Altitude-dependent Variations in Some Morphological and Anatomical Features of Anatolian Chestnut

Gizem Özdikmenli,[a] Nurcan Yiğit,[a] Halil Barış Özel,[b] and Hakan Şevik [c]

Morphological measurements of Anatolian chestnut (*Castanea sativa* Mill.) leaves were done within the borders of Abana district of Kastamonu province. The study was conducted using mixed (oak, beech, hornbeam, black pine, and yellow pine) medium (41% to 70%) and fully closed (71% to 100%) stands. Some leaf parameters, such as leaf blade width, petiole length, leaf blade length, leaf length, distance between lateral veins, teeth width, teeth length, the angle between the leaf base and the petiole, and the angle between the midrib and lateral veins, were measured. Moreover, stomata of the leaves picked up from precise altitudes were observed under a scanning electron microscope. The differences between fibre elevation, fibre wall thickness, elasticity coefficient, rigidity coefficient, Muhlstep rate, and Runkel ratio were found in the wood samples taken from different altitude zones. It was found that altitude did not affect leaf blade width, fibre length, fibre width, felting ratio, and lumen width. However, it was determined that altitude affected other studied characteristics.

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Contact information: *a: Department of Forest Engineering, Faculty of Forestry, Kastamonu University, Türkiye; b: Department of Forest Engineering, Faculty of Forestry, Bartın University, Türkiye; c: Department of Environmental Engineering, Faculty of Engineering and Architecture, Kastamonu University, Türkiye;*
** Corresponding author: nyigit@kastamonu.edu.tr*

INTRODUCTION

Anatolian chestnut (*Castanea sativa* Mill.) belongs to the Fagaceae family. They are long-lasting trees that can reach up to 30 to 35 m in height and can grow up to 1.5 to 2 m in diameter. They have a wide crown and a bark, which is smooth in juvenile trees and cracked in more mature trees (Yaltirik 1993; Subasi 2004).

Anatolian chestnut widely spreads in the highly precipitated and elevated places of the temperate zones in the northern hemisphere. Other locations where it spreads are Northern Africa, Southern Europe, East Asia, and South-West and North America. In terms of altitude, it can grow at 700 to 800 m, and can reach an altitude of 1700 to 1800 m in the Caucasus and Rize province. In addition, it locally spreads in the Aegean and the Mediterranean regions of Türkiye. The only chestnut species naturally grown in Türkiye is *Castanea sativa* species (Erdem 1951; Kayacik 1981; Yaltirik 1993; Yilmaz 2014).

Castanea sativa generally grows in places with good drainage located in the humid and temperate broad-leaved forests in the Black Sea region of Türkiye. They are rarely found in pure stands. Rather, they are often in mixed form with hornbeam (*Carpinus*), beech (*Fagus*), linden (Tilia), alder (*Alnus*), and ash (*Fraxinus*) trees. Among the other parts of Türkiye, they grow especially in the main Aegean part of the Aegean region, on the north-facing slopes of Boz Mountains, and in the river valleys of Aydin region. In these areas, they are found in the mixed form with black pine, Calabrian pine, and scrub communities (Özdikmenli 2019).

Plants that are grown in different environments undergo some anatomical and morphological changes adaptable to that environment. Environmental conditions affect all phenotypic characteristics of living organisms (Key *et al.* 2022). The most important environmental factors are temperature, precipitation, and humidity (Cetin *et al.* 2023; Dogan *et al.* 2023). Although other factors remain constant in the same region, climatic parameters change depending on altitude (Tekin *et al.* 2022; Zeren Cetin *et al.* 2023). For this, studies on the relationship between altitude and plant morphology can be beneficial for ecological researchers, and morphological knowledge is still very important in many fields of plant sciences, including: population variability (Zebec *et al.* 2015; Poljak *et al.* 2018), taxon delimitation (Sękiewicz *et al.* 2016), morphological and physiological seed characterization (Güney *et al.* 2015; Daneshvar *et al.* 2016; Atar *et al.* 2020), morphological involucre variation (Xue *et al.* 2020; Atar 2022), cultivar characterization (Ertan 2007; Poljak *et al.* 2016) and selection (Solar *et al.* 2005), and variation of macro- and micro-morphological leaf (Poljak *et al.* 2015; Güney *et al.* 2016; Bayraktar *et al.* 2018) and fruit traits (Atar and Turna 2018; Eminagaoglu and Ozcan 2018). Overall, morphological variability and plasticity can be used to predict population dynamics and the evolutionary adaptations of plants to a novel environment (Nicotra *et al.* 2010). For this reason, many changes are observed in their stomata. Most of the plants' water loss (about 85% to 90%) occurs through the stomata. Therefore, it is important to know the structure and number of stomata of each plant species (Dickison 2000; Sevik *et al.* 2017; Yiğit *et al.* 2023).

