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# The Leaf Properties, Stomatal Index and Chlorophyll Content of Turkish Hazelnut (Corylus avellana L.) Cultivars 

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#### Abstract

This study examined the leaf micromorphological properties, stomatal indexes, and chlorophyll contents of 20 Turkish hazelnuts (Corylus avellana) cultivars. The cultivars examined included the "Acı, Allahverdi, Cavcava, Çakıldak, Foşa, Giresun melezi, Incekara, Kalınkara, Kan, Karafındık, Kargalak, Kuş, Mincane, Okay 28, Palaz, Sivri, Uzunmusa, Tombul, Yassı Badem, and Yuvarlak Badem". The chlorophyll content was measured by a portable chlorophyll meter and the surface sections of leaves were excised by hand and all measurements were obtained by using imaging software (NIS - Elements, Version 3.00 SP5). The stomatal index per unit area ( $1 \times 10^{4} \mu \mathrm{~m}^{2}$ ) was calculated. For scanning electron microscope (SEM) imaging, the dried leaves were mounted on stubs using double-sided adhesive tape. The leaf samples were coated with $12.5-15.0 \mathrm{~nm}$ of gold and the coated leaves were photographed


using a Hitachi SU 1510 SEM. Three wax ornamentation types were found in the leaf samples (e.g., crust, smooth, and granules). The epidermal features, stomatal index, and chlorophyll quantities showed some differences among the C. avellana cultivars. The importance of stoma width and stoma length were determined for the "Palaz", "Kuş", "Yuvarlak Badem", and "Yassı Badem". The stomatal index and width and length of upper epidermis and lower epidermis were identified as distinctive properties for the "Allahverdi", "Kargalak", "Kara", and "Mincane". The chlorophyll density was identified as a distinctive feature of the "Sivri", "Çakıldak", "Incekara", and "Acı" cultivars. The highest correlation was found at a rate of 0.98 between "Okay 28 " and "Tombul" while the lowest correlation was found at a rate of 0.87 between "Sivri - Karafındık", "Sivri -Foşa", and "Sivri - Kargalak".

Keywords: Corylus avellana, Leaf micromorphology, Scanning electron microscopy, SPAD, Stoma

## 1. Introduction

The Betulaceae family consists of six genera and 120 species around the world (Hardin \& Bell 1986) and includes 5 genera and 12 species in Turkey (Güner et al. 2012). The Corylus L. genus belongs to the family Betulaceae. According to Davis (1982) and Güner et al. (2012), the genus is represented by three species in Turkey; C. avellana L., C. maxima Mill. and C. colurna L. Today, however, many researchers agree that the genus should be represented in Turkey by 2 species as $C$. colurna and C. avellana. According to these researchers, C. maxima species should be included in C. avellana species due to its continuous variation in morphology, hybridizes easily, and overlaps geographical distribution. In addition, DNA fingerprint dataset analysis supports a common origin for the $C$. maxima and C. avellana species (Mehlenbacher 1991; Rovira 1997; Botta et al. 2019; Erdogan \& Mehlenbacher 2000; 2002). The common hazelnut (C. avellana) is an important horticultural crop and is grown for consumption worldwide. There are 20 hazelnut cultivars in Turkey, of which 18 are registered and 2 are unregistered. The registered cultivars include "Allahverdi, Cavcava, Çakıldak, Foşa, Giresun Melezi, Incekara, Kalınkara, Kan, Karafindık, Kargalak, Mincane, Okay 28, Palaz, Sivri, Uzunmusa, Tombul, Yassı Badem, Yuvarlak Badem", "Acı" and "Kuş" are unregistered cultivars (Balık et al. 2016). "Kargalak" has the biggest nut and kernel size among the other Turkish hazelnut cultivars, while the "Tombul" is reported to be the highest quality and the most productive hazelnut in Turkey (Akçin \& Bostan 2018).

The leaf characteristics such as chlorophyll quantities, stomata and epidermal structures are effective on hazelnut yields, fruit quality and resistance to ecological conditions (Rong-hua et al. 2006). For this reason, it is important to determine the characteristics of cultivars such as stomatal characteristics and chlorophyll quantities. The ability of plants to adapt to an ecological environment is related to the processes of transpiration and photosynthesis that occur in the leaves. In addition, the number of stomata and stomatal properties affect gas exchange, photosynthesis production, drought resistance, and vegetative development (Çağlar \& Tekin 1999; Çağlar et al. 2004; Drake et al. 2013). The number of stomata per unit area, stomata, and epidermis properties varies according to species and cultivars (Çağlar et al. 2004; Akçin et al. 2013; Avcı \& Aygün 2014; Hurt \& Doğan 2020). Although leaf micromorphological features such as cuticular wax types, and epidermal and stomatal properties have been used in the identification of plants, the literature survey has shown that no comprehensive study has yet been conducted.

