

LEAF AND PETIOLE ANATOMY OF SOME GRAPEVINE (*Vitis vinifera* L.) CULTIVARS GROWN IN TÜRKİYE

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Keywords

Anatomical studies
Paraffin method
Transverse section
Superficial section
PCA

To cite this article

Biyiklioglu, O., Karabıcak, S., Ceter, T., Leaf and petiole anatomy of some grapevine (*Vitis vinifera* L.) cultivars grown in Türkiye.
Commun.Fac.Sci.Univ.Ank.Ser.C Biology, 34 (2) (2025), 243-259.
<https://doi.org/10.1111/communc.1817845>

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Abstract

The grapevine has been cultivated in almost every region of the world due to its commercial value and as a spread source of food, all which has allowed diversification of grape types. The distinction of grapevine varieties is usually made by examining their morphological features. However, it is known that anatomical studies are very valuable in plant taxonomy. In this study, the anatomical features of leaf and petiole sections were examined using three set of samples for eight different grapevine varieties (i.e., 'Öküzgözü', 'Narince', 'Syrah', 'Boğazkere', 'Kalecik Karası', 'Viognier', 'Malbec' and 'Sauvignon Blanc') grown in the vineyards of Toklumen village in the center of Kırşehir Province. Leaf blade sections are generally seen to consist of upper epidermis, palisade parenchyma, spongy parenchyma and lower epidermis layers. On the other hand, the petiole has a five-lobed appearance in cross-section, drawing attention to the accessory vascular bundle seen from the adaxial region along with large vascular bundles above them. According to the findings, it was determined that although the anatomical features may vary according to environmental effects, there were significant differences in different cultivars growing in the same area.

1. INTRODUCTION

The Vitaceae family consists of 910 species of about 14 genera of succulent plants that grow naturally in tropical and subtropical regions. In general, they are woody climbers, herbaceous vines or shrubby while rarely they can become in the form of small succulent trees. Flowers are hermaphrodite or unisexual, actinomorphic, small, inflorescence cymose (Ampelopsis) or paniculate (*Vitis*) [1-5]. The genus *Vitis*, which includes vines, is one of the most important genera of the Vitaceae family with its high economic value. *Vitis* generally spreads in tropical and subtropical regions in the northern hemisphere and includes tendril structures, all of which are climbing and winding. It is accepted that tendril structures are an altered form of the fruit stem that has evolved to enable climbing and hold [6].

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When historical sources and archaeological and micro-chemical data are examined, it is estimated that the grapevine was cultivated in the Transcaucasia region between 4000-8000 BC in the Neolithic Age and was used for wine production. It is characteristic that the grape clusters and tendrils always come out opposite to the leaves [7-9].

Grapevine is consumed not only for wine but also in many areas in today. Many varieties have been cultured and become common cultivars worldwide. According to 2018 data, the total vineyard area in the world was 7.4 million hectares, and the total grape production was 78 million tons. 50% of the grape production in the world are provided by five countries, including Türkiye [10].

It is thought that there are over 10 thousand grape varieties in the world, and 1200 of them are grown in Türkiye [11]. Ampelographic studies are carried out to ensure the separation of grape varieties. Ampelography is generally based on morphological characters [12]. However, there are also such studies showing that anatomical characters can be used in Ampelographic studies [4, 13- 21]. However, anatomical studies on the genus *Vitis* have generally been done to understand its biology [22- 34].

This study aimed to compare the leaf and petiole anatomies of eight grape varieties grown in Toklügen town of Kırşehir province and compare their differences and similarities. It is thought that the findings will support ampelographic studies and contribute to a better understanding of the anatomical features of the grapevine.

2. MATERIALS AND METHODS

All grapevine samples were collected from the vineyards of Kavaklıdere Winery Inc., located in Toklügen village of Kırşehir province. Three samples were collected from each variety. The samples used in the study were taken between the 4th and 6th nodes after harvesting and then preserved in 70% alcohol. Transverse sections were taken in the centre of the petioles and over the midvein of the leaf blade, and superficial sections were taken from the leaf surface (Figure 1). Transverse sections were taken in 10-15µm sections by modified Paraffin Sectioning Method using Thermo SHANDON FINESS325 microtome [35-38]. After removing the paraffin from the sections, safranin O and fast green dyes were applied [36]. Superficial sections were taken by hand method. Preparations were examined with a Leica DM3000 LM microscope and photographed with a Leica DFC295 camera. In order to determine the anatomical features, measurements were done on 13 characters for the leaf blade and seven characters

for the petiole. All the measurements were calculated using the ImageJ program (Figure 2-3 and Table 1-2).

