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Leaf Stomatal Characteristics of Some Species in Bush Form Used in Kastamonu Urban Landscape

Kastamonu Kent Peyzajında Kullanılan Çalı Formunda Bazı Türlerin Yaprak Stoma Karakteristikleri

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Öz

Günümüzde hızla artan nüfus, betonlaşma, hava kirliliği, trafik yoğunluğu gibi birçok sorunu da beraberinde getirmiştir. Kentsel peyzaj için önemli bir unsur olan park ve bahçelerin kentlerde insan-doğa ilişkisini dengelemekle kalmayıp kentsel yaşam koşullarını iyileştirdiği bilinmektedir. İnsanların rekreasyon ihtiyaçlarını karşılamak, kaybolan doğayı geri kazanmak ve sağlıklı yaşam alanları oluşturmak için kentsel alanları işlevsel ve estetik açıdan değerlendiren park ve bahçeler son derece önemlidir.

Bu çalışmada, Kastamonu ilinde park, bahçe ve merkezi refüj çevre düzenleme çalışmalarında kullanılan Buxus microphylla Siebold & Zucc., Prunus laurocerasus L, Ligustrum delavayanum Har., Berberis thunbergii DC., Ilex aquifolium L., Euonymus japonicus Wall. var. aureomarginatus Rehder, Cotoneaster lacteus W.W.Sm., Mahonia aquifolium (Pursh) Nutt., Pyracantha coccinea M. Roem. türlerinin yaprak stoma özelliklerinin belirlenmesi amaçlanmıştır.

Mikromorfolojik incelemeler ile çalışmada kullanılan türler üzerinde SEM ile stoma özellikleri belirlendi. Türler üzerinde birim alandaki stoma yoğunluğuna göre en yüksek stoma yoğunluğuna Pyracantha coccinea türünün (81), minimum stoma yoğunluğunun ise Cotoneaster lacteus türünün (10) sahip olduğu belirlenmiştir. Ayrıca ortalama en uzun stoma uzunluğuna Prunus laurocerasus (34.82 µm), en kısa stoma uzunluğuna Mahoni aquifolium'un (7.183 µm) sahip olduğu belirlendi.

Anahtar Kelimeler: Kastamonu, Mikromorfolojik Karakterler, SEM, stoma

ABSTRACT

Nowadays, the rapidly increasing population has brought many problems such as building concrete structures everywhere, air pollution, and traffic congestion. It is known that parks and gardens, which are an important element for the urban landscape, not only balance the human-nature relationship in cities, but also improve urban living conditions. Parks and gardens that utilize urban areas in a functional and aesthetic way to meet the recreational needs of people, to recover the lost nature and to create healthy living spaces are extremely important.

In this study, it was aimed to determine the leaf stomatal characteristics of Buxus microphylla Siebold & Zucc., Prunus laurocerasus L, Ligustrum delavayanum Har., Berberis thunbergii DC., Ilex aquifolium L., Euonymus japonicus Wall. var. aureomarginatus Rehder, Cotoneaster lacteus W.W.Sm., Mahonia aquifolium (Pursh) Nutt., Pyracantha coccinea M. Roem. species, one of the species used in the park, garden and central landscaping studies in Kastamonu province.

With micromorphological investigations, stomatal characteristics were determined by SEM on the species used in the study. With respect to stomatal density in the unit area on species, it was determined that Pyracantha coccinea species had the maximum stomatal density (81)

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while Cotoneaster lacteus species had the minimum stomatal density (10). Furthermore, it was determined that Prunus laurocerasus (34.82 µm) had mean the longest stomatal length and Mahoni aquifolium (7.183 µm) had the mean shortest stomatal length.

Keywords: Kastamonu, micromorphological characteristic, SEM, stomata

INTRODUCTION:

Plants and trees have attracted people's attention with their visuality since the early ages. (Dönmez and Türkmen, 2018). Nowadays, this attention attracting has developed in the form of vegetation and plantation works in large parks and gardens in urban areas (Dönmez et al., 2015; Dönmez and Türkmen, 2015; Ürgenç, 1998). Nowadays, green and open areas are highly needed in the cities where industrialization has become intense to increase the quality of life (Ozturk et al., 2019; Ozturk et al., 2020; Yılmaz & Irmak, 2004). Trees prevent air pollution, reduce noise, dust, wind, and greenhouse effect, preventing the reflections of light, balancing temperature, providing energy saving and moisture, and creating habitat (Gül et al., 2006). Many species are used in the plantation studies of the parks and gardens of the city of Kastamonu, as in many places, and each of these species forms a visual arrangement and also contributes to the urban appearance as a landscape value (Edeoga & Ikem,2001; Kong, 2001; Shi & Li, 2003; Carpenter, 2005; Zhou & Xia, 2012; Kumar & Murugan, 2013; Denk, Güner & Grimm, 2014; Ozturk et al., 2021).

