RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2024; 8(1): 78-87
DOI: https://doi.org/10.31015/jaefs.2024.1.9

Stomatal density, type and their relationships with leaf morphological traits in *Vitis vinifera* L. varieties

Sinem GÜLER[1] • **Birhan KUNTER**[2] • **Aysun ŞEHİT**[3]

[1] Graduate School of Natural and Applied Sciences, Ankara University, Türkiye
[2] Department of Horticulture, Faculty of Agriculture, Ankara University, Türkiye
[3] Department of Plant and Animal Production, Boğazlıyan Vocational School, Yozgat Bozok University, Yozgat, Türkiye

Citation: Guler, S., Kunter, B., Sehit, A. (2024). Stomatal density, type and their relationships with leaf morphological traits in *Vitis vinifera* L. varieties. International Journal of Agriculture, Environment and Food Sciences, 8(1), 78-87

Received: *December 29, 2023*
Accepted: *February 18, 2024*
Published Online: *March 25, 2024*

Correspondence: Birhan KUNTER
E-mail: marasali@agri.ankara.edu.tr

Available online at
https://dergipark.org.tr/jaefs
https://jaefs.com/

Abstract
This study was carried out to determine the relationships between stomatal density and type and some leaf morphological traits in 10 grapevine varieties (*Vitis vinifera* L.). The study was performed during the vegetation period of 2022-2023 in the vineyards of the Research Station for Viticulture of Ankara University Faculty of Agriculture in Kalecik-Ankara. 'Lival', 'Tekirdağ Seedless', 'Beauty Seedless', 'Michele Palieri', 'Cabernet Sauvignon', 'Narince', 'Syrah', 'Kalecik Karası', 'Alphonse Lavallée' and 'Hasandede' varieties, grafted on 5BB rootstock, were evaluated as experimental materials in the study. The results revealed that there have been significant variations among varieties in terms of stomatal density, distribution of stomatal types, the proportion of trichomes, and leaf thickness. In general, stomatal density ranged from 168.17 to 268.27 stomata mm^{-2} in the varieties. The presence of the three different stomatal types (same level, raised above, and sunken) was detected by SEM images. The percentage of stomatal types varied between 39-59 for the same level, 25-42 for the raised above and 10-26 for the sunken. The percentage of the same level stomatal type was found to be higher than the other two types in all varieties. In addition, the same level stomatal type exhibited negative correlations with the raised above and the sunken stomatal types. In terms of leaf characteristics, the proportion of trichomes showed a negatively significant correlation ($r = -0.309$; $p<0.01$) with stomatal density. However, the relationship between leaf thickness and stomatal density was not significant.
Keywords: Grapevine, Stomatal density, Stomatal type, Sunken stomata, Correlations

INTRODUCTION

Stomata, located in the epidermis of plant leaves, serve as the starting point for metabolic processes, acting as the primary units for exchanging oxygen and carbon dioxide. They are essentially defined by two specialized guard cells in the leaf epidermis with a pore between them.

The presence and structure of stomata in the leaves of different plant species, even within a single species, show different morphological and anatomical characteristics. Stomata, along with their properties, are crucial structures for plants to respond to environmental conditions. Recently, new perspectives have been incorporated to study stomatal density, size and distribution patterns on leaves to analyse plant adaptation and evolution. There is a growing interest in using leaf and stomatal traits as rapid and reliable criteria to express the adaptability of plants to their ecological conditions (Franks et al., 2009; Sack and Buckley, 2016; Liu et al., 2018; Liu et al., 2021). On the other hand, the importance of generating information on leaf morpho-anatomical characters is emphasized for evaluating the adaptability of genotypes against the possible consequences of climate change (IPCC 2013).

The measurable criteria for evaluating the adaptability of grapevines are mainly expressed in terms of developmental and yield characteristics. However, agricultural practices have an impact on the capacity of the vine, and therefore, the assessment of adaptation may be limited. The time-consuming criteria based on perennial grapevine development and yield contribute to the delay in recommending cultivars for ecology. However, there are not enough results on the functional effects of morpho-anatomical characteristics of leaves on the adaptation ability of grapevine. Recently, some approaches have been considered to understand the correlations with leaf traits (such as leaf shape, area, thickness, hairiness, cuticle, stomata, epidermis cell properties, and mesophyll anatomy) and stress conditions to develop alternative criteria for the assessment of adaptation (Boso et al., 2010; Samarth et al., 2021; MacMillan et al., 2021).

In particular, the characteristics of stomata in the species and the varieties of grapevine have been studied in relation to genotype, rootstock, and environmental conditions (Düzenli and Ağaoğlu, 1992; Shiraishi et al., 1996; Kara and Özeker, 1999; Marasalı and Aktekin, 2003; Gökbayrak et al., 2008; Rogiers et al., 2009; Keller, 2010; Hopper et al., 2014; İşçi et al., 2015; Boso et al., 2016; Uyak et al., 2016; Bodor et al., 2019; Doğan et al., 2020; Odabaşıoğlu, 2020; Candar et al., 2021; Copper et al., 2022). Currently, studies have been focused on the different stomatal types in grapevines, which have interested many plant species for decades. Thus, the role of stomata in the adaptability of genotypes in response to environmental conditions has been under investigation (Boso et al., 2011; Teixeira et al., 2018; Nassuth et al., 2021).

Studies on stomatal shape and the relationship between leaf morphological characteristics and stomata in grapevine species and cultivars are limited. Also, stomatal types of *Vitis vinifera* L. could not investigated in national viticultural literature. In this study, it was aimed to investigate the stomatal characteristics and their relations with some morphological traits of leaves in ten grapevine varieties of *Vitis vinifera* L. grown in Ankara-Kalecik conditions.