This study was carried out to determine the altitude-dependent variations of some morphological and micro-morphological characteristics of the leaves and some anatomical characteristics of the woods of Anatolian chestnut (*Castanea sativa* Mill.) species as well as the anatomical characteristics of its branches.

EXPERIMENTAL

In this study, the changes in morphological, micro-morphological, and anatomical characteristics of *Castanea sativa* depending on the altitude were investigated. Samples were taken from the branches and green leaves of the species. The study area is composed of the zones where Anatolia chestnut naturally spreads, within the borders of Abana Forest Sub-District Directorate of Bozkurt Forestry Operation Directorate.

In the studies, Kastamonu University Faculty of Forestry Bilgehan Bilgili Herbarium, Forest Industry Engineering Wood Chemistry Laboratory, and Kastamonu University Central Research Laboratory were utilized.

The study was carried out between 2013 and 2015, by the end of August beginning of September, when *Castanea sativa* completely stopped growing. Within the scope of the study, 3 different altitude zones were identified (0 to 200 m, 200 to 400 m, and 400 to 600 m), and samples were collected from 3 different points in each altitude zone. From these mentioned points, 5 leaves were collected from 20 trees. Mature leaves were collected from the central parts of the trees that were exposed to the sun from different directions. Their coordinate values were noted; they were kept separately after being numbered and then brought to the laboratory environment. These leaf samples were dried using standard pressing processes. The different altitude zones of each sample and the points from which they were taken were recorded. During this drying phase, the newspapers were periodically changed to avoid decay, fungus, *etc*. in the plants. The dried leaves were photographed by placing a ruler next to them to create a scale, and files with ".jpeg" extension were obtained.

Nine different morphometric parameters of the leaves were measured using "imageJ" computer measurement program over the scaled leaf photographs. These parameters were specified as;

- LW (blade width (cm)),
- PL (petiole length (cm)),
- LBL (leaf blade length (cm)),
- LL (leaf length (cm)),
- DBLV (distance between lateral veins (cm)),
- TW (teeth width (cm)),
- TL (teeth length (cm)),
- ABP (the angle between the leaf base and the petiole (°)), and the
- ABV (angle between the midrib and lateral veins (°))

Additionally, the stomata on the leaf samples collected from 3 different points in each of 3 different altitude zones (0 to 200 m, 200 to 400 m, and 400 to 600 m) were examined. Scanning electron microscopy (SEM) was used for the examination and the "ImageJ" computer measurement program was used on the obtained figures; the following characters were measured:

- SL (Stoma Length (µm): obtained by measuring the length of 10 stoma figures at each altitude zone),
- SW (Stoma Width (µm): obtained by measuring the width of 10 stoma figures at each altitude zone),
- SPL (Stoma Pore Aperture Length (µm): obtained by measuring the length of 10 stoma pore aperture figures at each altitude zone),
- SPW (Stoma Pore Aperture Width (µm): obtained by measuring the width of 10 stoma pore aperture figures at each altitude zone),
- SW/SL: (Stoma width/Stoma length): the value found by dividing stoma width to stoma length,
- SD (Stomatal density): (obtained by counting the stomata in mag*1000 per unit area)

The Spearin-Isenberg (sodium chlorite and acetic acid) method was used for the fibre-releasing process called maceration (Alkan *et al*. 2003). The leaves of the samples used for maceration were separated from their woody branches. The pieces of wood, which were brought to the size of the matchstick, were extracted from the last two-year ring of the trunk.