In the plant, the leaf veins, epidermal cells, stoma, trichomes, cuticular layer leaf epidermal characters have been used as taxonomic and phylogenetic characters that are used morphologically and micromorphologically.

Kastamonu province is located in the euxin zone of the European-Siberian floristic region. Kastamonu province, which is situated between latitıde of 41°21' north and longitude of 33° 46' east the Western Black Sea Region, constitutes 1.7% of the country with a surface area of 13.108.1 km². The long-term (1930- 2017) meteorological climate data of Kastamonu Province are presented in Table 1 (URL 1).

Table 1. The long-term (1930 - 2017) meteorological climate data of Kastamonu Province

Months	January	February	March	April	May	June	July	August	September	October	November	December
Mean temperature (°C)	-1,1	0,7	4,3	9,5	14,2	17,6	20,3	20	15,7	10,7	5,1	0,8
Maximum mean temperature (°C)	3,1	6	10,8	16,5	21,1	24,6	27,8	28	23,8	18	10,9	4,7
Mean lowest temperature (°C)	-4,6	-3,5	-0,9	3,3	7,5	10,4	12,3	12,2	8,8	5,1	0,8	-2,4
Average Sunbathing Time (hours)	2,3	3,6	4,6	5,8	7,3	8,6	9,9	9,5	7,4	5,6	3,8	2
Average of Rainy Days	12,4	11,3	12	12,9	14,5	11,8	6,2	5,7	6,5	9,1	9,6	11,9
Average Monthly Total Rainfall (mm)	29,9	27	34,5	51,8	74,1	71,4	31,8	31,2	30,1	35,3	29,1	33,8

According to Table 1, the average annual maximum temperature is 9.8 °C, and the average precipitation is 480 mm.

The stomata in the epidermis are usually found in all green parts of plants formed by two chlorophyllose bean-shaped cells, which are important in the gas exchange in plants and accelerate transpiration in the leaf tissue, by leaving aperture (Yentür, 2003). It is known that morphological characters vary among the species depending on many factors, especially the origin (Yigit et al., 2016a,b; Özel and Ertekin, 2012). In the studies carried out, it has been revealed that micromorphological characters vary significantly according to species and habitat differences (Galmés et al., 2007; Kravkaz and Vurdu, 2010, Kravkaz and Karaöz, 2016).

Stomata are located on both the lower and upper sides of the leaf according to their presence on the surface of the leaf. They are named as amphistomatic type on the upper side of the leaf, as hypostomatic type only on the lower side, and as epistomatic type only on the upper side (Akman & Güney, 2010). The leaf surface roughness shows many variations not only among plant species but also among abaxial and adaxial surfaces of the same species and depending on whether the leaf is old or young (Weyers & Lawson, 1997; Weyers et al., 1997). The underlying epidermal characteristics depend on the shape and size of cells, vascularization patterns, and especially the presence or absence of trichomes. All these characteristics can be explained in detail by examining with a scanning electron microscope (SEM) (Holloway, 1993).

This study was carried out on 9 different species that were used in the landscaping areas in Kastamonu city center. The stomatal characteristics “Stoma length (STL), stoma width (STW), stoma por length (PORL), stoma por width (PORW) and stoma density (SDEN)”, which are among leaf micromorphological characteristics, were determined by SEM.

MATERIAL AND METHOD:

The species preferred in the central refuge, park, garden and landscaping works in the city center of Kastamonu province were used in this study. The leaves of *Berberis thunbergii, Euonymus japonicus, Cotoneaster lacteus, Mahonia aquifolium, Ligustrum delavayanum, Buxus microphylla, Prunus laurocerasus, Ilex aquifolium, Pyracantha coccinea* species constitute the material of the study.