The quantity of chlorophyll in leaves is typically expressed in terms of either concentration or content and can vary significantly in value among different plant taxa and growing stages (Taiz et al. 2014).

There are some data on leaf epidermis micromorphologies of the Corylus species. Uzunova (1999) investigated the leaf epidermis in European Corylaceae while Avcı \& Aygün (2014) determined the stomata density and distribution in the leaves of 18 varieties of Turkish hazelnuts. There is, however, no data on the micromorphological properties of Turkish hazelnut cultivars.

This study aims to determine the differences between the stomatal index and chlorophyll content (SPAD value) of 20 Turkish hazelnut cultivars and determine the similarities and differences between them.

## 2. Material and Methods

The specimens of 20 hazelnut cultivars were collected from the Hazelnut Research Station (Giresun -Turkey- coordinate: $40^{\circ} 54,35.2^{\prime \prime} \mathrm{N}$, $38^{\circ} 21^{\prime} 09.7^{\prime}$ 'E), which sits at an altitude of 14 m , in 2021. The studied cultivars were "Acı, Allahverdi, Cavcava, Çakıldak, Foşa, Giresun melezi, Incekara, Kalınkara, Kan, Karafındık, Kargalak, Kuş, Mincane, Okay 28, Palaz, Sivri, Tombul, Uzunmusa, Yassı Badem, and Yuvarlak Badem". The experimental design was planned in a randomized manner with five replications ( 5 bushes with multi stems), and a plant represented by 5 leaves in each replication. A total of 10 measurements were obtained for each leaf. Leaves of the same size at the tips of south-facing branches were used for measurements. Chlorophyll measurements were conducted at 13:00-14:00 on 7 July. The SPAD value of each leaf was obtained by an average of 250 measurements. Chlorophyll content was measured through a portable chlorophyll meter (Minolta SPAD-502, Osaka, Japan). In each cultivar, the quantity of chlorophyll in the leaves was measured, after which the leaves were placed in a $70 \%$ alcohol solution to determine the stomatal index of the cultivars. The surface sections of leaves were excised by hand and they covered with glycerin-gelatin (Vardar 1987). All measurements were obtained using imaging software (NIS - Elements, Version 3.00 SP 5 ). The stomatal index per unit area ( $1 \mathrm{x} 10^{4} \mu \mathrm{~m}^{2}$ ) was calculated according to Meidner and Mansfield (1968). For scanning electron microscope (SEM) imaging, dried leaves were mounted on stubs using double-sided adhesive tape. The samples were coated with $12.5-15.0 \mathrm{~nm}$ of gold and the coated leaves were examined and photographed using a Hitachi SU 1510 SEM (Figures 1, 2).


Figure 1- Scanning electron micrographs of upper leaf surface of C. avellana cultivars. A: Acı, B: Allahverdi, C: Cavcava, D: Çakıldak, E: Foşa, F: Giresun Melezi, G: Incekara, H: Kalınkara, I: Kan, J: Kara, K: Kargalak, L: Kuş, M: Mincane, N: Okay 28, O: Palaz, P: Sivri, R: Tombul, S: Uzunmusa, T: Yassı Badem, U: Yuvarlak Badem


Figure 2- Scanning electron micrographs of leaf lower surface of C. avellana cultivars. A: Acı, B: Allahverdi, C: Cavcava, D: Çakıldak, E: Foşa, F: Giresun Melezi, G: Incekara, H: Kalınkara, I: Kan, J: Kara, K: Kargalak, L: Kuş, M: Mincane, N: Okay 28, O: Palaz, P: Sivri, R: Tombul, S: Uzunmusa, T: Yassı Badem, U: Yuvarlak Badem
Table 1- Some morphological properties of leaf epidermis, stomata and chlorophyll density in 20 cultivars of C. avellana species