First, the sample pieces, then 0.5 mL of sodium chloride, pure water exceeding the sample size, and then approximately 2 mL of acetic acid with the help of dropper were added in each glass test tube. Some water was added in an empty beaker, and the glass test tubes were placed in this beaker and allowed to boil in the laboratory furnace. For about 2.0 h, $NaClO_2$ and CH_3COOH were added in half an hour intervals until the lignin in the test tubes was softened.

The samples in the test tubes were washed with water and filtered to remove the solution. Afterwards, the samples were placed into an empty beaker and some water was added onto them. Their disintegration into fibres was observed in the laboratory mixer for 5 to 10 min, and when it was ready, a few drops of alcohol (C_2H_6O) were added onto the jars to prevent deterioration. The disintegrated fibres were observed on a micrometre calibrated SOIF brand binocular laboratory microscope to identify their properties and to perform measurements.

The samples were transferred to a computer by way of an MshOT microscope image transfer camera, and then measured *via* the program. Fibre length, fibre width, and lumen diameter width of the samples were measured when using a 4x objective glass. A total of 100 measurements were performed for the average fibre length (L), and 50 for fibre width (D) and lumen width (d). At least 150 measurements were carried out from each altitude zone. The fibre wall thickness (W) was calculated using the (D-d)/2 equation.

The cellulose content of the fibres, fibre sizes, and the ratios calculated based on these sizes are important in the determination of the plant's suitability to be turned into paper. The following equations were used in fibre sizes and the relationships between these sizes (Goksel 1986; Kirci 2006).

- Felting Ratio (FR) = Fibre Length (*L*) / Fibre Width (*D*) × 1000
- Elasticity Coefficient (EC) = Lumen Width (*d*) × 100 / Fibre Width (*D*)
- Rigidity Coefficient (RC) = Fibre Wall Thickness (*W*) × 100 / Fibre Width (*D*)
- Muhlstep Ratio (MR) = Fibre Wall Area (D_2 -d_2) × 100 / Fibre Cross Section Area (D_2)
- Runkel Ratio (RR) = 2 × Fibre Wall Thickness (*W*) / Lumen Width (*d*)
- "F" Factor (FF) = Fibre Length (*L*) × 100 / Fibre Wall Thickness (*W*)

The data obtained for all characters were evaluated using SPSS 20.0 package program, variance analysis was applied to the data, and the homogenous groups were formed by applying Duncan test to the data with statistically significant differences ($p < 0.05$). In addition, correlation analysis was conducted to determine the level of relationship between the characters in the study.

RESULTS AND DISCUSSION

In this study, the changes in morphological, micro-morphological, and anatomical characteristics of the chestnut species, which naturally spread in three different altitude zones, were evaluated based on these different altitude zones. Variance analysis was applied to the values measured from the samples taken from different altitude zones, and the analysis results are given in Table 1.

As a result of the variance analysis conducted according to different altitude zones, it was found that the altitude did not affect the lamina width (LW), one of the morphological characteristics. However, altitude zones were observed to affect (at the 99.9% confidence level) some other studied characters of PL, LBL, LL, TW, TL, ABP, and ABV. In terms of the distance between lateral veins (DBLV) character, a difference was determined at the 95% confidence level. The Duncan test was applied to the characteristics to determine this effect rate (Tables 1 and 2).