The leaf samples were pressed, and their images were taken in the scanning electron microscope to examine the micromorphological structure of the leaves of the species and to obtain information about their stomatal structures and epidermis. The images of the species in the study taken in SEM for their micromorphological measurements were analyzed by Digimizer image analysis program (Image 1).

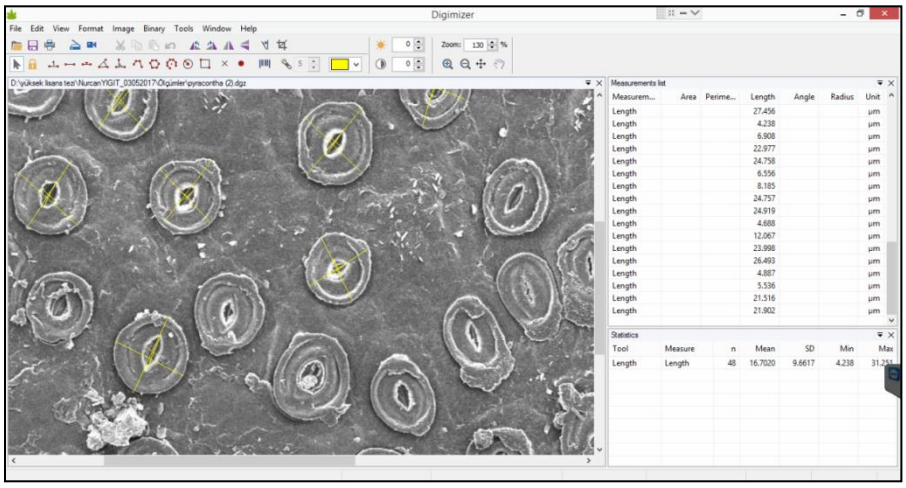


Image 1. Measurement of stoma data

The measurements of stoma images were performed in the sections taken from the leaves of the species with the Digimizer program.

RESULT AND DISCUSSION:

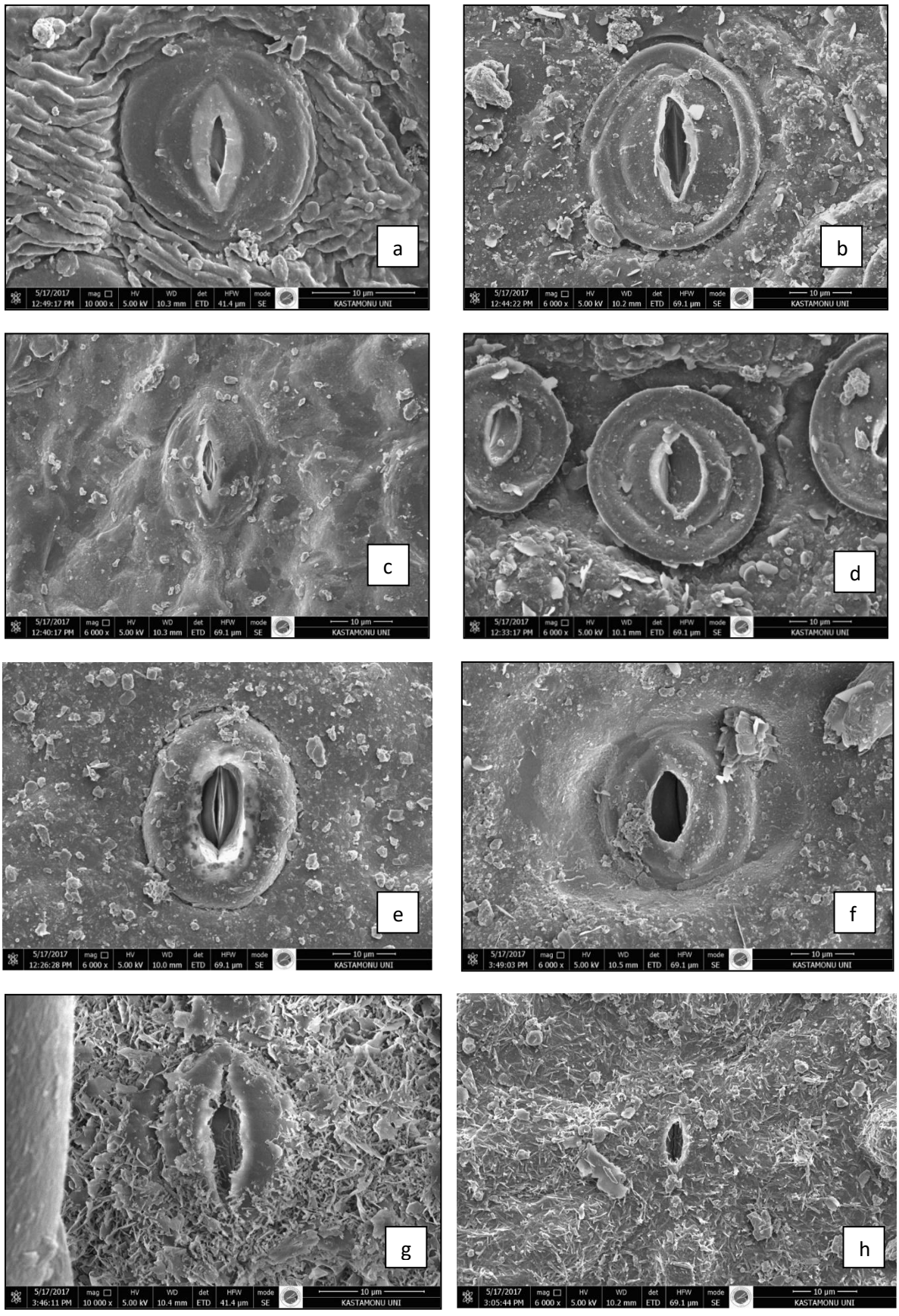
The average, minimum, maximum and standard deviation values of the stomatal characters of the species are presented in Table 1. The leaf micromorphological characters of the species were viewed and examined by SEM. The abaxial and adaxial surfaces of leaf samples of the species were observed, and their stomatal structures were viewed.