MATERIALS AND METHODS

The study was performed during the growing seasons of 2022-2023 on ten grapevine varieties cultivated in the vineyards of the Research Station for Viticulture of Ankara University Faculty of Agriculture in Kalecik-Ankara, Türkiye. The coordinates of the research area were 40°06´ 33.8´´ N 33°25´ 43.2´´ E, 670 m above sea level. The studied varieties were 'Lival', 'Tekirdağ Seedless', 'Beauty Seedless', 'Michele Palieri', 'Cabernet Sauvignon', 'Narince', 'Syrah', 'Kalecik Karası', 'Alphonse Lavallée', and 'Hasandede'. The grapevines were planted in 2005 and, after being grafted on 5 BB rootstocks, were spaced 1.5x3 m rows and were trained at a double cordon having 80 cm stem height. Drip irrigation was applied 4-5 times from bud-burst to pre-vérasion. The vineyard soil was clay-loam with a pH of 7.5. The research region has a continental climate. The experimental design consisted of three replications and randomly selected five vines for each replication per variety.

Collecting of leaf samples

Five leaf samples were hand-collected from the vines of each replication for stomatal and leaf morphological examinations. According to the OIV (2001) definition of mature leaves, the collection was performed between the berry set and the véraison period. The sampling was performed on July 18, 2022 and July 22, 2023. To ensure uniformity, leaves were collected from the 8th and 9th nodes in the same direction across all vines. To avoid dehydration and discolouring, leaf samples were transported in cooler bags and were transferred to the Cytology Laboratory of the Department of Horticulture, Ankara University.

Determination of stomatal density

The leaf print removal method was used to determine stomatal density in fresh leaf samples. Leaf prints were removed from the lower surface of the leaf blade using nail polish. Leaf prints were obtained from a total of 6 areas on a leaf blade, including around the petiole sinus, both sides of the main vein and lateral veins. These prints were prepared for examination using a light microscope (LM-Zeiss Axiolab). During the LM examination of the slides, microphotographs were taken to create the digital archive for further measurements. In microscopic studies, mainly 40x magnification was used. Stomatal density (stomata mm^{-2}) was determined in microphotograph areas of 0.037 mm^2.

Determination of stomatal type

The stomatal type was determined through LM and SEM (Scanning Electron Microscope) examinations. SEM studies were performed on well-dried leaf samples. The gold-coated process was provided by the supervision of the Science Application and Research Center of Van Yuzuncu Yıl University. The definitions were realized on the SEM microphotographs according to Monteiro et al. (2013), Teixeira et al. (2018), and Šantrůček et al. (2022).

Examination of leaf morphological traits

In the present study, leaf area (cm^2), the proportion of trichomes (%) and leaf thickness (mm) were examined.

Leaf areas were calculated from fresh leaf images, trichomes were examined using a stereo microscope (Olympus SZ40), and microphotographs were taken of the abaxial surfaces of fresh leaves. Here, it was preferred to use the term trichome as a collective term for two different hair structures, consisting of both prostrate and erect type hairs, rather than hairiness. Computations on all digitised observations were analysed using the ImageJ/IJ 1.46r program (https://imagej.nih.gov/ij/index.html). In addition to the digitised-based calculations, the OIV (2001) scale was used to score the density of the prostrate and erect hairs. Leaf thickness was measured using a digital micrometer device (INSIZE 3109-25A), and thickness values were grouped according to Bozkurt (2023).

Statistical analysis

Descriptive statistics for the continuous variables were presented as Mean and Standard error of mean. One-way ANOVA was used to compare group means. Following the ANOVA, Duncan multiple comparison test was performed to identify different varieties' means. Pearson correlation coefficients were computed to determine linear relationhips between the variables. In addition to correlation analysis, Linear regression analysis was performed to predict dependent variables with independent variables. Statistical significance level was considered as 5% and 1%. SPSS (ver: 26) statistical program was used for all statistical computations.

RESULTS AND DISCUSSION

Stomatal density (stomata mm^{-2})

Stomatal density varied significantly among all grapevine varieties, ranging from 168.2 to 268.3 stomata per mm^2. The varieties with the highest stomatal density were 'Lival' (268.3 stomata mm^{-2}), 'Tekirdağ Seedless' (265.3 stomata mm^{-2}), and 'Beauty Seedless' (258.3 stomata mm^{-2}). 'Hasandede' showed the lowest stomatal density, with 168.2 stomata per mm^2 (Figure 1). Many researchers reported that stomatal densities varied significantly among grapevine varieties. Keller (2010) stated a wide range of 50-400 stomata mm^{-2} for *Vitis* species. Previous studies investigating stomatal density under the influence of genotype and environmental conditions reported the following findings: Shiraishi et al. (1996), 136.1-302.6 stomata mm^{-2}; Kara and Özeker (1999), 208.3- 294.8 stomata mm^{-2}; Marasalı and Aktekin (2003), 156.1-269.5 stomata mm^{-2}; Gökbayrak et al. (2008), 190.9-220.6 stomata mm^{-2}; Rogiers et al. (2009), 162.0-232.6 stomata mm^{-2}; Monteiro et al. (2013), 206.7-285.7 stomata mm^{-2}; Bekişli (2014), 150.9-189.3 stomata mm^{-2}; İşçi et al. (2015), 67.2-188.9 stomata mm^{-2}; Monteiro et al. (2018), 179-256 stomata mm^{-2}; Teixeira et al. (2018), 170-250 stomata mm^{-2}; Bodor et al. (2019), 94.8-157.0 stomata mm^{-2}; Nassuth et al. (2021), 178.0-354.5 stomata mm^{-2} and Copper et al. (2022), 139.8-238.6 stomata mm^{-2}. Apart from genotype and environmental factors, variations in stomatal density were attributed to microscopic miscounting. Therefore, LM and SEM examinations were evaluated together in this study to prevent such errors. Figure 2 shows the image clarity for stomatal evaluation in this study.

Figure 1. Stomatal density of grapevine varieties (stomata mm^{-2}). Lival-L; Tekirdağ Seedless-TS; Beauty Seedless-BS; Michele Palieri- MP; Cabernet Sauvignon-CS; Narince-N; Syrah-S; Kalecik Karası-KK; Alphonse Lavallée-AL; Hasandede-H (Different lower cases represent statistically significant differences among the varieties).