| Cultivar | Epidermis cell (mean) |  |  |  |  |  | Stomata cell (mean) |  |  |  | Chloropyll content |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Length ( $\mu \mathrm{m}$ ) |  | Width ( $\mu \mathrm{m}$ ) |  | Number of epidermis cells ( $1 \times 10^{4} \mu^{2}$ ) |  | Length ( $\mu \mathrm{m}$ ) | Width ( $\mu \mathrm{m}$ ) | Number of stomata (1x 104 $\mu m^{2}$ ) | Stomatal index |  |
|  | Ue | Le | Ue | Le | Ue | Le |  |  |  |  |  |
| Acı | 17.99BCDEFG | 20.77ABCDEF | 20.50EFG | 34.83 ABCD | 31.22EF | 24.77 EFGH | 24.46BCDEFG | 20.81ABCDE | 2.33 AB | 8.60ABCD | 47.24 AB |
| Allahverdi | 14.74EFG | 26.04 AB | 20.83 EFG | 38.92 AB | 36.55 BCD | 22.33 GHI | 26.06 ABCDE | 18.59CDEF | 2.11 AB | 8.66 ABCD | 46.32 ABC |
| Cavcava | 17.21CDEFG | 22.91 ABCD | 26.67 BCDE | 37.64 ABC | 31.55 EF | 19.55IJK | 27.75 AB | 23.49 A | 1.77B | 8.26ABCD | 46.13 ABCD |
| Çakıldak | 18.69BCDEF | 16.16F | 24.09CDEFG | 28.4D | 27G | 24.77 EFGH | 23.53DEFG | 21.67 ABC | 2.55 AB | 9.33 ABCD | 47.18AB |
| Foşa | 20.66 ABC | 24.87 ABC | 31.43B | 39.45A | 16.77K | 16.44K | 27.21 ABC | 22.19 AB | 2.33 AB | 12.44 A | 41.18 FG |
| Giresun <br> Melezi | 15.90DEFG | 19.52CDEF | 22.50DEFG | 33.18 ABCD | 40.55 AB | 38A | 21.69 G | 17.58EF | 2.66 AB | 6.56CD | 39.58 G |
| İncekara | 14.25 FG | 18.62DEF | 20.33EFG | 29.87 CD | 40.44 AB | 26.66CDEF | 26.39ABCDE | 22.47 AB | 2.44 AB | 8.39ABCD | 46.44ABC |
| Kalınkara | 16.56 DEFG | 20.93 ABCDEF | 23.58 CDEFG | 30.85 BCD | 31.55 EF | 29.77 BC | 24.62BCDEFG | 19.28BCDEF | 2.88 AB | 6.15D | 45.91 ABCD |
| Kan | 13.62 G | 18.04DEF | 21.80 EFG | 30.02 CD | 35 CDE | 30.77B | 23.11 EFG | 18.63 CDEF | 3.22 A | 9.46 ABCD | 41.97EFG |
| Kara | 22.10 AB | 22.01 ABCDEF | 30.06BC | 33.70 ABCD | 21.11IJ | 24.88DEFG | 26.35ABCDE | 20.04BCDE | 2.77 AB | 10.04 ABCD | 42.68DEFG |
| Kargalak | 20.95 ABC | 22.65 ABCDE | 26.95BCDE | 36.63 ABCD | 22.88 HI | 28.33 BCD | 26.82 ABCD | 21.62 ABC | 2.55 AB | 8.25 ABCD | 30.39 H |
| Kuş | 17.38CDEFG | 21.80 ABCDEF | 18.65 G | 37.32 ABC | 37BCD | 17.66JK | 26.83 ABCD | 20.51 ABCDE | 2.11 AB | 10.60 ABC | 47.69A |
| Mincane | 23.42 A | 26.40 A | 25.63BCDEF | 35.79 ABCD | 26.11 GH | 20.22IJ | 23.55DEFG | 16.70F | 2.22 AB | 9.86 ABCD | 44.02 BCDEF |
| Okay 28 | 14.69 EFG | 21.87 ABCDEF | 24.55 CDEFG | 31.90 ABCD | 42.22 A | 28.77 BC | 22.32 FG | 17.87DEF | 3.33 A | 10.42 ABC | 45.60 ABCD |
| Palaz | 20.42ABCD | 20.15BCDEF | 24.88BCDEFG | 30.16CD | 25.88 GH | 25.88 HI | 25.36ABCDEF | 19.91BCDEF | 2.77 AB | 11.34 AB | 47.03 AB |
| Sivri | 14.89 EFG | 16.97EF | 18.90FG | 28.61D | 37.44 BC | 29.88BC | 25.22ABCDEFG | 19.43BCDEF | 2.11 AB | 6.50CD | 45.51 ABCD |
| Tombul | 15.27EFG | 19.73CDEF | 24.13CDEFG | 31.39 ABCD | 33.11 DE | 28.22BCDE | 22.08 G | 17.9EF | 2.33 AB | 7.65BCD | 46.37ABC |
| Uzunmusa. | 17.86BCDEFG | 20.54 ABCDEF | 26.72BCDE | 32.01 ABCD | 27.55 FG | 24.55 FGH | 23.80CDEFG | 18.66CDEF | 2.44 AB | 8.95ABCD | 44.89 ABCDE |
| Yassı <br> Badem | 18.89ABCDE | 20.94ABCDEF | 38.76A | 38.82 AB | 18.77JK | 20.11IJ | 28.37 A | 20.87ABCDE | 2.11 AB | 9.31 ABCD | 43.29 CDEF |
| Yuvarlak Badem | 18.26BCDEFG | 22.43 ABCDE | 29.12BCD | 33.78ABCD | 21.66IJ | 22.66 GHI | 25.90ABCDEF | $21.17 \pm$ ABCD | 3 AB | 11.56 AB | 43.36CDEF |
| P value | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