Table 1. Effects of 3 Altitude Zones on the Leaf Morphological Parameters

Parameters		Sum of Squares	df	Mean of Squares	F	Significance Level
LW	Between-groups	4.539	2	2.269	2.171	0.117
	Within-groups	185.027	177	1.045		
	Total	189.566	179			
PL	Between-groups	2.011	2	1.006	9.902	0.000
	Within-groups	17.976	177	0.102		
	Total	19.987	179			
LBL	Between-groups	414.841	2	207.421	16.375	0.000
	Within-groups	2242.036	177	12.667		
	Total	2656.877	179			
LL	Between-groups	440.512	2	220.256	15.156	0.000
	Within-groups	2572.199	177	14.532		
	Total	3012.710	179			
DBLV	Between-groups	0.880	2	0.440	3.293	0.039
	Within-groups	23.662	177	0.134		
	Total	24.543	179			
TW	Between-groups	0.212	2	0.106	50.461	0.000
	Within-groups	0.371	177	0.002		
	Total	0.582	179			
TL	Between-groups	0.254	2	0.127	48.620	0.000
	Within-groups	0.463	177	0.003		
	Total	0.717	179			
ABP	Between-groups	4586.325	2	2293.163	10.287	0.000
	Within-groups	39456.905	177	222.920		
	Total	44043.230	179			
ABV	Between-groups	1026.152	2	513.076	20.057	0.000
	Within-groups	4527.715	177	25.580		
	Total	5553.867	179			

According to the results of the Duncan test conducted in accordance with the variance analysis applied to the characteristics with at least 95% confidence of difference between them, it was found that a single class was established in terms of LW characteristics, and that the LW characteristics in the 1st, 2nd, and 3rd altitude zones were in the same class.

As a result of variance analysis, the morphological characteristics related to the altitude zones were not found to be statistically significant ($p = 0.117$). For this reason, the Duncan test was not applied to the LW character.

Table 2. Duncan Test Results Regarding the Effect of 3 Altitude Zones on the Leaf Morphological Parameters

Measured Characters	Altitude Zones	N	Subset for Alpha = 0.05		
			1	2	3
PL (cm)	1	60	1.051a		
	2	60		1.207b	
	3	60		1.308b	
	Sig		1.000	0.085	
LBL (cm)	1	60	14.567a		
	3	60	15.433a		
	2	60		18.132ba	
	Sig		0.184	1.000	
LL (cm)	1	60	15.601a		
	3	60	16.742a		
	2	60		19.340b	
	Sig		0.103	1.000	
DBLV (cm)	3	60	0.853a		
	2	60	0.950a	0.950b	
	1	60		1.023b	
	Sig		0.145	0.275	
TW (cm)	2	60	0.194a		
	1	60		0.229b	
	3	60			0.278c
	Sig		1.000	1.000	1.000
TL (cm)	2	60	0.185a		
	1	60		0.254b	
	3	60		0.272b	
	Sig		1.000	0.052	
ABP (º)	2	60	107.344a		
	3	60		113.027b	
	1	60			119.695c
	Sig		1.000	1.000	1.000
ABV (º)	2	60	50.119a		
	3	60		54.878b	
	1	60		55.442b	
	Sig		1.000	0.542	

According to the Duncan test results given in Table 2, it was found that two different classes were formed in terms of PL characteristics and that the PL characteristics in the 2nd (1.207 cm) and 3rd (1.308 cm) altitude zones were in the same groups. In addition, it was found that two different classes were formed in terms of LBL characteristics, that the LBL characteristics in the 1st and 3rd altitude zones were in the same class, but the value measured in the individuals in the second altitude zone was 18.1 cm. It was found that two different classes were formed in terms of LL characteristics, and that the LL characteristics in 1st and 3rd altitude zones were in the same class. It was also seen that there were two different classes formed in terms of DBLV characteristics, and that the DBLV characteristics in the 3rd (400 to 600 m) and 1st (0 to 200 m) altitude zones were in totally different classes in terms of measured values. In terms of TW characteristics, it was found that 3 different classes were formed and that the TW characteristics in the 1st, 2nd, and 3rd altitude zones were in separate classes. It was also found that the highest TW value was measured at 400 to 600 m, which was the 3rd altitude zone. When the TL characteristic was examined, two different classes were formed and the TL characteristic in the 1st and 3rd altitude zones were in the same class (Table 2).

When examined in terms of ABP and ABV characteristics, it is apparent that both characteristics formed the first and a different class with the lowest value compared to the characteristics measured at 200 to 400 m altitude zones. The ABP and TW characteristics were in three different classes in terms of measured values.

As a result of the variance analysis performed in accordance with different altitude zones, it was found that the altitude zones affected (with at least 99.9% confidence) 9 characteristics, and the Duncan test was applied to the characters to determine the effect (Table 3).