Table 1. *Stomatal character values of the species*

Species		PORW (µm)	PORL (µm)	STW (µm)	STL (µm)	SDEN (1000 mag)
Buxus microphylla	Mean	2,816	7,304	17,626	20,226	80
	Maximum	4,09	8,753	20,553	23,745	
	Minimum	1,092	4,004	15,947	17,77	
	Standard deviation	0,865	1,549	1,475	1,91	
Prunus laurocerasus	Mean	4,047	15,618	30,085	34,82	20
	Maximum	7,79	17,953	35,016	39,186	
	Minimum	1,96	12,766	25,953	31,673	
	Standard deviation	1,739	1,65	2,77	2,419	
Ligustrum delavayanum	Mean	1,768	9,094	16,525	22,733	27
	Maximum	2,298	14,744	19,881	25,346	
	Minimum	0,784	4,232	14,838	19,65	
	Standard deviation	0,472	3,171	1,427	1,699	
Berberis thunbergii	Ortalama	4,953	11,525	26,37	29,289	45
	Maksimum	7,105	17,144	30,201	36,67	
	Minimum	3,433	8,224	20,094	20,551	

	Standart sapma	1,252	2,446	3,387	3,826	
Ilex aquifolium	Mean	4,882	10,799	22,276	29,402	23
	Maximum	8,86	16,008	25,139	32,634	
	Minimum	3,012	6,088	18,702	26,415	
	Standard deviation	1,592	3,57	2,246	2,203	
Euonymus japonicus	Mean	2,985	7,492	22,859	24,716	42
	Maximum	5,882	10,771	26,873	27,414	
	Minimum	1,567	5,406	20,992	22,506	
	Standard deviation	1,148	1,682	1,896	1,273	
Cotoneaster lacteus	Mean	2,383	9,331	10,541	14,994	10
	Maximum	3,48	13,725	13,199	18,628	
	Minimum	0,614	3,302	8,093	11,107	
	Standard deviation	0,956	3,207	1,783	2,514	
Mahonia aquifolium	Mean	1,838	4,858	3,896	7,183	30
	Maximum	2,886	6,357	4,701	8,679	
	Minimum	0,925	2,736	2,296	4,48	
	Standard deviation	0,546	1,342	0,608	1,406	
Pyracantha coccinea	Mean	5,623	9,657	24,843	27,409	81
	Maximum	8,257	13,16	27,745	31,251	
	Minimum	4,238	6,908	21,876	23,892	
	Standard deviation	1,126	2,028	1,571	2,497	

When the leaf micromorphological character of *Buxus microphylla* species was examined, it was determined that the stomata were on the abaxial surface of the leaf. The stoma type of the species is amaryllis type stoma which is known by its similarity to bean. Image 2a shows that the stoma is open.