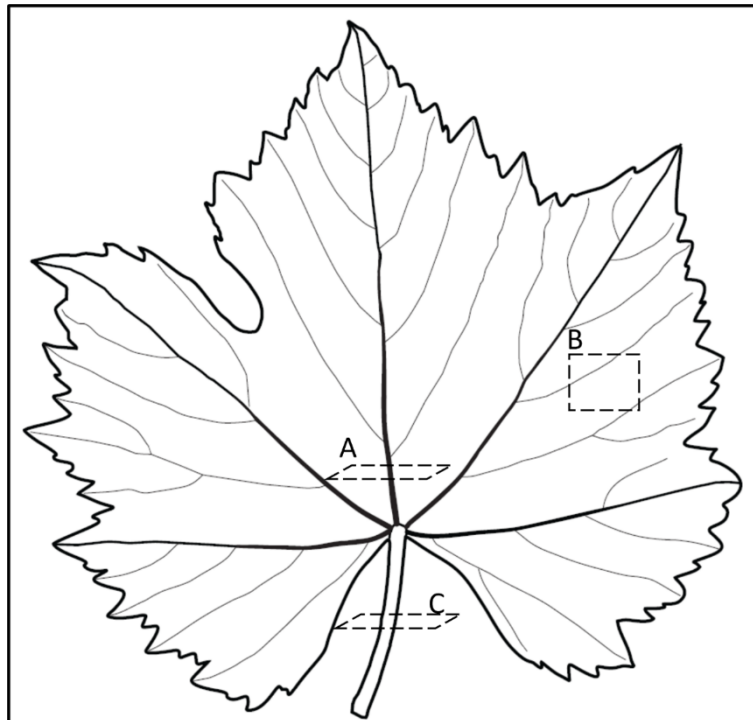


FIGURE 1. Areas where microscopic sections were taken from the leaf blade and petiole (A and C: transverse sections, B: Superficial sections areas)

TABLE 1. Characters examined in leaf section of *Vitis vinifera*.

Character Code	Description	Measurement Unit
L1	Thickness of the upper epidermis layer	μm
L2	Thickness of the palisade parenchyma layer	μm
L3	Thickness of the sponge parenchyma layer	μm
L4	Thickness of the lower epidermis layer	μm
L5	Stomata length	μm
L6	Stomata width	μm
L7	Leaf vein vascular bundle width	μm
L8	Leaf main vein vascular bundle height	μm
L9	Leaf vein epidermis thickness	μm
L10	Wall thickness of leaf vein tracheal cells	μm
L11	Stomatal density	Piece / mm^2
L12	Stomatal index	Ratio (%)
L13	Leaf thickness	μm

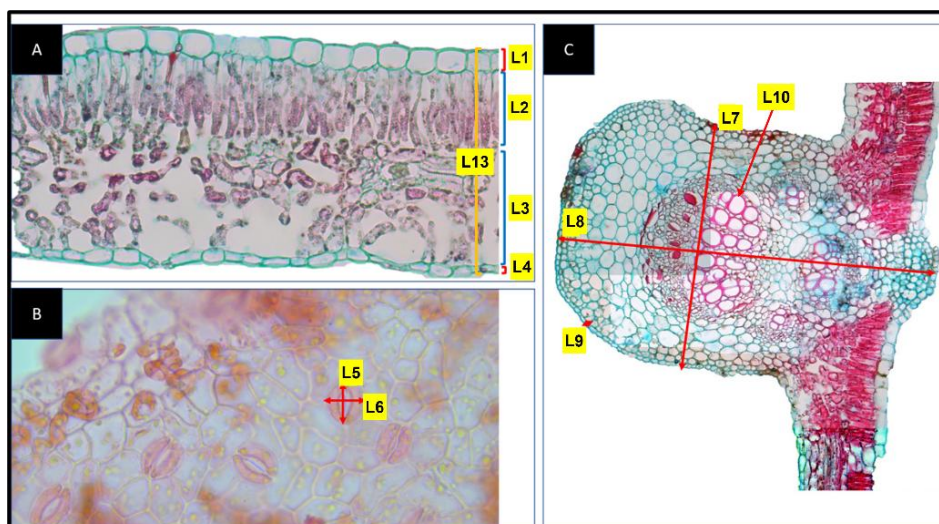


FIGURE 2. Characteristics examined in *Vitis vinifera* leaf blade transverse (A, C) and superficial (B) sections

TABLE 2. Characters examined in leaf section of *Vitis vinifera*.