When the stomata in the leaf samples collected from three different points in three different altitude zones (0 to 200 m, 200 to 400 m, and 400 to 600 m) were examined, it was found that the stomata were hypostomatic type because of the fact that the stomata were present only on the abaxial surface of the leaves.

Table 3. Mean Values of Micro-morphological Characters According to Altitudes

Altitude Zone	SL (µm)	SW (µm)	SPL (µm)	SPW (µm)	SD (mag*1000)	SW/SL
0 to 200 m	25.857a	19.191a	8.503a	3.946a	28a	0.742a
200 to 400 m	22.133b	20.703b	8.293a	3.488a	33a	0.935b
400 to 600 m	25.521a	21.024c	12.071b	5b	42b	0.823c
F Value	30,972**	31,257***	4,406**	2,936**	9,586***	6,416***

**significant at 0.01 level; ***significant at 0.001 level; The letters a, b, c, etc. means, according to Duncan test results, show that the group is located. It is statistically different from the values contained in different groups, starting with the letter a numerical value grows

As a result of the measurements made, it was found that SL, SW, SPL, and SPW characteristics had the lowest values in chestnut individuals located in an altitude zone of 200 to 400 m. In terms of micro-morphological characteristics, it was determined that the highest values were found in chestnut individuals at an altitude of 400 to 600 m; whereas the minimum stoma density value was found at an altitude zone of zero to 200 m.

The stoma images obtained from the leaf samples collected from the trees at different altitude zones are shown in Figs. 1, 2, and 3.