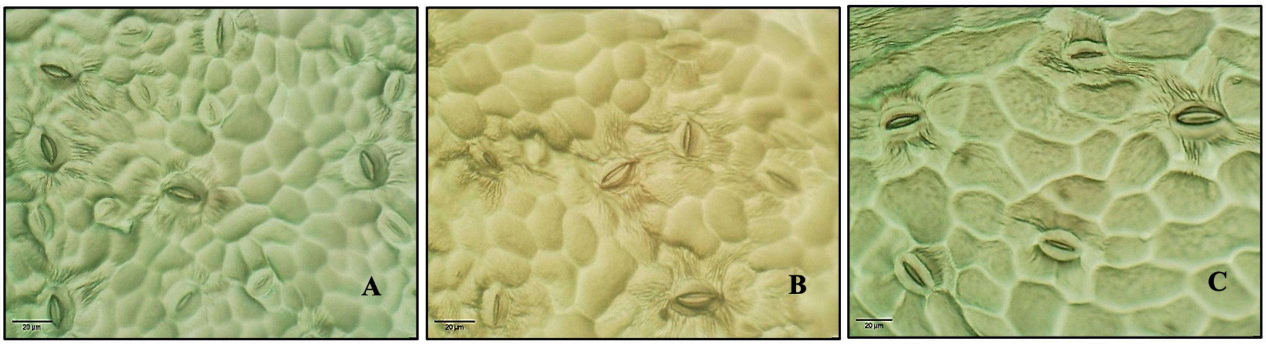

Figure 2. Images of stomata with different densities under a light microscope. A-'Beauty Seedless'; B-'Kalecik Karası'; C-'Hasandede' (Scale bar=20 µm).

Stomatal types and distribution in grapevine varieties

Three different stomatal types reported for *Vitis* species were detected in the leaves of ten grapevine varieties. These types were classified as (1) same level, (2) sunken and (3) raised above, depending on the structural height of the guard cells on the leaf surface relative to subsidiary cells (Swanepoel and Villers, 1987; Monteiro et al., 2013; Teixeira et al., 2018). SEM images used to determine the stomatal types were given in Figures 3, 4 and 5. The proportion of the same level stomata varied between 39.12% (Syrah) and 59.41% (Alphonse Lavallée) in evaluating the distribution of stomatal types in the varieties. The proportion of the raised-above stomata ranged from 25.39% (Alphonse Lavallée) to 42.63% (Hasandede), while the proportion of the sunken stomata ranged from 10.53% (Beauty Seedless) to 26.47% (Cabernet Sauvignon) (Figure 6). Based on the visual representation in Figure 6 and the statistical interpretation of the results in Table 1, it was determined that the distribution of stomatal types within the same cultivar was significant. The percentage of the same level stomata in all varieties was higher than the other two types. Hasandede was the only variety that deviated from this rule. There was a minimal and statistically insignificant difference between the same level and the raised above stomata in this variety. In addition, the sunken stomata showed the lowest percentage of all varieties. Monteiro et al. (2018) emphasized that stomatal types differ among varieties. In this study, it was observed that the sunken stomatal type was smaller than the other two types. This type was characterized by guard cells buried between the subsidiary cells. Monteiro et al. (2018) and Teixeira et al. (2018) emphasized that genotypes with high stomatal density and the sunken stomata may be more advantageous against abiotic stress conditions, especially in hot and arid ecologies. Furthermore, Nassuth et al. (2021) reported that sunken stomata can also be advantageous at low temperatures and are present in cold-tolerant varieties. Jones (2014), Serra et al. (2017), and Teixeira et al. (2018) stated that high stomatal density and sunken stomata are important in reducing plant transpiration and that these traits play a role in the adaptation of varieties to water stress.

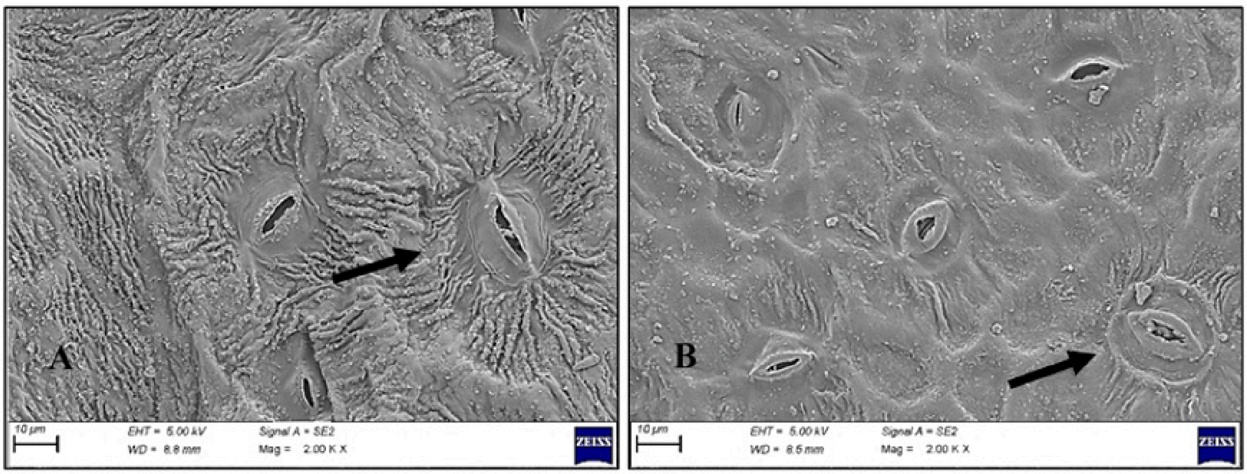

Figure 3. SEM image of the raised above stomata. A-'Narince'; B-'Tekirdağ Seedless'