Character Code	Description	Measurement Unit
P1	Petiole height	µm
P2	Petiole width	µm
P3	Accessory vascular bundles length	µm
P4	Large vascular bundle length	µm
P5	Tracheal cell wall thickness in large vascular bundles	µm
P6	Epidermis layer thickness	µm
P7	Petiole height/width	-

The means of the measurements were analyzed using SPSS v23, and the differences were compared at the 0.05 significance level. Differences between varieties were determined by ANOVA followed by Tukey's test, and significant differences are indicated with superscript letters. The PCA (Principal Component Analysis) was performed with R, and the graphs were created by grouping them according to each grape variety using the ggbiplot library. The contributions of the examined characters to the PCA components were computed using the factoextra library. The PCA results were interpreted based on PC1 and PC2, which explained the highest proportion of variance [39-40].

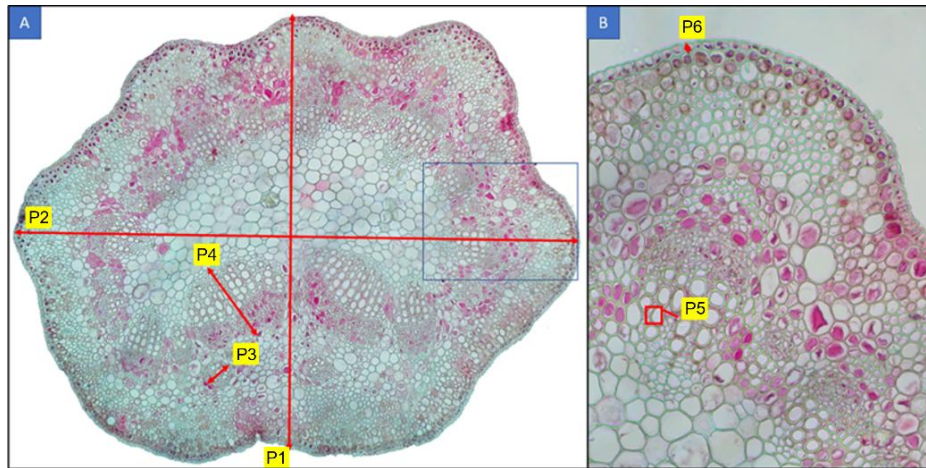


FIGURE 3. Characters examined in *Vitis vinifera* petiole section (A: General view, B: Detailed view)

3. RESULTS AND DISCUSSION

In the cross-section of the leaf blade of the examined grapevine varieties, upper epidermis, generally single-row palisade parenchyma, spongy parenchyma consisting of several rows of cells and a single-row lower epidermis layers were observed. Both the upper and lower epidermis are tapered on the leaf vein. Stomata in all varieties were found only in the lower epidermis (hypostomatic). They were of the amaryllis type, while they were observed to be of the anomocytic type according to the arrangement of the adjacent cells. Leaf blade thicknesses were determined between 124.42-199.15 μm . The midrib is similar in all cultivars, and prominent vascular bundles are conspicuous. Generally, six distinct vascular bundles can be distinguished, but this number can vary between 4 and 8 even within the same cultivar. In addition, smaller vascular bundles were observed in all cultivars, with these prominent bundles nearby. Generally, vascular bundles on the main vessel are more developed in the adaxial and abaxial regions than in the lateral region. Raphide bundles and druse crystals were frequently encountered on the leaf blade and leaf vein in all cultivars (Table 3 and Figure 4-6).

TABLE 3. Data from leaf sections.