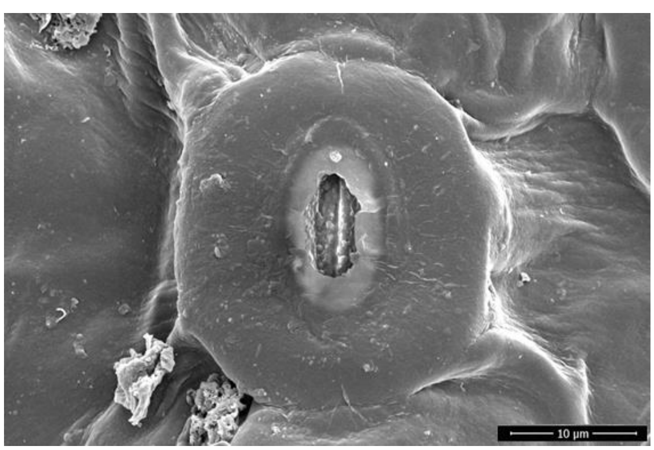

Fig. 1. Stoma figure in the zero to 200 m altitude zone

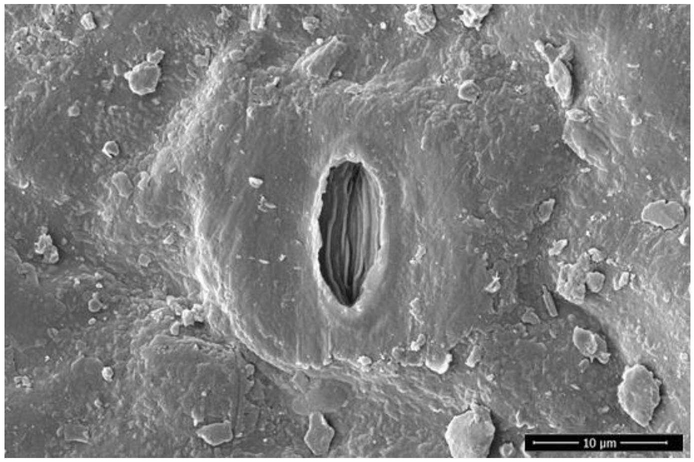

Fig. 2. Stoma figure in the 200 to 400 m altitude zone

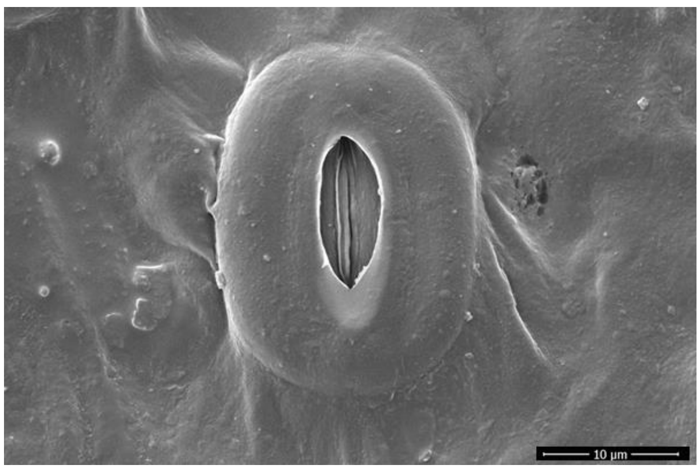

Fig. 3. Stoma figure in the 400 to 600 m altitude zone

The abaxial surface stoma images obtained from the leaf samples collected from the chestnut trees at different altitude zones are shown in Figs. 4, 5, and 6.

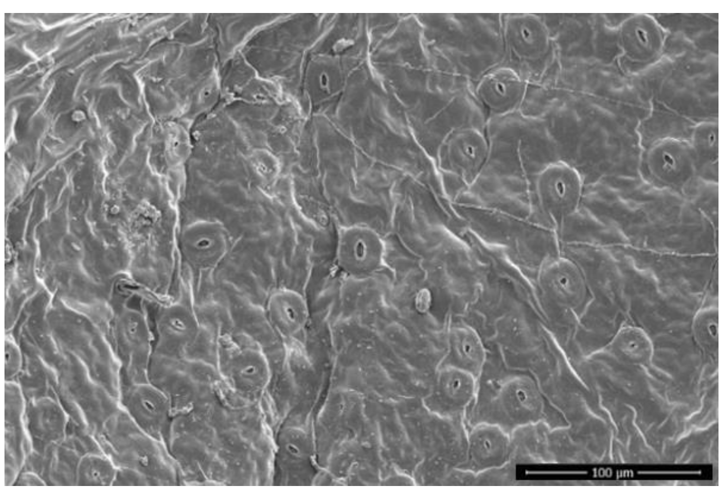

Fig. 4. Abaxial surface stoma in the 0-200 m altitude zone

Variance analysis was applied to the characters measured to determine the altitude-related differences demonstrated by the anatomical characteristics. The results of variance analysis are shown in Table 4.

Table 4. Results of Variance Analysis Applied to the Anatomical Characteristics

Characteristics		Sum of Squares	df	Mean of Squares	F Value	Signifi-cance
L (mm)	Between-groups	0.289	2	0.145	3.084	0.047
	Within-groups	20.982	447	0.047		
	Total	21.272	449			
D (µm)	Between-groups	0.000	2	0.000	1.537	0.216
	Within-groups	0.051	447	0.000		
	Total	0.051	449			
d (µm)	Between-groups	0.000	2	0.000	1.809	0.165
	Within-groups	0.045	447	0.000		
	Total	0.046	449			
W (µm)	Between-groups	0.000	2	0.000	7.721	0.001
	Within-groups	0.001	447	0.000		
	Total	0.001	449			
FR	Between-groups	10674	2	5337	1.692	0.185
	Within-groups	1410135	447	3155		
	Total	1420810	449			
EC	Between-groups	2028	2	1014	7.900	0.000
	Within-groups	57358	447	128.3		
	Total	59386	449			
RC	Between-groups	507	2	253.4	7.900	0.000
	Within-groups	14340	447	32.08		
	Total	14846	449			
MR	Between-groups	3873	2	1937	7.922	0.000
	Within-groups	109274	447	244.5		
	Total	113148	449			
RR	Between-groups	0.921	2	0.461	5.480	0.004
	Within-groups	37.57	447	0.084		
	Total	38.49	449			
"F" Factor	Between-groups	1.67×10^9	2	8.35×10^8	1.258	0.285
	Within-groups	2.97×10^{11}	447	6.64×10^8		
	Total	2.98×10^{11}	449			