Table 2- Some micromorphological and morphological properties of leaf epidermis and stomata in 20 cultivars of C. avellana species

| Cultivars | Epidermis cell |  |  |  | Stomata cell |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Shape |  | Anticlinal | walls | Outer <br> stomatal rim | Peristomatal rim | Aperture | Membrane - wax ornamentation |
|  | $\boldsymbol{U e}$ | Le | $\boldsymbol{U e}$ | Le |  |  |  |  |
| Acı | Rec. | Irr. | Undulate | Sinuous | Raised | Evident | Long and wide | Striated-crust |
| Allahverdi | Irr. | Irr. | Sinuous | Sinuous | Raised | Stout and raised | Long and narrow | Striated -crust |
| Cavcava | Rec.-pol. | Irr. | Undulate | Sinuous | Raised | Evident | Long and narrow | Striated-smooth |
| Çakıldak | Rec.-pol. | Irr. | Str-cur | Undulate | Raised | Raised, wide and amorphous | Long and narrow | Smooth- granules |
| Foşa | Rec.-irr. | Rec. | Undulate | Undulate | Raised and wide | Barely perceptible | Short and wide | Striated-smooth or crust |
| Giresun Melezi | Rec.-pol. | Rec.-pol. | Str-cur | Str-cur | Raised | Raised and double ring | Short and wide | Striated-smooth |
| Incekara | Rec.-irr. | Irr. | Str-cur | Sinuous | Raised | Raised | Long and wide | Smooth or striated-granules |
| Kalınkara | Rec.-pol. | Rec.-pol. | Undulate | Sinuous | Raised | Evident | Long and wide | Striated-smooth or granules |
| Kan | Rec.-irr. | Rec.-irr. | Str-cur | Undulate | Raised | Overlapping | Long and narrow | Striated- crust |
| Kara | Rec. | Rec. | Str-cur | Undulate | Raised and wide | Raised | Short and narrow | Striated-smooth |
| Kargalak | Rec. | Irr. | Str-cur | Sinuous | Raised | Barely perceptible | Short and narrow | Smooth-crust |
| Kuş | Rec.-pol. | Rec.-pol. | Str-cur | Undulate | Raised | Evident and raised | Short and narrow | Smooth or striated- crust |
| Mincane | Rec.-pol. | Irr. | Undulate | Sinuous | Raised | Evident and raised | Long and wide | Striated-smooth |
| Okay 28 | Rec.-pol. | Rec.-pol. | Str-cur | Str-cur | Raised | Raised | Long and narrow | Smooth-striated |
| Palaz | Pol.-rec. | Irr. | Str-cur | Undulate | Raised | Barely perceptible | Short and narrow | Granular-crust |
| Sivri | Rec.-pol. | Irr. | Undulate | Undulate | Raised | Raised | Long and wide | Striated-granules |
| Tombul | Rec.-pol. | Irr. | Str-cur | Sinuous | Raised | Overlapping and raised | Long and narrow | Smooth- granules or crust |
| Uzunmusa. | Pol. | Irr. | Str-cur | Sinuous | Raised | Evident and not raised | Long and wide | Striated-smooth |
| Yassı Badem | Pol. | Irr. | Str-cur | Sinuous | Raised | Very evident, stout, raised and amorphous | Long and narrow | Granular-smooth |
| Yuvarlak Badem | Rec.-pol. | Irr. | Str-cur | Sinuous | Raised | Stout, wide, raised and amorphous | Long and narrow | Granular-smooth |

[^0]Analysis of variance, Tukey multiple comparison tests and the principal component analysis (PCA) methods were used for statistical analysis of the obtained data. The significance level ( $\alpha$ ) was determined as 0.05 in calculations and interpretations. The Minitab 17 statistical package program was used for statistical analysis.