	Öküzgözü	Narince	Syrah	Boğazkere	Kalecik Karası	Viognier	Malbec	Sauvignon Blanch
L1	18.34±3.35 ^{ae}	21.93±2.8 ^b	16.44±2.77 ^{acd}	14.51±3.72 ^d	15.95±3.47 ^{ad}	19.16±3.33 ^e	16.74±2.35 ^{ade}	15.86±2.92 ^{cdf}
L2*	71.65±11.54 ^a	69.65±10.66 ^{ad}	50.82±9.72 ^b	39.26±6.85 ^c	61.75±10.48 ^{de}	65.18±12.44 ^{ade}	76.65±14.73 ^a	59.51±9.51 ^e
L3*	96.18±14.61 ^a	90.12±7.67 ^a	61.89±10.89 ^b	49.98±9.29 ^c	62.41±10.41 ^b	73.13±10.05 ^{de}	79.59±11.98 ^d	70.81±8.43 ^e
L4*	12.61±2.91 ^{ac}	18.22±3.14 ^b	17.43±2.54 ^b	10.98±1.97 ^a	17.05±3.73 ^{bd}	16.99±3.11 ^{bd}	16.01±2.86 ^{bd}	14.92±3.31 ^{cd}
L5*	22.24±3.09 ^{ace}	24.18±2.12 ^{ab}	21.88±1.96 ^c	24.26±4.24 ^{ace}	23.39±2.54 ^{ace}	21.89±2.74 ^{cde}	22.9±2.84 ^{ace}	23.54±1.57 ^{be}
L6*	17.26±3.02 ^{acd}	16.52±2.31 ^{ab}	17.53±1.67 ^{acd}	16.68±3 ^{acd}	17.01±1.78 ^{acd}	18.49±2.17 ^c	16.67±1.62 ^{bd}	17.01±2.11 ^{acd}
L7	938.5±283.45	978.87±99.68	736.03±183.29	823.5±31.36	1223.13±526.66	939.17±199.9	1173.83±220.91	697.2±94.71
L8	759.5±244.03	1100.3±110.23	1171.73±230.47	942.27±23.02	1533±369.99	980.9±304.89	1302.77±319.77	904.7±25.66
L9*	11.73±3.32 ^{ab}	9.65±2.54 ^{abc}	10.29±2.62 ^{abc}	9.53±2.1 ^{ac}	10.51±2.91 ^{abc}	11.54±2.32 ^b	9.22±2.21 ^c	9.13±1.58 ^{cd}
L10*	2.69±0.56 ^{ab}	2.58±0.67 ^{ab}	2.56±0.65 ^{ab}	2.33±0.89 ^{ab}	2.74±0.78 ^{ab}	2.38±0.53 ^a	2.91±0.64 ^b	2.46±0.58 ^{ab}
L11	203.9±24.08	199.44±29.78	280.37±38.78	261.63±85.3	244.29±30.92	268.51±84.34	281.76±47.67	236.25±40.51
L12*	9.24±1.18 ^{ab}	8.56±0.63 ^{ab}	8.07±1.24 ^{ab}	10.69±1.6 ^a	8.39±1.37 ^{ab}	8.05±1.05 ^b	7.93±1.03 ^{bc}	9.72±0.14 ^{ab}
L13*	196.14±14.77 ^{ae}	199.15±11.86 ^a	156.68±7.53 ^b	124.42±14.1 ^c	147.52±16.66 ^b	180.22±15.85 ^d	189.19±11.63 ^{de}	159.1±10.9 ^b

*There are statistically significant differences in the 95% confidence rate.

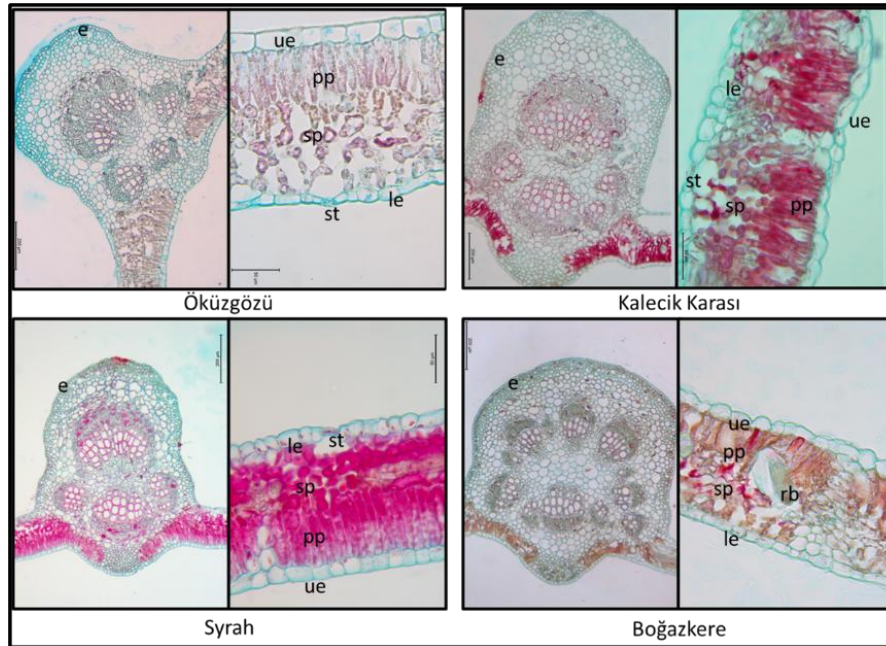


FIGURE 4. Leaf blade cross-section images (e: epidermis, le: lower epidermis, ue: upper epidermis, st: stoma, sp: sponge parenchyma, pp: palisade parenchyma, rb: raphide bundles)

