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Variation of some micro-morphological characters of leaves of *Aesculus hippocastanum* based on growing environment

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Abstract

Buckeye (*Aesculus hippocastanum* L.) is a member of the family Sapindaceae and is an exotic species for Turkey. It is a kind of tree that can grow up to an average of 15-20 m and is deciduous, which we often see in parks, gardens and roadsides. Although it is not in the natural flora of Turkey due to the use of different areas especially in the city centers, it is being used in the areas where many different climate types dominate. In this study, it was aimed to determine the variation of some micromorphological characters of the leaves of *Aesculus hippocastanum* L. depending on the growing environment. Samples were grown on 6 different cities which are dominated by different climate types. With this purpose, leaf samples were collected from *Aesculus hippocastanum* L. individuals growing in 6 different cities (Rize, Samsun, İzmir, Antalya, Sivas and Ankara) in the Central and Western Anatolia regions where the Black Sea, Mediterranean and continental climate types predominate. Scale images were obtained with the help of electron microscope (SEM = Scanning Electron Microscope) of the collected leaf samples and stoma length (µm), stoma width (µm), por length (µm), por width and stoma density (per mm^2 field) were determined. Variance analysis and Duncan test were applied to the obtained data with the help of SPSS package program and the variation of the indicated characters according to the growing environment was evaluated statistically. As a result of the study, it was determined that there was a statistically significant difference in at least 95% confidence level among the growth environment conditions for all characters except for stoma width.

Keywords *Aesculus hippocastanum* L., buckeye, micro-morphological character, SEM, Stoma

Introduction

Due to the rapidly growing population in the world, the green spaces in cities are under constant threat, which is leading to various environmental problems including noise, air pollution and unplanned urbanization [1-4]. Gradually congested large settlements are increasing the need of people for natural and green areas. Accordingly, the importance of the landscape design in the city centers and plants existence have increased and started to be accepted as a sign of the quality and livability of cities [3, 5-11]. The plants are able to adapt to different growing environments around the world, especially due to the varying size, density and distribution of stoma on the leaf surfaces [12-14].

Just like in natural areas, plants perform many functions in the city centers, such as being an economic resource, decreasing the speed

of the wind and supporting the wild life [15-17]. In addition to these, they add aesthetic value to the growing areas [18-19]. Therefore, the use of plants outside the natural environment is a common occurrence in landscape studies.

Table 1. Some climate data of the sample areas

Annual Avarage Values	Rize	Samsun	İzmir	Antalya	Sivas	Ankara
Average Temperature (°C)	14.3	14.5	17.8	18.6	8.9	11.9
Average Maximum Temperature (°C)	18.0	18.2	22.6	24.1	15.3	17.8
Average Minimum Temperature (°C)	11.1	11.0	13.4	13.7	2.8	6.2
Average Sunshine Time (hours)	49.4	61.0	94.5	100.3	80.5	80.3
Average Number of Rainy Days	172.5	135.6	77.7	75.1	112.5	102.3
Total Rainfall Amount (kg/m2)	2304.1	717.5	695.9	1066.9	429.2	387.2
Highest Temperature (°C)	38.2	39.0	43.0	45.0	40.0	41.0
Lowest Temperature (°C)	-7.0	-9.8	-8.2	-4.6	-34.6	-24.9

In landscaping studies, plants are often left alone with the stress factors (water, light, temperature, etc.), depending on whether the plants are used outside the natural spreading area or not. These stress factors are likely to affect the morphological and micro-morphological properties of the plants [19-21].

One of the first places where people and trees are closest and where they are most useful to each other is the roadside planting areas. In fact, trees, which are a part of the forest ecosystem, are under the influence of various pressures on roadsides where urban ecosystem and urban constructions are dominant. Due to these pressures, they grow more slowly and live shorter than their homologues in natural life [22].