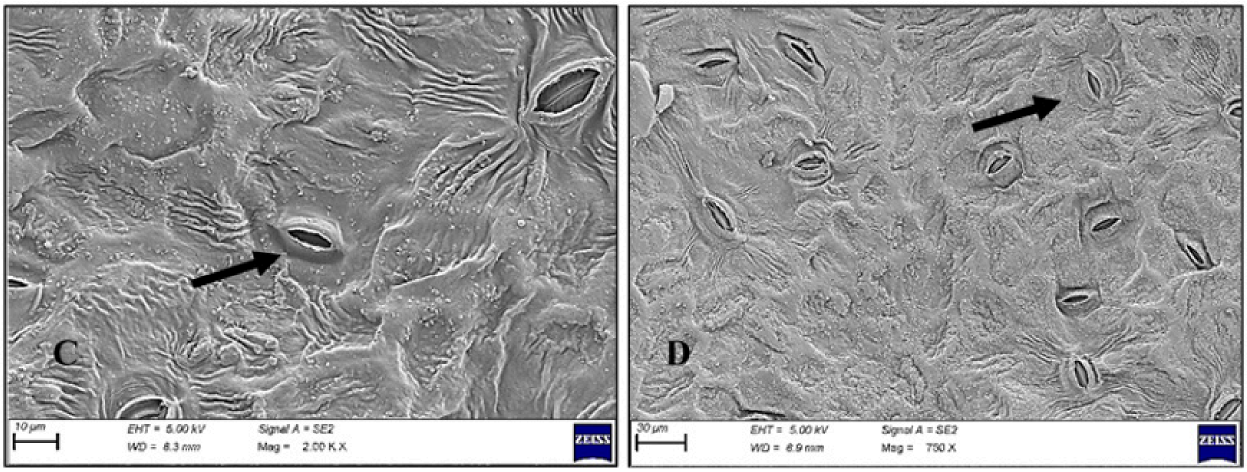

Figure 4. SEM image of the same level stomata. C-'Michele Palieri'; D-'Cabernet Sauvignon'

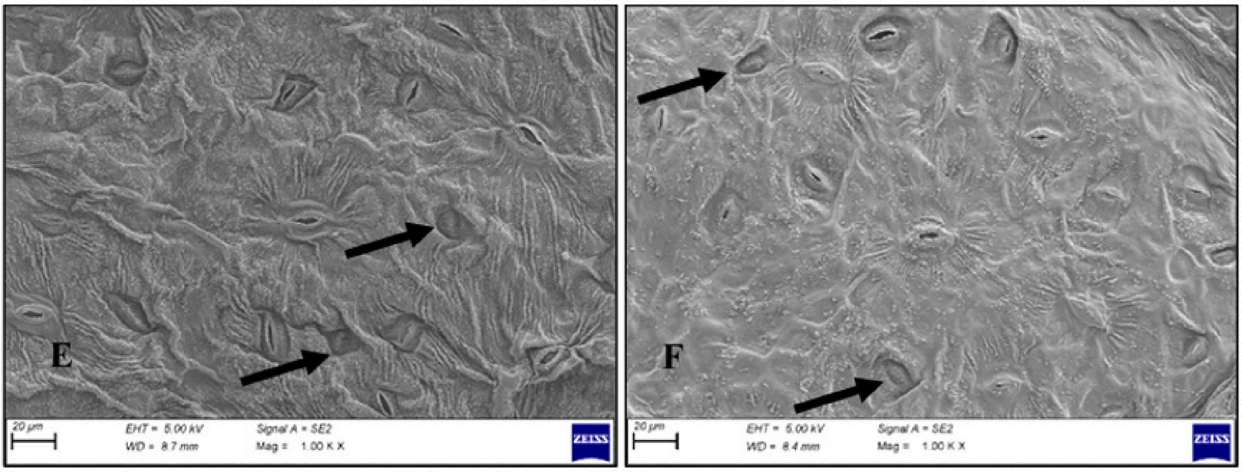

Figure 5. SEM image of the sunken stomata. E-'Alphonse Lavallee'; F-'Beauty Seedless'

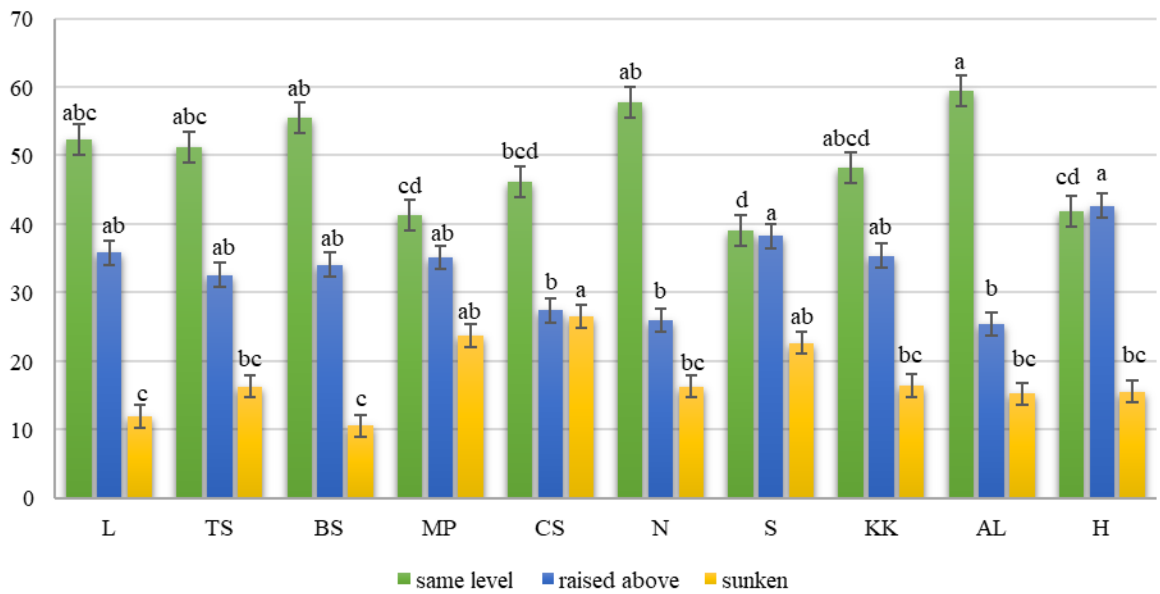

Figure 6. The distribution of stomatal types among grapevine varieties (%). Lival-L; Tekirdağ Seedless-TS; Beauty Seedless-BS; Michele Palieri- MP; Cabernet Sauvignon-CS; Narince-N; Syrah-S; Kalecik Karası-KK; Alphonse Lavallée-AL; Hasandede-H (Different lower cases represent statistically significant differences among the varieties).