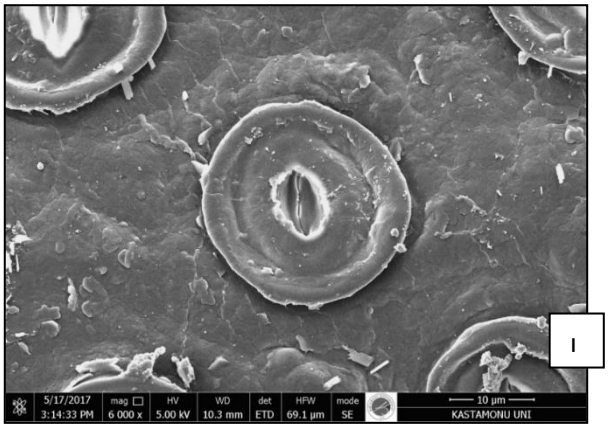


Figure 2. Buxus microphylla (a), *Prunus laurecerasus* (b), *Ligustrum delavayanum* (c), *Berberis thunbergii* (d), *Ilex aquifolium* (e), *Euonymus japonicus* (f), *Cotoneaster lacteus* (g), *Mahonia aquifolium* (h), *Pyracantha coccinea* (ı)

The data on the stoma values of *Buxus microphylla* species are presented in Table 1. According to Table 1, in *Buxus microphylla* species, it was found out that stomatal pore aperture width was 2.816 µm and the standard deviation was 0.865 µm. It was found out that stomatal pore aperture length was 7.304 µm on average, maximum 8.753 µm and minimum 4.004 µm, and the standard deviation was 1.549 µm. The mean stomatal width was 17.626 µm, and the mean stomatal length was 20.226 µm. The stomatal density in the unit area (on 1000 mag scale) was found to be 80 pieces.

The abaxial and adaxial surface stomatal structures of the leaf sample of *Prunus laurecerasus* species were viewed by SEM. It was observed that stomata were on the abaxial surface of the leaf. The stoma type of the species is amaryllis type stoma which is known by its similarity to bean. Image 2b shows that the stoma is open.

According to Table 1, in the *Prunus laurecerasus* sample, it was found out that PORW was 4.047 µm on average, maximum 7.790 µm and minimum 1.960 µm, and the standard deviation was 1.739 µm. While PORL and STW were 15.618 µm and 30.085 µm on average, STL was found to be 34.820 µm on average. SDEN was found to be 20 pieces on 1000 mag scale.

The abaxial and adaxial surfaces of the leaf sample of *Ligustrum delavayanum* species were observed by SEM. It was observed that stomata were on the abaxial surface of the leaf. The stoma type of the species is the amaryllis type (Image 2c). According to Table 1, in the *Ligustrum delavayanum* sample, it was found out that PORW was 1.768 µm on average and the standard deviation was 0.472 µm. It was found out that STWL was 9.094 µm on average and the mean STW value was 16.525 µm. STL was 22.733 µm on average, and SDEN was found to be 27 pieces (on 1000 mag scale).

The stoma type of *Berberis thunbergii* species is called amaryllis type stoma. Image 1d shows that the stoma is open. According to Table 1, in the *Berberis thunbergii* sample, it was found out that PORW was 4.953 µm on average, PORL was 11.525 on average, STW was 26.370 µm on average, STL was 29.289 on average. The stomatal density was found to be 45 pieces on 1000 mag scale.

According to Table 1, in the *Ilex aquifolium* sample, it was found out that PORW was 4.882 µm on average, PORL was 10.799 on average, STW was 22.276 µm on average, STL was 29.402 µm on average, SDEN was found to be 23 pieces on 1000 mag scale. In Image 1e of *Ilex aquifolium* species, it is observed that the stoma is open and it is the amaryllis type stoma.

According to Table 1, in the *Euonymus japonicus* sample, it was found out that PORW was 2.985 µm on average and the standard deviation was 1.148 µm. It was measured that PORL was 7.492 µm on average, STW was 22.859 µm on average, and STL was 24.716 µm on average. SDEN was found to be 42 pieces on 1000 mag scale. In Image 1f, it is observed that the stoma is open and it is the amaryllis type stoma.