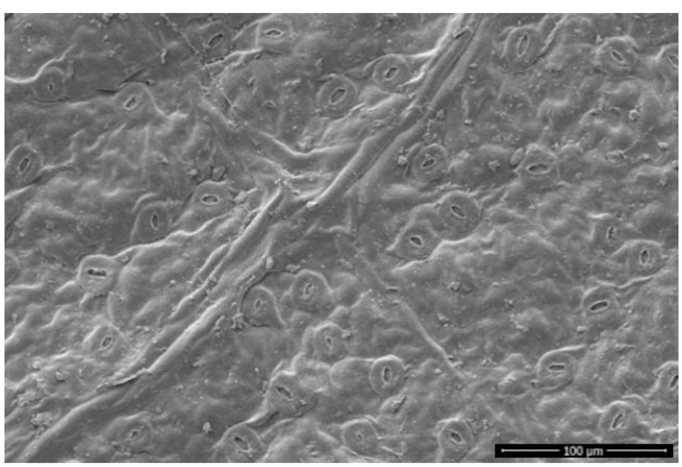

Fig. 5. Abaxial surface stoma in the 200 to 400 m altitude zone

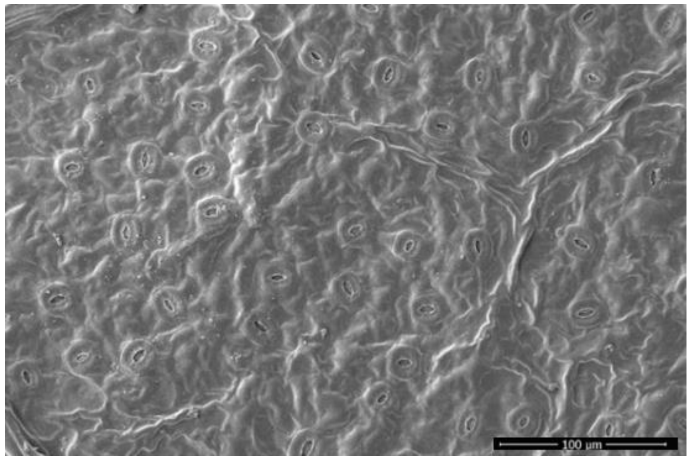

Fig. 6. Abaxial surface stoma in the 400 to 600 m altitude zone

When examining Table 4, it can be seen that there were statistically significant differences between L, W, Elasticity Coefficient (EC), Rigidity Coefficient (RC), Muhlstep ratio (MR), and Runkel ratio (RR), and that difference was significant at 99.9% confidence level for all characteristics. The Duncan test was applied to the characteristics that had significant differences as a result of the analysis. Duncan test results are given in Table 5.

Table 5. Duncan Test Results for Anatomical Characters

Altitude Zone	L	W	EC	RC	MR	RR
0 to 200 m	0.868b	0.0034a	67.233a	16.383b	53.511b	0.531b
200 to 400 m	0.806a	0.0028b	70.213b	14.893a	46.353a	0.472a
400 to 600 m	0.834ab	0.0030b	72.412b	13.793a	46.372a	0.421a

Examining Table 5 according to Duncan test results for anatomical characteristics, it was observed that chestnut trees, which were found at an altitude of 0 to 200 m, formed a class by themselves in terms of fibre wall thickness character, and that no difference was observed between the altitude zones in terms of the rigidity coefficient character. Different classes were formed in individuals at an altitude of 0 to 200 m in terms of Muhlstep ratio (MR) and Runkel ratio (RR) values.

Correlation analysis is a statistical analysis that reveals the size, direction, and significance of the relationship between two variables. The relationship that tried to be measured in correlation analysis is related to the linear part of the relationship between the variables. The correlation coefficient calculated as a result of correlation analysis is denoted with r, and it can take values between –1 and +1. The coefficient being close to + 1 indicates that there is a good relationship between the two variables, and if it is close to -1, it means that there is still a good but an opposite relationship where one of the variables increases when the other one decreases.

As a result of the study, a correlation analysis was applied to the data with the purpose of determining the relationship levels between the altitude zones and the measured anatomical characters. Results are given in Table 6.