Table 1. The distribution of stomatal types in grapevine varieties (%)

Variety	Stomatal Type		
	Same level (Mean ± SEM)	Raised above (Mean ± SEM)	Sunken (Mean ± SEM)
Lival	52.28 ± 3.68 a	35.82 ± 3.17 b	11.90 ± 2.70 c
Tekirdağ Seedless	51.13 ± 1.38 a	32.57 ± 2.03 b	16.29 ± 3.20 c
Beauty Seedless	55.45 ± 5.23 a	34.02 ± 2.14 b	10.53 ± 3.35 c
Michele Palieri	41.21 ± 3.18 a	35.10 ± 4.32 ab	23.69 ± 3.84 c
Cabernet Sauvignon	46.18 ± 2.35 a	27.36 ± 0.93 b	26.47 ± 2.43 b
Narince	57.79 ± 3.98 a	25.93 ± 1.70 b	16.29 ± 2.28 b
Syrah	39.12 ± 3.28 a	38.25 ± 3.99 a	22.63± 0.85 b
Kalecik Karası	48.26 ± 3.99 a	35.33 ± 5.12 a	16.41 ± 2.04 b
Alphonse Lavallée	59.41 ± 4.09 a	25.39 ± 3.58 b	15.20 ± 1.55 b
Hasandede	41.84 ± 4.32 a	42.63 ± 3.06 a	15.54 ± 1.87 b

* Different lowercase letters on the same line indicate significant differences of stomatal types in each variety (p<0.05). SEM: Standard error of Mean

Leaf morphological traits and stomatal relationships

The average size of the leaves collected during the period from berry set to veraison in Kalecik conditions is shown in Table 2. The leaf area ranged from 72.21 cm^2 (Syrah) to 95.09 cm^2 (Tekirdağ Seedless), with no statistically significant difference. This result allowed us to analyse stomatal density on comparable leaf areas. Bekişli (2014) found the relationship between leaf area and stomatal density at a value of $R^2 = 0.0498$. Thus, the correlation between leaf area and stomatal density was not statistically significant. Similarly, Boso et al. (2016) reported that this relationship was not significant. The study investigated the relationships between the proportion of trichomes and leaf thickness, stomatal density, and the distribution of density by stomatal type. The results are presented in Table 5 and Figure 7. The proportion of trichomes (%) was found to be significant among the varieties. 'Kalecik Karası' exhibited the highest percentage at 39.75%, followed by 'Syrah' at 16.29% and 'Alphonse Lavallée' at 14.91%. 'Beauty Seedless', 'Michele Palieri', and 'Tekirdağ Seedless' exhibited the lowest percentages (Table 2). In addition, the density of prostate and erect hairs between the main veins on the lower side of blade in the varieties was classified according to the OIV 84 and OIV 85 codes (Table 3). The trichome percentage values (the sum of prostrate and erect hairs) of the varieties were in line with the scoring results obtained according to OIV (2001). Monteiro et al. (2018) and Gago et al. (2019) reported differences in leaf anatomical tissue thickness among grapevine varieties. Additionally, Gago et al. (2016) found variations in spatial density and relative abundance of trichomes among grapevine varieties.

There was a significant difference in leaf thickness among the varieties. Regarding the leaf thickness, higher values were found in 'Kalecik Karası' (0.73 mm), 'Narince' (0.63 mm) and 'Michele Palieri' (0.61 mm). The lowest thickness value (0.45 mm) was found for 'Tekirdağ Seedless' (Table 2). According to the method described by Bozkurt (2023), 'Kalecik Karası' was placed in the thick group, while 'Narince', 'Beauty Seedless', 'Michele Palieri', and 'Hasandede' were placed in the medium group. 'Alphonse Lavallée', 'Lival', 'Cabernet Sauvignon' and 'Syrah' were classified in the thin group, and 'Tekirdağ Seedless' was placed in the very thin group (Table 4).

The correlation analysis showed that there was a significant negative correlation ($r = -0.309$; $p<0.01$) between the stomatal density and the proportion of trichomes (%). However, there was no significant relationship with leaf thickness. A significant positive correlation was found between the proportion of trichomes (%) and leaf thickness (mm) ($r = 0.528$; $p<0.01$) (Table 5). It was possible to interpret from this study that trichome density was generally lower or absent in the varieties with a high stomatal density. Boso et al. (2011) found similar results and emphasized the importance of the relationship between stomatal density and trichome density. 'Kalecik Karası' showed the highest values in the proportion of trichomes (39.75%) and leaf thickness. This result confirmed the relationship obtained.

In the evaluation of the relationships by the stomatal types, negative correlations were found between the same level stomata and the raised above stomata ($r = -0.704$, $p<0.01$), as well as between the sunken stomata and the same level stomata ($r = -0.574$, $p<0.05$). However, no such association have been reported for stomatal types in previous studies.