Among the countries in the temperate zone, Turkey is one of the countries with the richest flora in the world in terms of number of species [23]. In Turkey, plant species that are grown in three different phytogeographic regions can be used in landscape designs. These plants generally do not differ significantly from one another morphologically at first glance. Nevertheless, there is not enough information about how they differ at the micro-morphological level. However, the changes that occur at fairlysmall levels can give many ideas, from the stress level of the plant to the level of adaptation to their growth environment [24].

Aesculus hippocastanum L. is one of the tree species that spread in areas where different climate types dominate. Buckeye (*Aesculus hippocastanum* L.) is a member of the family Sapindaceae and is deciduous. Although their main homeland is the Balkan peninsula, it also grows naturally in Albania and northern Greece. Today, it is widely used as a shadow and alley tree. This species that can grow up to 20-30 m, has 5-7 pieces of fuzzy leaves and standing upright flowers. Buckeyes are often grown in parks, gardens and roadsides as ornamental plants because of their beautiful and eye-catching flowers and large leaves. In this study, it was aimed to determine the changes in micro-morphological characters of leaves of *Aesculus hippocastanum* L. individuals, which were grown in areas that are dominated by different climate types (Black Sea, Mediterranean and continental).

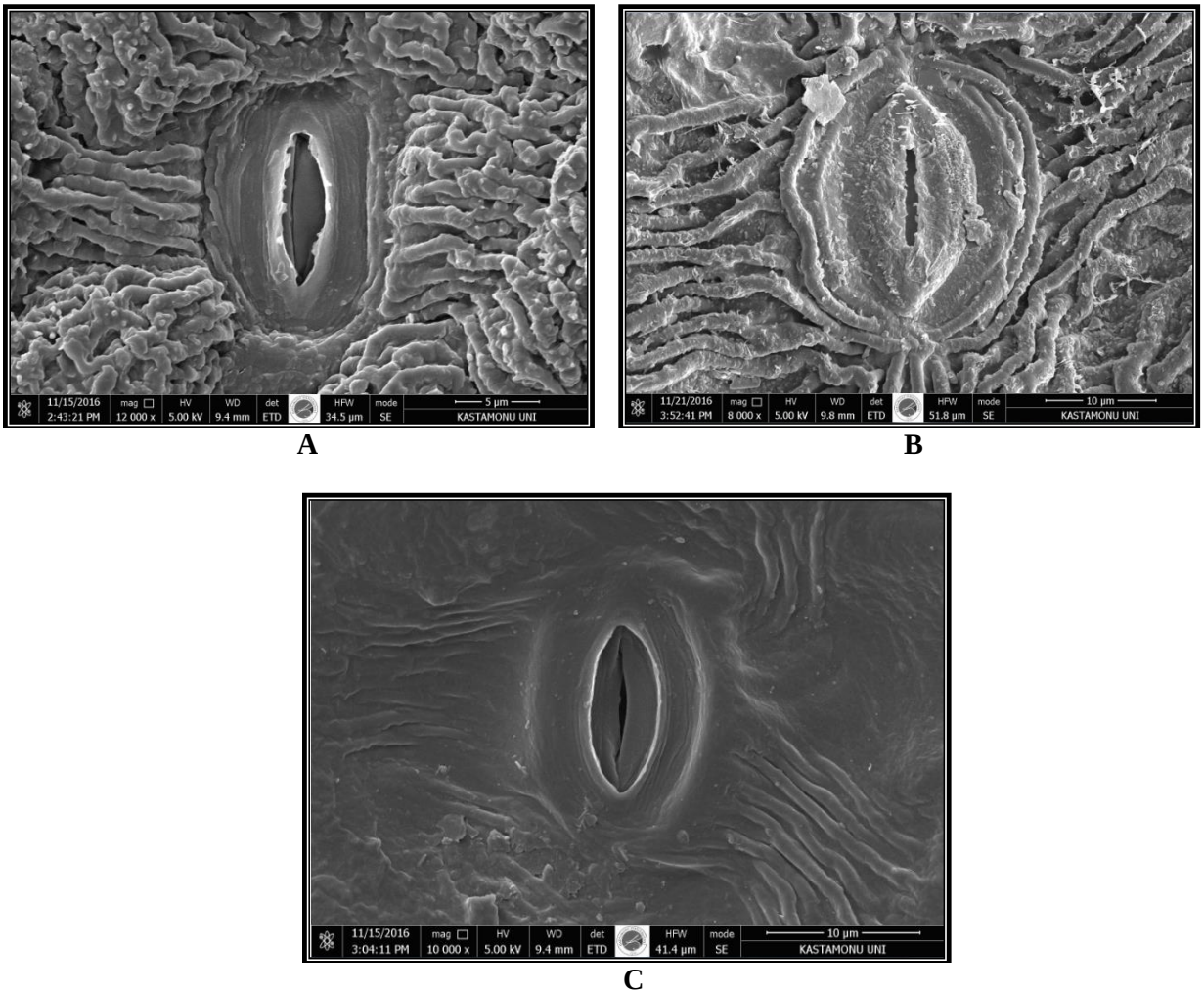
Table 2. Variation of leaf micromorphological characters depending on growth environment