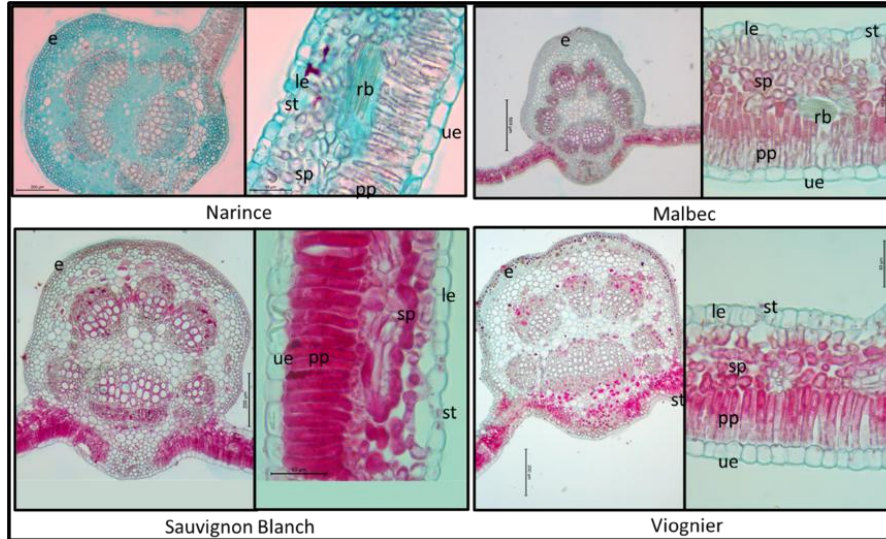


FIGURE 5. Leaf blade cross-section images (e: epidermis, le: lower epidermis, ue: upper epidermis, st: stomata, sp: sponge parenchyma, pp: palisade parenchyma, rb: raphide bundles)

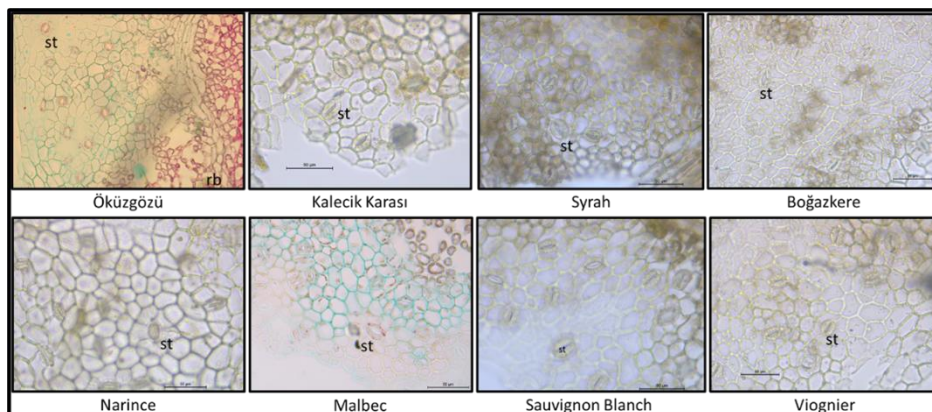


FIGURE 6. Leaf blade superficial section images (st: stomata, rd: raphide bundles)

The largest leaf thickness was seen in the 'Narince' variety, with 199.15 μm , while the variety with the narrowest leaf blade was determined as the 'Kalecik Karası', with 147.52 μm . The thickness of the upper epidermis was measured the most in the 'Narince' cultivar (21.93 μm) while the least in the 'Boğazkere' cultivar (14.51 μm). The thickness of the lower epidermis was determined the most in the Narince variety (18.22 μm) and the least in the 'Öküzgözü' (12.61 μm). The largest palisade parenchyma layer (76.65 μm) was seen in 'Malbec', while 'Öküzgözü' had the thickest sponge parenchyma layer (96.18 μm). The smallest palisade parenchyma thickness was 39.26 μm in 'Boğazkere', and the smallest sponge parenchyma was again 49.68 μm in the 'Boğazkere' variety. Similarly, in the study conducted by Monteiro et al. (2013), distinctive differences were found between different grape varieties in the thickness of the total lamina, lower and upper epidermis, palisade, and sponge parenchyma tissue [16].