Table 2. Leaf morphological traits in grapevine varieties

Variety	Leaf area (cm^2) (Mean ± SEM)	Proportion of trichomes (%) (Mean ± SEM)	Leaf thickness (mm) (Mean ± SEM)
Lival	87.73 ± 5.30	14.04 ± 2.85 bc	0.54 ± 0.02 cd
Tekirdağ Seedless	95.09 ± 4.85	0.41 ± 0.12 e	0.45 ± 0.00 e
Beauty Seedless	91.06 ± 8.81	0.00 ± 0.00 e	0.57 ± 0.01 bc
Michele Palieri	92.81 ± 3.03	0.00 ± 0.00 e	0.60 ± 0.03 bc
Cabernet Sauvignon	90.86 ± 8.87	7.42 ± 0.21 d	0.51 ± 0.14 d
Narince	83.21 ± 2.55	11.03 ± 0.28 cd	0.63 ± 0.03 b
Syrah	72.21 ± 0.84	16.29 ± 1.14 b	0.51 ± 0.14 d
Kalecik Karası	82.43 ± 0.04	39.75 ± 2.80 a	0.73 ± 0.03 a
Alphonse Lavallée	85.19 ± 3.21	14.91 ± 1.13 bc	0.48 ± 0.02 de
Hasandede	75.91 ± 2.52	1.98 ± 0.93 e	0.58 ± 0.01 bc

*Different lowercase letters in the same column indicate significant differences among varieties for each trait ($p<0.05$). SEM: Standard error of Mean

Table 3. Density of prostrate and erect hairs between main veins on the lower side of the blade: 1 = none or very low, 3 = low, 5 = medium, 7 = high, 9 = very high (OIV 2001)

Variety	Density of prostrate hairs (OIV 84)	Density of erect hairs (OIV 85)
Lival	3	3
Tekirdağ Seedless	1	1
Beauty Seedless	1	1
Michele Palieri	1	1
Cabernet Sauvignon	3	3
Narince	3	3
Syrah	3	3
Kalecik Karası	5	5
Alphonse Lavallée	3	3
Hasandede	1	5

Table 4. Range values for the average leaf thickness class of the varieties (mm)

Very thin	Thin	Medium	Thick	Very thick
0.356-0.448	0.449-0.541	0.542-0.634	0.635-0.727	0.728-0.821
Tekirdağ Seedless	Alphonse Lavallée Lival Cabernet Sauvignon Syrah	Narince Beauty Seedless Michele Palieri Hasandede	Kalecik Karası	

Table 5. Correlation analysis between stomatal density, type and leaf morphological traits

		Stomatal density (stomata mm^{-2})	Proportion of trichomes (%)	Leaf thickness (mm)	Stomatal type		
					Raised above	Same level	Sunken
Stomatal density (stomata mm^{-2})		1					
Proportion of trichomes (%)		-0.309*	1				
Leaf thickness (mm)		-0.284	0.528**	1			
Stomatal type	Raised above	-0.074	-0.041	0.124	1		
	Same level	0.154	0.081	0.016	-0.704**	1	
	Sunken	-0.128	-0.065	-0.165	0.174	-0.574*	1

*: $p<0.05$ **: $p<0.01$

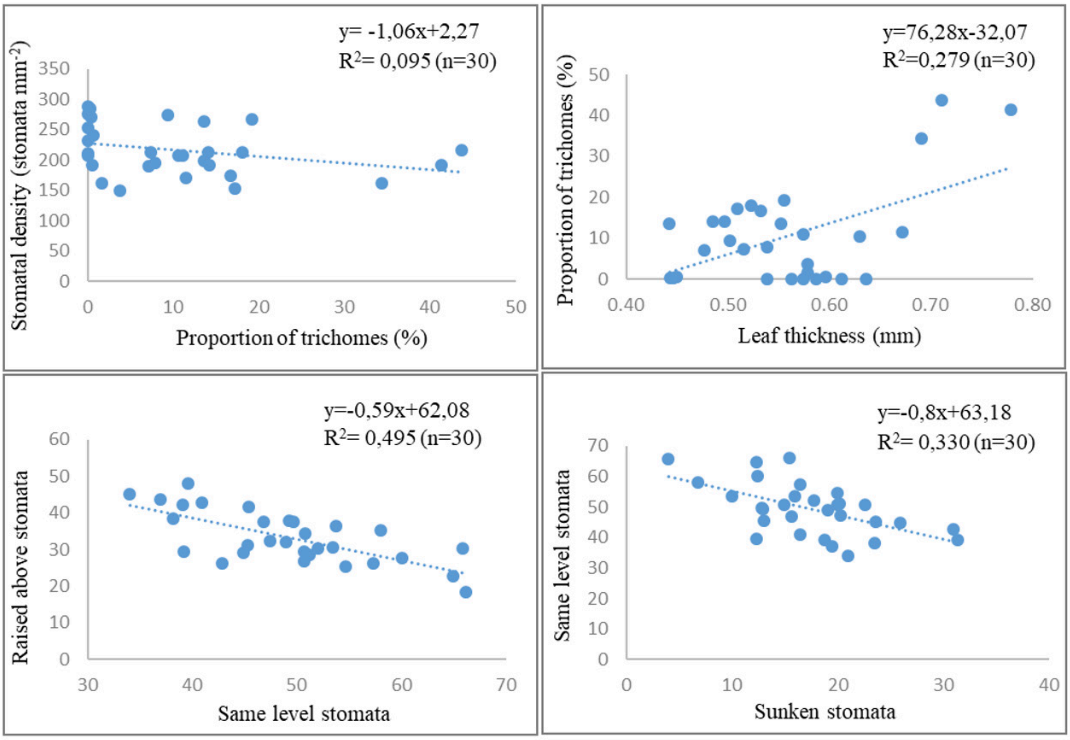

Figure 7. Regression graphs for stomatal density, type and leaf morphological traits.

CONCLUSION

The study revealed stomatal density was a cultivar-specific characteristics for grapevine varieties (*Vitis vinifera* L.). This result is consistent with previous findings that grapevines grown in different ecological conditions. It is thought that, variations in stomatal density among grapevine varieties were probably caused by the intrinsic characteristics of the cultivars in relation to the conditions of their growing ecologies. The relationship between stomatal characteristics and adaptation ability of varieties is discussed. One of the aims of this study is to draw attention to this argument. It was thought that the percentage of sunken stomatal types could be used to evaluate the adaptation of varieties to the semi-arid conditions of Kalecik. In addition, correlation analysis revealed interest in trichome proportion, leaf thickness and stomatal characteristics. To improve vineyard management, it is necessary to gather more information on the impact of climate change on viticulture. Research that examines stomatal and leaf characteristics can provide a new approach to adaptation studies. Finally, the study is the first report on stomatal types in the viticultural literature of Türkiye.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

All authors declare that they have no conflict of interest.

