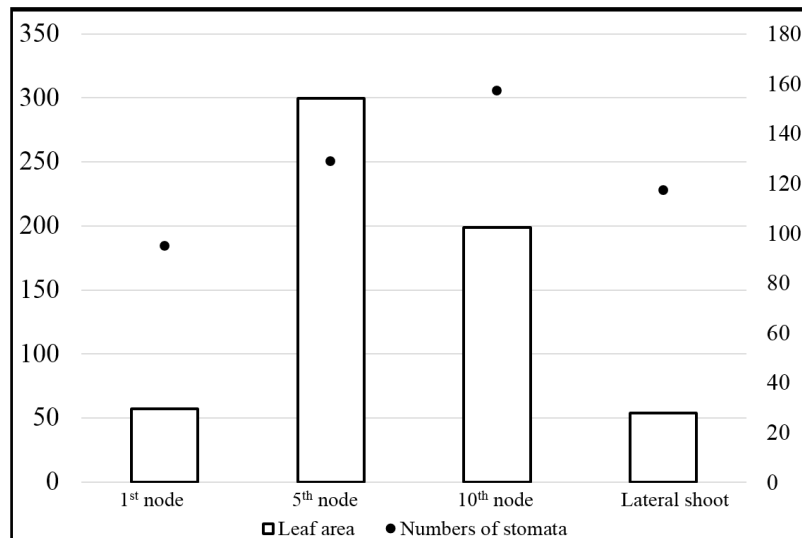


Figure 2: Leaf area and number of stomata of 'Kékfrankos' grapevine cultivar on the 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup> nodes and on the lateral shoots

**Discussion**

Leaf area, leaf thickness and number of stomata showed significant difference among the samples collected from the 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup> nodes of the main shoot and from the lateral shoots of the 'Kékfrankos' grapevine cultivar. Leaf area was 57.11 cm<sup>2</sup> on the abaxial leaves while 299.47 cm<sup>2</sup>

on the 5<sup>th</sup> node. This morphological alteration along the shoot is in accordance with the literature. Previously Demaria and Leardi (1875) have already published that leaf morphology of the grapevine is not constant, and there is a notable diversity. Thus not only the alteration of the canopy levels, but the variability within the samples collected from the same position

have importance. Relative standard deviations were calculated and these data showed that the variability of the leaf size is the lowest on the 5<sup>th</sup> node (rel. st. dev.: 24.29) and highest on the 10<sup>th</sup> node (rel. st. dev.: 43.13). Our previous study showed that leaf morphology is the most typical for a cultivar on the 9-12<sup>th</sup> nodes (Bodor et al., 2018). This is the reason why international standards also recommend leaf sampling from the middle third of several shoots, since these represent the genotype the best (OIV, 2009). The present study is in contrast with the earlier results and highlights that more cultivars in our future observations should be involved.

The values of leaf thickness were also differing among the samples, decreasing from the 1<sup>st</sup> leaf to the 10<sup>th</sup> nodes and the leaves from the lateral shoot were the thinnest. Variability was higher on the 10<sup>th</sup> node than on the 5<sup>th</sup> (rel. st. dev.: 18.22 and 10.45 respectively).

Stomatal number was the lowest on the 1<sup>st</sup> node and the highest on the 10<sup>th</sup>, with 94.75/mm<sup>2</sup> and 156.98/mm<sup>2</sup> respectively. The variability in stomatal number was the lowest at the 10<sup>th</sup> node (rel. st. dev.: 9.85), while it proved to be the highest on the samples collected from laterals (rel. st. dev.: 32.01) and from the 1<sup>st</sup> node (rel. st. dev.: 22.87). Earlier Rogiers et al. (2011) published that the position of the leaves alongside the shoot has an effect both on leaf size and stomatal density. They pointed out that leaves collected from lower nodes have less stomata than the ones higher on the shoot. These previous results are in accordance with our observations.