City	micromorphological characters				
	STU (µm)	STG (µm)	PORU (µm)	PORG (µm)	STY (number)
Ankara	16,388 a	9,709 a	6,713 a	2,041 a	288 d
Antalya	31,222 b	23,689 b	**14,976 c**	**5,480 c**	208 c
Samsun	18,304 a	10,237 a	12,184 b	4,026 c	139 b
İzmir	**43,355 c**	**30,362 b**	14,798 c	1,920 a	145 b
Rize	19,769 a	12,023 a	11,362 b	2,679 ab	97 a
Sivas	16,714 a	9,387 a	6,340 a	2,175 ab	309 e
F Value	40,766***	59,711***	24,770***	47,222***	594,840***

Methodology

The study was carried out on leaves of *Aesculus hippocastanum* L. that were collected from different cities. *Aesculus hippocastanum* L. is an exotic species in Turkey that can be grown in areas dominated by different types of climate. The leaf samples used in the study were collected from Samsun and Rize that are dominated by Black Sea climate type; Izmir and Antalya that are dominated by Mediterranean climate type; and Ankara and Sivas provinces that are dominated by continental climate type. Even when these cities are in the same regions and dominated by the same climate types, there is a great difference between the climate data of the cities. Some of the climate data of the cities from where the leaf samples were collected are given in Table 1

Mature leaf samples from *Aesculus hippocastanum* L. individuals, which were located in parks, gardens and roadsides in urban centers from İzmir, Antalya, Samsun, Rize, Ankara and Sivas were collected in 2016. They were collected late in the vegetation season, and were pressed dried by standard pressing process and drying method. Further, leaf samples were brought to Central Research Laboratory of Kastamonu University, examined with electron microscopy and necessary images were taken. With the help of SEM, scaled images were obtained from the lower surface of the leaf and near the middle parts. The files were created with the extension ".jpeg" and by the "ImageJ" program;STU: Stoma Length, STG: Stoma Width, PORU: Pore length, PORG: Pore width and STY: Stomach Density (1 mm^2 field) were measured.



A **B**

C

Figure 1. In general adaxial surface of *Aesculus hippocastanum* L. individuals from different Phytogeographic region (A) Europe-Siberia (Bar 5 µm) (B) Iran-Turan (Bar 10 µm) (C) Mediterranean Phytogeographic region Bar 10 µm

Aesculus hippocastanum L. leaf samples were measured after the images were obtained from the lower surface and the obtained data were evaluated with SPSS package program and variance analysis and Duncan test were applied to the data. Correlation analysis was applied to the data with the help of SPSS package program in order to determine the existence of statistically significant relationship between the examined micro-morphological characters.