## 3. Results and Discussion

Table 1 shows some morphological properties of leaf epidermis and stomata, stomatal index, and chlorophyll contents in 20 cultivars of C. avellana species. The micromorphological characteristics of leaf epidermal cells such as shape, the structure of the anticlinal walls, outer stomatal rims, peristomal rims, apertures, wax ornamentation, and membrane ornamentation are summarized in Table 2. Some significant differences were found among cultivars in terms of the epidermal properties, stomatal index, and chlorophyll contents.

### 3.1. Epidermis cells

Statistically significant differences were found in the width, length, and number of epidermis cells on the upper and lower surfaces of the leaves in the 20 hazelnut cultivars examined ( $\mathrm{p}<0.000$ ) (Table 1). The highest values of upper and lower epidermis lengths were determined in "Mincane" with 23.42 and 26.40, respectively. The smallest epidermis length value was found in "Kan" with 13.62 for the upper epidermis and in "Çakıldak" with 16.16 for the lower epidermis. The largest upper epidermis width was measured in "Yassı Badem" (38.76), and the smallest width was measured in "Kuss". It was determined that "Foşa" has the highest value in the lower epidermis (39.45). The number of epidermis cells in the leaves varies between 16.77-42.22 on the upper surface and 16.44-38 on the lower surface of the examined hazelnut cultivars. The lowest number of the epidermis was found in the "Foşa" on both surfaces.

Leaf anatomy, leaf epidermis morphology, and micromorphology and stomata properties provide relative taxonomic data (Uzunova 1999; Nabin et al. 2000; Chen 2008; Akçin et al. 2013; Razaz et al. 2015) Uzunova (1999) stated that there are differences in the epidermal structures of taxa belonging to the Corylaceae family. Various studies have been conducted on the determination of the leaf anatomical and morphological structures of the cultivars and thus a better recognition of the cultivars was defined (Sagaram \& Lambardini 2007; Nur Fathhah et al. 2014; Najmaddin \& Saeed 2020). The anatomical and palynological structures of Bougainvillea glabra cultivars were examined and it was determined that there were differences among leaf characteristics. (Najmaddin \& Saeed 2020). In our study, statistically significant differences were found among the sizes of epidermis cells, the sizes of stomatal cells, the stomatal index, and the number of stomata and epidermis cells in hazelnut cultivars.

The micromorphological features of epidermis cells are shown in Table 2. The epidermal cell shapes on both surfaces of the hazelnut cultivars are rectangular, polygonal, rectangular-polygonal, or irregular. The irregular epidermis is the most common shape on the lower surface. There are usually rectangular-polygonal cells on the upper surface. The "Allahverdi" has an irregular epidermis shape on the upper surface while the "Foşa", "Incekara", and "Kan" cultivars have rectangular-irregular shapes. The cells on the upper surface of "Acı", "Karafindık" and "Kargalak" are rectangular in shape. The anticlinal walls of the epidermis cells show some differences in the examined specimens. The anticlinal walls of epidermis cells are sinuous and undulate on the lower surface. Eleven cultivars have sinuous anticlinal walls. Undulate, sinuous and straight to curved anticlinal walls are present on the upper surfaces of leaves of the cultivars examined. Straight to curved walls are the most common type on the upper surface of leaves. "Allahverdi" has a sinuous type, and "Foşa" and "Sivri" have undulate type anticlinal walls on both surfaces of a leaf (Figures 1, 2).

There are different opinions about the systematic importance of the shapes of epidermis cells. Chen et al. (2008) stated that the shapes of epidermal cells were not useful in the systematic of the Salix genus or Salicaceae family. Cheng (2006) noted that some epidermal characteristics such as the shape of epidermal cells, type of stomata, and cuticular ornamentation in the Schisandraceae family are usually constant within species and this factor is useful in defining the relationship between species. According to present study, anticlinal walls of leaf epidermal cells show differences among the studied cultivars; three cultivars (Allahverdi, Foşa, and Sivri) have the same anticlinal walls on both upper and lower surfaces. In other specimens differences are apparent between the surfaces. These properties can help determine the boundaries of the cultivars "Allahverdi", "Foşa", and "Sivri". Yang and Lin (2005) and Zamani et al. (2015) reported that the properties of an anticlinal wall can be regarded as a diagnostic feature at the species level.