The difference between the size and the shape of the stomata was not significant. It suggests that this characteristic is regulated genetically while leaf position on the shoot and age of the leaf have less influence on them. On the other hand, several previous studies about the size of the stomata found significant differences among cultivars (Eris and Soyulu, 1990, Boso et al., 2016), which alludes that this character is possibly not uniform. Moreover, it requires further investigations on more cultivars.

Morphological inequality among leaf samples collected from distinct nodes of the shoot can be explained with two biological reasons, namely ontogeny and heteroblasty. The first reason

(*ontogeny*) explains the morphological variability with the age difference among the leaves, while the second one, i.e. *heteroblasty* (morphological) relates to the phenotypical differences of the leaves with their position on the shoot.

Regarding *ontogeny* a rather long timeframe has to be considered. New leaves arise constantly on the vine. Main leaves on the primer shoot can occur until the first trimming, while lateral shoots arise almost constantly throughout the growing season (Lőrincz and Barócsi, 2010). So the age difference of leaves can be even more than 100 days, giving significant time for ontogeny. Moreover, if phenological stages are discussed, requirements for abiotic factors and differences in ecological conditions have to be considered as well. The basal leaf is the oldest on the shoot arising at the beginning of the vegetation period, leaves in the middle of the shoot are younger, and apical leaves on the shoot top are the youngest. Beside the main shoot laterals are arising from the lateral buds. It is caused by many reasons, for example the injury/removal of the main shoot top or high vegetative performance, etc. (Kozma, 1991). The age of the leaves on the laterals is hard to defined because their formation and growing are different from the main shoots (Zufferey, 2016). This phenological difference between the oldest and youngest leaf inside the canopy can be 2 months or even more. If we consider the ecological circumstances of these phases, the alteration among the samples is not surprising. Generally, the oldest leaves (1<sup>st</sup> node) arise in April when the temperature is usually low and humidity is high, thus high evaporation is not significant. Middle leaves (5<sup>th</sup> and 10<sup>th</sup> nodes) develop days or weeks later on the same shoot when both temperature and radiation are increasing, so the environment is changing. In this study the investigated laterals had arisen approximately 1-2 weeks before sampling (middle of May). It has to be emphasised that the leaves collected from the 1<sup>st</sup>, 5<sup>th</sup> and 10<sup>th</sup> nodes were fully developed, while those collected from the laterals would increase in size, probably changing the distribution of the stomata later. As mentioned above, stomatal shape and size require further studies with more genotypes (cultivars, clones) at more phenological stages. But the obvious correlation between ecological

conditions with phenology and morphology suggests, we should complete our studies and sampling in different vineyards, wine regions, possibly in other phenological stages with more frequent “collection”.

Zotz et al. (2011) concluded that *heteroblasty* has many functional reasons, such as the different light conditions, water relations or nutrient supply. In its natural circumstances grapevine is a liana like plant (Mullins et al. 2003) climbing up to the tree canopy to reach optimal light conditions. In those cases basal leaves are usually in the shade, while apical leaves reach higher radiation. In contrast with this in the vineyard cultivated plants do not have any competitors, and growers aim to provide the highest radiation to the whole canopy with minimized self-shading. In this way the initial canopy can get high radiation i.e. low self-shading in the beginning of the growing season.

Lee and Richards (1991) explained vine heteroblasty with other reasons. According to their concept (similarly to other lianas) vines have to find support during the initial phase of the growing season, consequently plants invest less source to the development of individual organs than to the apical growing in this stage.

This is in accordance with our previous (Bodor et al. 2018) and present findings: basal leaves are smaller and less differentiated than those arising from above in the middle of the shoots.

Based on the present study it can be concluded that leaf morphology and stomatal characteristics still have several unanswered questions. Further investigations are required to detect correlations of leaf morphology and stomatal characteristics with ecological conditions, phenological stages and genotypes.

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