Stomata widths and lengths were also found to differ between the examined cultivars. Numerous studies have investigated stomatal density in grapevine leaves. Some of these studies showed that stomatal densities changed significantly according to environmental factors, while others did not show significant differences. According to Manoj et al. (2006), the length of the palisade layer was determined as 112.00 μm and 71.00 μm , and the sponge parenchyma layer was 99.00 μm and 113.00 μm , respectively, in 'Syrah' and 'Sauvignon Blanc' grapevine cultivars [41]. In a study conducted by Gago et al., (2019), it was reported that the leaf blade thickness of the 'Syrah' variety was $193.01 \pm 10.55 \mu\text{m}$. Stomatal density was 148.80 ± 7.36 , stomatal index was 8.24 ± 0.81 , and stomatal length was 28.21 ± 0.51 [20]. It is seen that the stomatal index is largely consistent with our study. However, it is seen that the

values of some characters such as leaf blade thickness measured in the same grape varieties are quite different. These differences are thought to be due to the effects of environmental factors. In a study conducted by Marasalı and Aktekin (2002), the variation of stomatal density depending on the irrigation regime on 17 different cultivars was examined, and significant differences were observed on only seven cultivars [15]. These values also differ from the measurements we obtained (199.44 pieces/mm² for 'Narince' and 244.29 pieces/mm² for 'Kalecik Karası'). It has been reported that while the mesophyll tissues and lamina thickness increase in some grape varieties exposed to heat stress, the stomatal density remains constant [42]. However, environmental factors were reported to be effective on stomatal density by 12 cultivars studied by Boso et al. (2016) and four different cultivars studied by Monteiro et al. (2013) [16, 17]. In addition, in these studies, it is seen that stomata densities show differences between cultivars. Also, Nassuth et al. (2021), it has been reported that the stomatal density and the number of small-sized stomata are higher in varieties that are more resistant to cold. Najmaddin et al. (2011) reported that raphide bundles, druse crystals, and secretion ducts were observed in the leaves of 3 different grape varieties [4]. The same situation was observed in the leaf samples we examined.

The petiole exhibits a five-lobed structure, and two accessory vascular bundles are typically present in the adaxial region. These two bundles are distinctly more developed than the others. In the microscopic examination, raphide bundles and druse crystals are frequently encountered in pith and phloem tissues. The pith region consists of highly prominent parenchymatous cells (Table 4 and Figure 7).

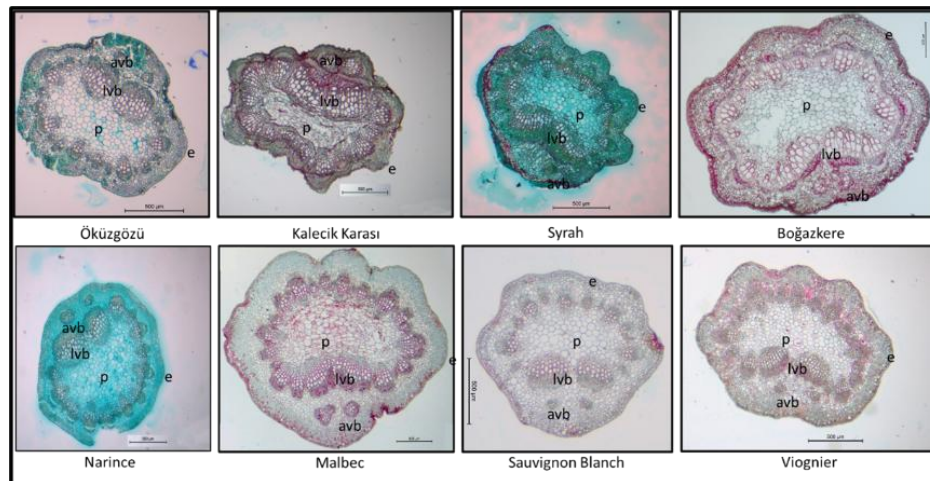


FIGURE 7. Images obtained from petiole sections (p: pith, lvb: large vascular bundles, avb: accessory vascular bundles, e: epidermis)

TABLE 4. Data from petiole sections.