Author contribution

Sinem Güler and Birhan Kunter were responsible for the experimental design, data acquisition and analysis, and manuscript drafting. Aysun Şehit contributed to data analysis. All authors read and approved the final version of the manuscript.

Ethics committee approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

This study consists of a part of the results of Sinem Güler's PhD thesis. The authors would like to thank Assoc. Prof. Yüksel Akınay and Res. Asst. İhsan Nuri Akkuş (Science Application and Research Center at Van Yuzuncu Yıl University) for their assistance with SEM imaging studies.

REFERENCES

Bekişli, İ. (2014). Determination of leaf and stomatal characteristics of some grape varieties and rootstocks grown in the Harran plain conditions. Harran University, Graduate School of Natural and Applied Sciences, Department of Horticulture, MSc Thesis, Şanlıurfa, Turkiye, 96pp (in Turkish with English abstract).

Bodor, P., Szekszárdi, A., Varga, Z., & Bálo, B. (2019). Investigation of the stomata size and frequency of grapevine (*Vitis vinifera* L.) cultivar 'Kékfrankos'. Columella- Journal of Agricultural and Environmental Sciences, 6 (1), 29-34. https:// doi.org/ 10.18380/ szie.colum.2019.6.29

Boso, S., Alonso-Villaverde, V., Santiago, J.L., Gago, P., Dürrenberger, M., Düggelin, M., Kassemeyer, H. H., & Martínez, M.C. (2010). Macro- and microscopic leaf characteristics of six grapevine genotypes (*Vitis* spp.) with different susceptibilities to grapevine downy mildew. Vitis 49, 43–50.

Boso, S., Gago, P., Alonso-Villaverde, V., Santiago, J. L., Mendez, J., Pazos, I., & Martínez, M. C. (2011). Variability at the electron microscopic level in leaves of members of the genus *Vitis*. Scientia Horticulturae, 128 (3), 228-238. https://doi.org/10.1016/j.scienta.2011.01.022

Boso, S., Gago, P., Alonso-Villaverde, V., Santiago, J.L., & Martinez, M.C. (2016). Density and size of stomata in the leaves of different hybrids (*Vitis* sp.) and *Vitis vinifera* varieties. Vitis, 55 (1), 17-22. https://dx.doi.org/10.5073/vitis.2016.55.17-22

Bozkurt, A. (2023). Improve of new powdery mildew tolerant grape cultivars for stuffe vine leaf. Tokat Gaziosmanpaşa University. Graduate School of Natural and Applied Sciences, Department of Horticulture, PhD Thesis, Tokat, Türkiye, 307pp (in Turkish with English abstract).

Candar, S., Açıkbaş, B., Korkutal, İ., & Bahar, E. (2021). The effects of water deficit on leaf and stoma morphological properties of wine grapes in thrace region. KSU Journal of Agriculture and Nature, 24 (4), 766-776 (in Turkish with English abstract). https://doi.org/10.18016/ksutarimdoga.vi.738285

Copper, A.W., Koundouras, S., Bastian, S.E., Johnson, T.E., & Collins, C. (2022). Assessing the response of *Vitis vinifera* L. cv. Xynisteri to different irrigation regimes and its comparison to cvs. Maratheftiko, Shiraz and Sauvignon Blanc. Agronomy, 12 (3), 634. https://doi.org/10.3390/agronomy12030634

Doğan, A.,Uyak, C., Akçay, A., Keskin, N., Şensoy, R. İ. G., Çelik, F., Kunter, B., Çavuşoğlu, Ş., & Özrenk, K. (2020). Determination of chlorophyll amounts and stoma densities of grape cultivars grown in Hizan (Bitlis) conditions. YYU Journal of Agricultural Science, 30(4), 652-665 (in Turkish with English abstract).

Düzenli, S., & Ağaoğlu, Y. S. (1992). Effect of leaf age and leaf positions on stomatal density in some varieties of *Vitis vinifera* L. Doğa–Turkish Journal of Agriculture and Forestry, 16, 63-72 (in Turkish).

Franks, P.J., Drake, P.L., & Beerling, D.J. (2009). Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using *Eucalyptus globulus*. Plant Cell & Environment, 32, 1737–1748. https://doi.org/10.1111/j.1365-3040.2009.002031.x

Gago, P., Conejero, G., Martínez, M.C., Boso, S., This, P., & Verdeil, J. L. (2016). Microanatomy of leaf trichomes: opportunities for improved ampelographic discrimination of grapevine (*Vitis vinifera* L.) cultivars. Australian Journal of Grape and Wine Research, 22 (3), 494-503. https://doi.org/ 10.1111/ajgw.12226

Gago, P., Conejero, G., Martínez, M. C., This, P., & Verdeil, J. L. (2019). Comparative anatomy and morphology of the leaves of grenache Noir and Syrah grapevine cultivars. South African Journal of Enology and Viticulture, 40 (2), 1-9. https://doi.org/10.21548/40-2-3031

Gökbayrak, Z., Dardeniz, A., & Bal, M. (2008). Stomatal density adaptation of grapevine to windy conditions. Trakia Journal of Sciences, 6 (1), 18-22.

Hopper, D.W., Ghan, R., & Cramer, G.R. (2014). A rapid dehydration leaf assay reveals stomatal response differences in grapevine genotypes. Horticulture Research, 1 (2). https://doi.org/10.1038/hortres.2014.2

IPCC (2013). Climate change 2013: The physical science basis. Working group I contribution to the fifth assessment report of the invergovernmental panel on climate change. https://www.ipcc.ch/site/assets/uploads/2018/02/WG1AR5_all_final.pdf. [Access date: May 8, 2022].