Results and Discussion

The mean values of the micro-morphological characters in the leaves of *Aesculus hippocastanum* L. collected from İzmir, Antalya, Samsun, Rize, Ankara and Sivas which were located in the different phyto-geographical regions; and the variance analysis and Duncan test results applied to this data are given in Table 2. As the result of the variance analysis, it was determined that there was a statistically significant difference of 99.9% confidence level among the individuals which were grown in different cities in terms of all the investigated micro-morphological characters. According to the Duncan test results, the data were collected in three homogeneous groups as STG, PORU and PORG, two STG and five STY homogeneous groups. Duncan test results showed that Ankara and Sivas were in the first homogeneous groups in all the characters other than STY and they usually had the lowest values. For the other characters outside the STY, the highest values were obtained in Izmir and Antalya samples. The lowest values for STY were measured in Rize and Samsun. The change in stoma density can be considered as one of the clearest indicators of the environmental changes.

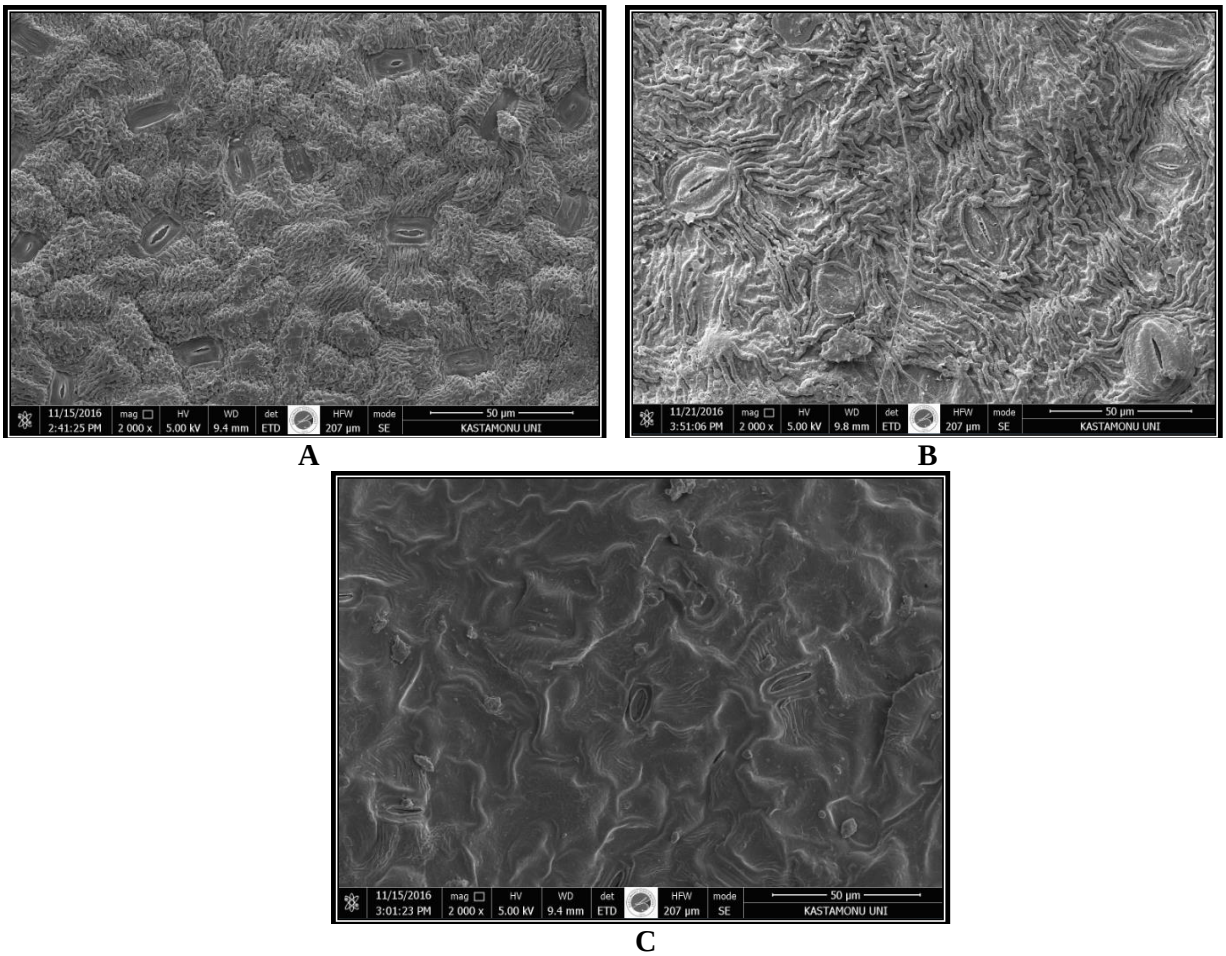


Figure 2. Mature leaf samples from *Aesculus hippocastanum* L. individuals from different phytogeographic region (A) Europe-Siberia (Bar 50 µm) (B) Iran-Turan (Bar 50 µm) (C) Mediterranean Phytogeographic region Bar 50 µm

Table 3. Results of correlation analysis

	STU	STG	PORU	PORG
STG	,909**			
PORU	,769**	,741**		
PORG	,073	,197	,479**	
STY	-,301*	-,319*	-,567**	-,167

Mature leaf samples from *Aesculus hippocastanum* L. individuals from different phytogeographic regions have been demonstrated in Figure 1. Epidermal cells of the abaxial surface were striated at the same level. Stoma appeared normal and were clearly visible with guard cells that were generally lacking folds (Figure 1). Adaxial surface of mature leaves of *Aesculus hippocastanum* L. individuals from different phytogeographic regions have been demonstrated in Figure 2.

Correlation analysis was applied to the data to determine the statistical significance of the obtained data (Table 3). According to the results of the correlation analysis, it was determined that the relationship between PORG and STU, STG and STY was not statistically significant; while there was a statistically significant relation between all the other characters. The relationship between the two variables according to Cohen [25], the correlation coefficient value was low between 0.10-0.29, medium level between 0.30-0.49 and high level between 0.50-1.0. Accordingly, the strongest correlations were found between STU and STG (0.909) and PORU (0.769). There was also a moderately strong relationship between PORU and STG and STY at high level, PORU with PORG (0.479) and STY with STU (0.301) and STG (0.319). All relations between STY and other characters were negative. Several studies have already reported that the size of the stoma is inversely proportional to the number [26-27].