### 3.2. Stomata

All hazelnut cultivars have stoma only on the lower surfaces of the leaves. Leaves are hypostomatic. The stoma sizes, the number of stomata, and stomatal index were statistically significant in hazelnut cultivars ( $\mathrm{p}<0.000$ ) (Table 1). Uzunova (1999) reported
that $C$. avellana and C. colurna L. have stomata only on a lower surface of a leaf. The widest stomata were determined in the "Cavcava" cultivar (23.49) while the longest stomata were found in "Yassı Badem" (28.37). Avcı and Aygün (2014) stated that the stomatal characteristics of Turkish hazelnut cultivars are unique and can be used for cultivar identification. Their study results from 18 hazelnut cultivars showed that the average stomatal width was $20.02 \mu \mathrm{~m}$ among the cultivars and varied between $17.00 \mu \mathrm{~m}$ (Sivri) and $22.61 \mu \mathrm{~m}$ (Yassı Badem). It was found that "Yassı Badem" has the widest stoma both in the present study and in Avcı and Aygün's (2014) study. Avcı and Aygün (2014) stated that the number of stomata varied between 83.08-117.73 in $1 \mathrm{~mm}^{2}$ and the highest number of stomata were determined in "Sivri". In our study, it was determined that the number of stomata varied between 1.77-3.33 per area ( $1 \times 10^{4} \mu \mathrm{~m}^{2}$ ). While the highest number of stomata was found in the "Okay 28 ", the lowest number of stomata was found in "Cavcava". In a study performed on 11 hazelnut cultivars and genotypes, it was determined that hazelnut cultivars and genotypes had different stomatal characteristics such as stomatal number and stomatal size (Hurt \& Doğan 2020). In previous studies, it was observed that as the stomatal width in leaves increased, the stomatal density decreased (Mert et al. 2009; Avcı \& Aygün 2014; Hurt \& Doğan 2020). Our results generally support this statement. While "Cavcava" had the widest stomata with 23.49, it was also found to be the lowest cultivar in terms of stomatal density.

The highest stomatal index was found in "Foşa" with 12.44 and the lowest in "Kalınkara" with 6.15. Avcı and Aygün (2014) reported that the stomatal index values in hazelnut cultivars varied between 10.55 and 17.15. Their study found that "Sivri" had the highest stomatal index and "Kalınkara" had the lowest stomatal index. The lowest stomatal index in "Kalnnkara" is in line with our findings. The difference in the stomatal index in cultivars can be explained by differences in the water uptake capacity, light requirement level, and plant growth rate (Warrit et al. 1980; Mert et al. 2009; Avcı \& Aygün 2014). Metcalfe and Chalk (1979) stated that changes in the stomatal index may be caused by factors such as humidity and nutritional conditions.

According to the micromorphological features of stomata given in Table 2, the outer stomatal rims are raised in all examined specimens. Wide outer stomatal rims are found in "Foşa" and "Karafindık". The peristomal rims are stout, raised, overlapping, and amorphous in all hazelnut cultivars. In "Foşa", "Kargalak", and "Palaz", the peristomal rim is barely perceptible while "Çakıldak", "Yassı Badem" and "Yuvarlak Badem" have amorphous peristomal rims. "Giresun Melezi" has a raised and double ring rim. Wilkinson (1979) reported that peristomatal rims may vary in different plants.
In present study the stomata aperture is usually long. While "Karafindık", "Kargalak", "Kuş", and "Palaz" have short and narrow apertures, "Foşa" and "Giresun Melezi" have short and wide apertures (Figures 1, 2).

### 3.3. Cell membrane and wax ornamentation

Three wax ornamentation types are recognized: crust, smooth, and granules in the present study. All hazelnut cultivars. The crust type is the most common wax ornamentation type on both surfaces of hazelnut cultivars. The cell membrane ornamentation types are striated or smooth. Most cultivars have roughly striated cuticles around their stomata which is evident in the "Allahverdi", "Foşa" and "Mincane" cultivars (Table 2, Figures 1, 2). Previous studies have emphasized that wax ornamentations are important in epidermal micromorphological characters (Sonibare et al. 2005; Akçin et al. 2013; Zamani et al. 2015).