İşçi, B., Altındişli, A., & Kaçar, E. (2015). Investigations on stomatal distribution of different grape cultivars grafted on different rootstocks. COMU Journal of Agriculture Faculty, 3 (1), 35–39 (in Turkish with English abstract).

Jones, H.G. (2014). Drought and other abiotic stresses. In: Plants and microclimate: A quantitative approach to environmental plant physiology (3rd ed., pp. 255-289). Cambridge University Press., Cambridge, UK.

Kara, S., & Özeker, E. (1999). Studies on leaf characteristics and stomatal distribution of round seedless grape cultivars grafted on different rootstocks. Anadolu Journal of Aegean Agricultural Research Institute, 9 (1), 76-85 (in Turkish with English abstract).

Keller, M. (2010). The science of grapevines: Anatomy and physiology (2nd ed.). Academic Press/Elsevier.

Liu, C., He, N., Zhang, J., Li, Y., Wang, Q., Sack, L., & Yu, G. (2018). Variation of stomatal traits from cold temperate to tropical forests and association with water use efficiency. Functional Ecology, 32 (1), 20-28. https://doi.org/10.1111/1365-2435.12973

Liu, C., Li, Y., Xu, L., Li, M., Wang, J., Yan, P., & He, N. (2021). Stomatal arrangement pattern: a new direction to explore plant adaptation and evolution. Frontiers in Plant Science, 12, 1-7. https://doi.org/10.3389/fpls.2021.655255

MacMillan, P., Teixeira, G., Lopes, C. M., & Monteiro, A. (2021). The role of grapevine leaf morphoanatomical traits determining

capacity for coping with abiotic stresses: a review. Ciência E Técnica Vitivinícola, 36 (01), 75-88. https://doi.org/10.1051/ctv/ctv2021360175
Marasalı, B., & Aktekin, A. (2003). Comparative study on stomatal density of grape cultivars grown under dry and irrigated vineyard conditions. Journal of Agricultural Sciences, 9 (3), 370-372 (in Turkish with English abstract). https://doi.org/ 10.1501/Tarimbil_0000000839
Monteiro, A., Teixeira, G., & Lopes, C.M. (2013). Comparative leaf micromorphoanatomy of *Vitis vinifera* ssp. vinifera (*Vitaceae*) red cultivars. Ciência e Técnica Vitivinicola, 28 (1), 19-28.
Monteiro, A., Teixeira, G., Santos, C., & Lopes, C.M. (2018). Leaf morphoanatomy of four red grapevine cultivars grown under the same terroir. E3S Web of Conferences, 50, 01038. https://doi.org/10.1051/e3sconf/20185001038
Nassuth, A., Rahman, M.A., Nguyen, T., Ebadi, A., & Lee, C. (2021). Leaves of more cold hardy grapes have a higher density of small, sunken stomata. Vitis, 60 (2), 63-67. https://doi.org/10.5073/vitis.2021.60.63-67
Odabaşıoğlu, M.İ. (2020). Determination of yield, quality and seed characteristics and stoma morphology of table grape varieties grafted on different rootstocks in semi-arid conditions. Harran University, Graduate School of Natural and Applied Sciences, Department of Horticulture, PhD Thesis, Şanlıurfa, Turkiye, 307pp (in Turkish with English abstract).
OIV (2001). The 2nd edition of the OIV descriptor list for grape varieties and *Vitis* species. https://www.oiv.int/public/medias/2274/code-2e-edition-finale.pdf. [Access date: May 8, 2022].
Rogiers, S.Y., Greer, D.H., Hutton, R.J., & Landsberg, J.J. (2009). Does night-time transpiration contribute to anisohydric behaviour in a *Vitis vinifera* cultivar?. Journal of Experimental Botany, 60 (13), 3751-3763. https://doi.org/ 10.1093/jxb/erp217
Sack, L., & Buckley, T.N. (2016). The developmental basis of stomatal density and flux. Plant Physiology, 171 (4), 2358–2363. https://doi.org/ 10.1104/pp.16.00476
Samarth, R. R., Shetty, D., Saha, S., & Sawant, I. (2021). Leaf micro-morphological diversity in *Vitis* species and its association with resistance to *Plasmopara viticola*. Research on Crops, 22 (2), 334-341. https://doi.org/10.31830/2348-7542.2021.076
Šantrůček, J. (2022). The why and how of sunken stomata: does the behaviour of encrypted stomata and the leaf cuticle matter?. Annals of Botany, 130 (3), 285-300. https://doi.org/10.1093/aob/mcac055
Serra, I., Strever, A., & Myburgh, P., Schmeisser, M., Deloire, P.A. (2017). Grapevine (*Vitis vinifera* L. 'Pinotage') leaf stomatal size and density as modulated by different rootstocks and scion water status. Acta Horticulturae, 1157, 177-181. https://doi.org/10.17660/ActaHortic.2017.1157.26
Shiraishi, S.I., Hsiung, T.C., & Shiraishi, M. (1996). Preliminary survey on stomatal density and length of grapevine. Journal of Faculty of Agriculture Kyushu University, 41 (1-2), 11-15. https://doi.org/10.5109/24124
Swanepoel, J.J., & Villiers, C.E. (1987). A numerical-taxonomic classification of *Vitis* spp. and cultivars based on leaf characteristics. South African Journal of Enology and Viticulture, 8 (2), 31-35. https://doi.org/10.21548/8-2-2311
Teixeira, G., Monteiro, A., Santos, C., & Lopes, C.M. (2018). Leaf morphoanatomy traits in white grapevine cultivars with distinct geographical origin. Ciência e Técnica Vitivinícola, 33 (1), 90-101. https://doi.org/10.1051/ctv/20183301090
Uyak, C., Keskin, N., Doğan, A., Şensoy Gazioğlu, R. İ., & Başdinç, A. (2016). Determination of stomatal densities and chlorophyll amounts in some grape varieties grown in Van ecology. Bahçe, 46, 738-742 (in Turkish with English abstract). https://doi.org/10.29133/yyutbd.698508