It is noteworthy that among most of the characters studied other than the STY, the highest values were from Izmir and Antalya. Ankara and Sivas are dominated by continental climate and İzmir and Antalya are in the areas dominated by the Mediterranean climate. The lowest average temperature values were found in Ankara and Sivas, and the highest average temperature values were found in Izmir and Antalya. This leads to the conclusion that the stoma characters were related to the climate and especially to the temperature. Stoma characters have been associated with the climate type in the studies of different species [28-30].

The study results showed that the stoma characters were closely related to each other. Phenotypic characters appeared by the interaction of the genetical and environmental changes [31] and they were formed by the effect of many environmental factors [32-34] and genetical factors [35-36]. However, it has been determined that the morphological characteristics of the species vary depending on many factors, especially the origin [37-39].

Studies have shown that micro-morphological characters vary significantly from species to species [26]. The stoma control the CO_2 and water vapor entry to the leaves and are significantly affected by the environmental conditions [40-43]. When plants are cultivated in an area outside the natural growth environment, some changes in their own structure occur to get them adapted to the growing environment. Plant species used in the landscape designs usually also deal with the stress factors, depending on the usage of plants outside the natural spreading areas. It is known that these stress factors affect some morphological properties of the plants. For morphological and physiological reasons, structural features of stoma, the number and the distribution of stomas on leaf surface have been the subject of many researches. Studies have shown that the plant morphological characteristics are influenced by many stress factors such as light, salt stress [44-45], and water stress [46]. Therefore, it is possible that stoma characters are affected by environmental conditions and especially stress factors [47-48]. Several studies have addressed that among different stoma characters, stoma density is highly affected by these factors [28-30].

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