### 3.4. Chlorophyll content (SPAD values)

The chlorophyll content of the 20 hazelnut cultivars of C. avellana species is shown in Table 1. The chlorophyll contents were statistically significant in the hazelnut cultivars ( $\mathbf{p}<0.000$ ) in which the chlorophyll content of the investigated cultivars varies between 47.69-30.39 values. While the highest chlorophyll content was detected in "Kuş", the lowest value was found in "Kargalak".

Recent studies have shown that the use of physiological characteristics such as chlorophyll content as selection criteria affect yield. Statistically significant correlations were found between the chlorophyll contents and main yield components in wheat where an increase in the amount of chlorophyll affected the yield positively. The photosynthetic pigment concentration in the leaf is related to the amount of sunlight absorbed by the leaf. Therefore, low chlorophyll concentration directly limits photosynthetic potential and primary production (Fillella et al. 1995; Bahar 2015). The most important factor in differentiating the chlorophyll levels of plants is the genetic structure. (Taner \& Sade 2005). The amount of chlorophyll varies between species as well as within species according to subspecies, varieties, and forms (Canova et al. 2008; Cetin 2017). It is known that one of the important factors determining the amount of chlorophyll is the leaf structure (Taner \& Sade 2005; Atar et al. 2020). In this study, chlorophyll contents were statistically very significant in hazelnut cultivars ( $\mathrm{p}<0.000$ ). Chlorophyll SPAD $>30$ in hazelnut plants was indicated as having a high chlorophyll
Table 3- Similarity rates of cultivars according to Bray-Curtis similarity index

|  | TMBL | KRFK | ÇKDK | FSS | $\boldsymbol{K S}$ | CVCV | UNMS | YSBM | PLZ | KLKR | KGLK | MNCN | $\boldsymbol{Y V K B ~}$ | INKR | KN | SVR | $A C$ | ALVD | OK28 | GMLZ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| TMBL | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| KRFK | 0.89 | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ÇKDK | 0.95 | 0.89 | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| FSS | 0.89 | 0.93 | 0.90 | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| KS | 0.93 | 0.91 | 0.93 | 0.92 | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| CVCV | 0.93 | 0.92 | 0.93 | 0.95 | 0.96 | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| UNMS | 0.97 | 0.92 | 0.95 | 0.92 | 0.94 | 0.95 | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| YSBM | 0.90 | 0.96 | 0.90 | 0.95 | 0.92 | 0.94 | 0.93 | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |
| PLZ | 0.96 | 0.92 | 0.96 | 0.92 | 0.95 | 0.94 | 0.96 | 0.92 | 1.00 |  |  |  |  |  |  |  |  |  |  |  |
| KLKR | 0.97 | 0.90 | 0.95 | 0.90 | 0.94 | 0.94 | 0.97 | 0.91 | 0.96 | 1.00 |  |  |  |  |  |  |  |  |  |  |
| KGLK | 0.89 | 0.91 | 0.89 | 0.94 | 0.91 | 0.94 | 0.92 | 0.92 | 0.91 | 0.90 | 1.00 |  |  |  |  |  |  |  |  |  |
| MNCN | 0.93 | 0.92 | 0.92 | 0.93 | 0.92 | 0.93 | 0.95 | 0.91 | 0.94 | 0.93 | 0.92 | 1.00 |  |  |  |  |  |  |  |  |
| YVKB | 0.93 | 0.95 | 0.93 | 0.96 | 0.95 | 0.96 | 0.96 | 0.95 | 0.95 | 0.94 | 0.94 | 0.94 | 1.00 |  |  |  |  |  |  |  |
| INKR | 0.95 | 0.89 | 0.95 | 0.89 | 0.95 | 0.94 | 0.94 | 0.90 | 0.95 | 0.96 | 0.89 | 0.90 | 0.93 | 1.00 |  |  |  |  |  |  |
| KN | 0.96 | 0.89 | 0.95 | 0.89 | 0.92 | 0.91 | 0.95 | 0.89 | 0.94 | 0.95 | 0.88 | 0.91 | 0.93 | 0.96 | 1.00 |  |  |  |  |  |
| SVR | 0.95 | 0.87 | 0.95 | 0.87 | 0.93 | 0.91 | 0.94 | 0.88 | 0.94 | 0.96 | 0.87 | 0.89 | 0.91 | 0.97 | 0.95 | 1.00 |  |  |  |  |
| AC | 0.95 | 0.91 | 0.95 | 0.91 | 0.97 | 0.95 | 0.96 | 0.92 | 0.96 | 0.96 | 0.91 | 0.93 | 0.95 | 0.96 | 0.94 | 0.94 | 1.00 |  |  |  |
| ALVD | 0.94 | 0.89 | 0.91 | 0.92 | 0.96 | 0.95 | 0.94 | 0.92 | 0.93 | 0.94 | 0.90 | 0.94 | 0.93 | 0.94 | 0.93 | 0.93 | 0.96 | 1.00 |  |  |
| OK28 | 0.98 | 0.91 | 0.94 | 0.90 | 0.94 | 0.94 | 0.97 | 0.91 | 0.96 | 0.97 | 0.90 | 0.94 | 0.95 | 0.94 | 0.96 | 0.94 | 0.95 | 0.94 | 1.00 |  |
| GMLZ | 0.96 | 0.89 | 0.92 | 0.89 | 0.92 | 0.91 | 0.95 | 0.89 | 0.92 | 0.95 | 0.90 | 0.92 | 0.92 | 0.93 | 0.96 | 0.93 | 0.94 | 0.92 | 0.95 | 1.00 |