In the *Cotoneaster lacteus* sample, it was found out that PORW was 2.383 µm on average, PORL was 9.331 µm on average, STW was 10.541 µm on average, STL was 14.994 µm on average. The stomatal density was found to be 10 pieces on 1000 mag scale. In Image 1, it is observed that the stoma is open and it is the amaryllis type stoma.

When Table 1 was examined, in the *Mahonia aquifolium* sample, it was found out that PORW was 1.838 µm on average, maximum 2.886 µm and minimum 0.925 µm, and the standard deviation was 0.546 µm. It was found out that PORL was 4.858 µm on average and the standard deviation was 1.342 µm. It was found out that STW was 3.896 µm on average and STL was 7.183 µm on average. SDEN was found to be 30 pieces on 1000 mag scale.

According to Table 1, in the *Pyracantha cocinea* sample, it was measured that PORW was 5.623 µm on average. It was found out that PORL was 9.657 µm on average, STW was 24.843 on average, and the standard deviation value was 1.571 µm. STL was 27.409 µm on average, and the stomatal density was found to be 81 pieces on 1000 mag scale.

Since the plants are used outside of their natural distribution areas in landscaping studies, they often face many stress factors such as light, salt stress and water stress that affect the morphological characteristics of the plant (Xu & Zhou, 2008). In particular, the stomatal density is highly influenced by these factors (Şevik et al., 2017). As it was proposed by Çağlar et al., the difference between stomatal densities could be caused by ecological conditions as well as genetic formation (Çağlar et al., 2004).

In the study carried out, it was observed that the number of stomata in the unit area on the leaves was different when stomatal measurements were performed on the leaf samples taken from 9 different species. The number of stomata in the unit area was measured to be 80 in *B. microphylla*, 20 in *P. laurecerasus*, 27 in *L. delavayanum*, 45 in *B. thunbergii*, 23 in *İ. aquifolium*, 42 in *E. japonica*, 10 in *C. lacteus*, 30 in *M. aquifolium,* 81 in *P. coccinea*.

Şevik et al. (2017), carried out a study on *Euonymus japonica* species by taking samples from different provinces, and they found out the results suggesting that stoma sizes could be proportional to the amount of precipitation. The amount of precipitation of Ankara province where the lowest values are obtained is also the lowest.

Along with the examination of the leaves of the samples by SEM, it was observed that the bean-shaped amaryllis type stoma seen in monocotyl and dicotyl plants was dominant in all species in the study.

The differences in the number of stomata in plants show that the difference of the genetic structure among the plants is due to species. To date, many studies have been carried out especially on environmental factors that affect the stomatal density (Liu et al., 2006; Yang & Wang, 2001; Zhang et al., 2006). In the studies carried out, it has been determined that stomatal density or sizes vary depending on many factors such as water stress, plant density (Yang et al., 2007; Gazanchian et al., 2007), light (Sevik et al., 2016) and salt (Zhao et al., 2001) stress.

As a result of this study, micromorphological characteristics of the species, which were obtained from the central refuge, park, garden and pavement plantations used in the landscape of Kastamonu, were determined by various analyses in the laboratory environment, and the differences between species were determined. The same studies can be carried out on the species other than the species that constitute the subject of the study, and a general conclusion can be made about these species used in the landscape. Both environmental and genetic factors are effective in the shaping of morphological and micromorphological characters (Sevik & Cetin, 2015; Sevik et al., 2016; Topacoglu et al., 2016; Sevik, 2012). The phenotypic features usually emerge as the interaction between genetics and environment and are shaped by these environmental and genetic factors (Kaya et al. 2009; Dempsey & Hook, 2000; Sevik & Cetin 2015). It also refers how different species react differently to the climatic conditions (Galmés et al., 2007; Maiti et al., 2016). Stomata are micromorphological characters that reflect the stress factors that plants are exposed to (water stress, drought stress, etc.) and are too small to be seen with the naked eye. Some plant species used in landscape studies are also plant species that are generally used outside of their natural distribution areas. For this reason, the effects of the stress factor show themselves in these plants sometimes in short periods and sometimes in long periods.

Micromorphological comparisons can be made on the same species by taking samples from different regions of the province for different species. Thus, it can be determined whether the location of the species has an effect on these characteristics. As a result of the study, it was determined that the plant species used in the parks, gardens and medians in Kastamonu city center were not affected by stress factors, according to the micromorphological features examined, and were suitable for use for the city at the plant design stage.

Compliance with Ethical Standard

Conflict of Interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

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