	Öküzgözü	Narince	Syrah	Boğazkere	Kalecik Karası	Viognier	Malbec	Sauvignon Blanch
P1 *	1020±856 ^{aef}	1403±252 ^{aef}	1505±159 ^{ab}	919±167 ^{ac}	1476±508 ^{aef}	1652±278 ^{ad}	2690±216 ^e	1339±103 ^{bcd}
P2 *	2136±767 ^{acd}	1751±167 ^{acd}	2272±515 ^{acd}	1218±40 ^{ab}	1779±336 ^{acd}	1871±174 ^c	3036±284 ^d	1476±99 ^{bce}
P3 *	224.63±102 ^{afg}	116.31±23.79 ^{ab}	131.6±24.09 ^{ac}	113.36±34.45 ^{ad}	195.84±81.64 ^{afg}	116.81±17.82 ^{ae}	212.98±32.84 ^f	128.54±39.3 ^{bcdeg}
P4 *	476.93±155 ^{afg}	357.2±68.85 ^{ab}	387.39±49.45 ^{ac}	218±36.26 ^d	365.69±317.88 ^{adf}	343.61±66.72 ^{ae}	531.72±59.97 ^f	318.5±81 ^{bcdeg}
P5 *	2.56±0.56 ^{abcd}	2.29±0.5 ^a	2.74±0.66 ^{abcd}	2.41±0.58 ^{ab}	2.39±0.69 ^{abc}	2.89±0.84 ^{bcd}	3.05±0.87 ^{cd}	2.98±0.64 ^d
P6 *	9.89±1.8 ^a	10.34±1.77 ^{ac}	10.3±1.74 ^{ac}	8.34±1.21 ^b	10.8±2.87 ^{ac}	11.58±2.11 ^c	10.15±2.22 ^{ac}	9.4±1.56 ^{ab}
P7	0.93±0.06	0.8±0.1	0.68±0.12	0.75±0.11	0.82±0.15	0.88±0.1	0.89±0.01	0.91±0.02

*There are statistically significant differences in the 95% confidence rate.

When the petiole sections of the studied cultivars were examined, 'Malbec' was the cultivar with the largest petiole diameter (2690 x 3036 μm) and 'Boğazkere' with the least petiole diameter (919 x 1218 μm). While 'Viognier' had the largest epidermis thickness, the narrowest epidermis layer was detected in the 'Boğazkere' cultivar. Najmaddin et al. (2011) reported that the shape of the petiole varies according to the cultivars, and the presence of trichomes on the surface varies. It has been determined that the vascular bundles are in the form of closed vascular bundles surrounded by sclerenchyma fibers [4]. These findings are similar to the cultivars we investigated. In addition, it has been reported that the higher number of xylem elements in the petiole provides higher water-holding capacity [43]. Therefore, it can be thought that cultivars with larger vascular bundles in their petioles are more resistant to exposure to water stress.

Although the general appearances of both leaf and petiole structures are similar between cultivars, statistically significant differences were found in many anatomical characteristics within the cultivars studied. Statistically significant ($p < 0.05$) differences were found between cultivars in 10 (L1, L2, L3, L4, L5, L6, L9, L10, L12, and L13) of 13 characters examined in leaf sections and 6 (P1, P2, P3, P4, P5 and P6) of 7 characters in petiole sections. PCA positioned cultivars in partially separate regions (Table 5, Figure 8).

TABLE 5. Effects of study characters on PCA components.

Leaf blade			Petiole		
Character Code	PC1	PC2	Character Code	PC1	PC2
L1	-0.79	0.20	P1	-0.96	0.09
L2	-0.78	0.10	P2	-0.85	0.40
L3	-0.83	0.31	P3	-0.77	0.36
L4	-0.48	-0.28	P4	-0.93	0.05
L5	0.30	0.40	P5	-0.52	-0.21
L6	0.17	0.32	P6	-0.55	-0.47
L7	-0.47	-0.65	P7	-0.58	-0.66
L8	-0.23	-0.79			
L9	-0.33	0.05			
L10	-0.18	-0.40			
L11	0.40	-0.50			
L12	0.46	0.27			
L13	-0.90	0.31			