 Incekara, KN: Kan, SVR: Sivri, AC: Acı, ALVD: Allahverdi, OK28: Okay 28, GMLZ: Giresun Melezi
content (Hand \& Reed 2014). In our study, the chlorophyll content of the examined cultivars was high, and the chlorophyll SPAD values varied 30.39 and 47.69. The highest chlorophyll content was detected in "Kuş", the lowest value was found in "Kargalak". Atar et al. (2020) reported that $C$. avellana has 30.6-48.9 SPAD values.

According to the Bray-Curtis similarity index (Table 3), the highest correlation was found between "Okay 28 " and "Tombul" cultivars with a ratio of 0.98 in terms of the traits examined. The lowest correlation was found between "Sivri - Karafindık", "Sivri - Foşa", and "Sivri - Kargalak" with a 0.87 ratio. The correlation ratio between "Giresun Melezi and Tombul" was 0.96 , and the correlations between "Giresun Melezi and Kargalak" and "Okay 28 and Kargalak" were 0.90 .

It was determined that the examined epidermal features, stomatal index, and chlorophyll quantities according to the PCA showed some differences among hazelnut cultivars. Stoma width and stoma length were determined to be significant for "Palaz, Kuş, and Yuvarlak Badem" and "Yassı Badem" (Figure 3). However, no statistical correlation was found between the amount of chlorophyll and the stomatal characteristics.


Figure 3- Principal component analysis of investigated traits in hazelnut cultivars. TMBL: Tombul, KRFK: Kara, ÇKDK: Çakıldak, FŞ: Foşa, KŞ: Kuş, CVCV: Cavcava, UNMS: Uzunmusa, YSBM: Yassı Badem, PLZ: Palaz, KLKR: Kalınkara, KGLK: Kargalak, MNCN: Mincane, YVKB: Yuvarlak Badem, INKR: Incekara, KN: Kan, SVR: Sivri, AC: Acı, ALVD: Allahverdi, OK28: Okay 28, GMLZ: Giresun Melezi, SE: Stoma width, SB: Stoma lenght, SI: Stomatal index, UEE: Upper epidermis width, UEB: Upper epidermis lenght, AEE: Lower epidermis width, AEB: Lower epidermis lenght, KY: Chlorophyll content

## 4. Conclusions

There are 20 hazelnut cultivars in Turkey, 18 of them are registered and 2 of them are unregistered. The determination of hazelnut cultivars is typically performed according to their pomological characteristics. Recently, it has been used in some molecular studies to determine hazelnut varieties. It is crucial to know the anatomical and micromorphological characteristics of the plants to recognize the cultivars better and increase the yield. For this reason, studies have been carried out to better understand the anatomical and micromorphological structures of cultivars in many agricultural products. In our study, the leaf epidermis and stomata characteristics and chlorophyll quantities of 20 hazelnut cultivars were determined in comparatively and in detail. Our study's findings show that the epidermal features, stomatal index, and chlorophyll quantities can be used as distinguishing features in the identification of cultivars.

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[^0]:    Ue: Upper epidermis surface, Le: Lower epidermis, Rec.: Rectangular, Irr.: Irregular, Pol.: Polygonal, Str-cur: Straight to curved