*The effects of >0.49 on PCA' components are written in bold

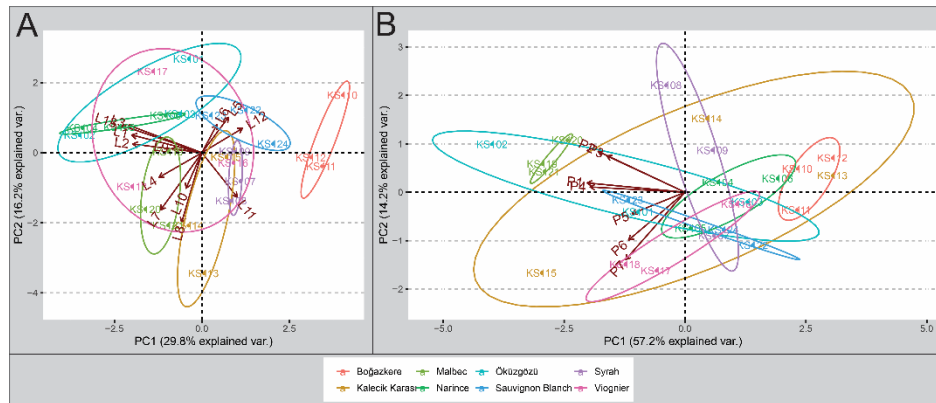


FIGURE 8. PCA results based on anatomical section data (A: Leaf, B: Petiole)
 KS101-103: Öküzgözü, KS104-105: Narince, KS07-KS109: Syrah, KS110-KS112:
 Boğazkere, KS113-KS115: Kalecik Karası, KS116-KS118: Viognier, KS119-KS121:
 Malbec, KS122-KS124: Sauvignon Blanc

In leaf sections, leaf thickness (L13), upper epidermis layer thickness (L1), sponge parenchyma layer thickness (L3), and palisade parenchyma layer thickness (L2) are the characters that affect the first component of PCA the most, while leaf main vein vascular bundle height (L8), leaf vein vascular bundle height (L7), and stomatal density (L11) had the most significant effect on the 2nd component. In petiole sections, the most significant characteristics affecting the 1st component of PCA are petiole height (P1), accessory vascular bundle length (P3), and petiole width (P2), while Epidermis layer thickness (P6) and petiole height / Petiole width ratio (P7) had a more significant effect on the second component. However, leaf characters seem to discriminate more successfully than petiole characters for PCA. In PCA, PC1 and PC2 components explained the variation between cultivars at the rate of 46% in leaf sections and 71.4% in petiole sections. In PCA graphs, the 'Boğazkere' cultivar differed significantly from other cultivars in leaf and petiole sections. 'Malbec', 'Syrah', 'Sauvignon Blanc', and 'Narince' cultivars showed a relatively narrow distribution in both sections. While 'Viognier' and 'Öküzgözü' showed the widest distribution in leaf sections, 'Kalecik Karası' and 'Öküzgözü' varieties showed the widest distribution in petiole sections. It is thought that studying cultivars such as 'Kalecik Karası', 'Viognier', and 'Öküzgözü' with a larger number of samples will contribute to a more specific grouping of these cultivars and explain the variation between cultivars at higher rates.

4. CONCLUSIONS

This study and the existing studies revealed that the anatomical features of grape varieties are statistically different from each other. The fact that anatomical characters are affected by environmental conditions and genetic effects contributes negatively to the studies carried out for the diagnosis of grapevine varieties with anatomical methods. However, it is thought that both leaves' and petioles' micromorphological features may be effective in diagnosing different grapevine species, with studies to be carried out in more varieties and different ecological conditions. In addition, anatomical features can show the adaptation capacity of plants to different ecological conditions, and the effect of environmental factors on micromorphological features can contribute to the determination of the environmental stresses of existing plants. In addition, keeping world grape production statistics more comprehensive can contribute to the use of future anatomical studies to indicate the success of grapevine varieties in adapting to the environment.

Acknowledgment We would like to thank Ahmet GÜRBÜZ, Vineyard Director of "Kavaklıdere Wines Inc", Ergün GÜMÜŞ, Ankara Vineyard Manager, Özgür MERT, Kırşehir Vineyard Manager and Hâlis CAMUŞ, Vineyard Worker, who contributed to the procurement of grapevine samples. We would also like to thank Mr Kutlu SALİHOĞLU for collecting grapevine samples through field work, Muhammet Ali KARAKAYA and Furkan İPAR for their contributions to laboratory and literature studies.

Author Contribution Statements TÇ provided the samples. OB and TÇ designed the study. OB performed the laboratory work and analyses. OB, SK, and TÇ wrote and revised the manuscript. All authors approved the final version of the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest.

Ethical Statement This research did not involve human participants or animals. Therefore, no ethical approval was required.

Use of Artificial Intelligence No artificial intelligence-based tools or applications were used in the preparation of this study. The entire content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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