Table 6. Results of Correlation Analysis of Anatomical Characteristics

Measured Characteristics	Altitude	*L* (mm)	*D* (µm)	*d* (µm)	*W* (µm)	FR	EC	RC	MR	RR	"F" Factor
***L* (mm)**	-0.05	1	-0.272**	-0.282**	-0.022	0.767**	-.1960**	0.196**	0.200**	0.178**	0.337**
***D* (µm)**				0.972**	0.340**	-0.593**	0.290**	-0.290**	-0.307**	-0.240**	-0.252**
***d* (µm)**					0.109*	-0.552**	0.482**	-0.482**	-0.500**	-0.413**	-0.096*
***W* (µm)**						-0.300**	-0.703**	0.703**	0.705**	0.637**	-0.680**
FR							-0.252**	0.252**	0.250**	0.253**	0.452**
EC								-1.000**	-0.992**	-0.944**	0.520**
RC									0.992**	0.944**	-0.520**
MR										0.898**	-0.555**
RR											-0.410**

** $P < 0.01$

When Table 6 is examined, it is seen that the characteristics had a statistically significant, strong, and positive relationship with each other. For instance, the relationship between elasticity coefficient and rigidity coefficient was strong, but in negative directions. A positive relationship with a quite high effect level was observed between rigidity coefficient and MR. Additionally, a strong but negative effect was observed between MR and elasticity coefficient.

In this study, the changes in morphological, micro-morphological, and anatomical characteristics of the Anatolian chestnut, which naturally spread in three different altitude zones, were evaluated based on these different altitude zones and the results of the analyses conducted were given in the findings and discussion section. According to the results obtained, it was found that altitude did not affect LW. However, it was determined that altitude affected (at least at the 99.9% confidence level) other studied characteristics of PL, LBL, LL, TW, TL, ABP, and ABV. In terms of the distance between lateral veins (DBLV) characteristic, difference was found at 95% confidence level.

When the stomata in the leaf samples collected from 3 different points in 3 different altitude zones (0 to 200 m, 200 to 400 m, and 400 to 600 m) were examined, it was found that the stomata were of hypostomatic type because the stomata were present only on the abaxial surface of the leaves.

It was found that there were statistically significant differences between fibre length, fibre wall thickness, elasticity coefficient, rigidity coefficient, MR, and RR, and that difference was significant at the 99.9% confidence level for all characteristics. These values formed a single class at 0 to 200 m altitude zones, whereas fibre length formed a single class in individuals found in the 400 to 600 m altitude zone. Sampling error can be shown as the reason for this.

The previous studies showed that the size and density of the stomata varied depending on the species, varieties, and different growing conditions of plants (Misirli and Aksoy 1994; Caglar *et al*. 2004; ILWin and Caglar 2009). Plants grown in different environments undergo some anatomical and morphological changes (Mert *et al*. 2009). Some intrinsic and extrinsic factors affect the density and movements of the stomata, sometimes alone and sometimes together. These factors include air and soil moisture, carbon dioxide (CO_2), water content of the plant, wind, temperature, light intensity, cultural applications, growth hormones, and enzymes (Sahin 1989; Gokbayrak *et al*. 2008).

Caglar *et al*. (2004) observed a positive relationship between the elevation of the places, where walnut species grew, from sea level and stoma densities. As a result of this study, it was found that the number of stomata increased as the elevation from sea level increased. Baas and Schweingruber (1987), Carlquist and Hoekman (1985) stated that multiple perforation plates were rare in tropical forests with low altitude, while they were common in very cold regions and tropical high mountain flora.

In fact, altitude has an effect on all environmental conditions (Varol *et al.* 2022; Isinkaralar *et al.* 2024). In general, as the altitude increases, the air temperature decreases (Cetin *et al.* 2023). Vegetation period shortens. Therefore, climate parameters change. Climate parameters affect all anatomical and morphological characteristics of plants (Ozel *et al*. 2021; Çobanoğlu *et al.* 2023). This is because all phenotypic characteristics of living organisms are shaped depending on the interaction between their genetic structures (Hrivnak *et al.* 2023; Kurz *et al.* 2023) and environmental conditions (Aricak *et al.* 2019; Kuzmina *et al.* 2023; Tandoğan *et al.* 2023). Among the environmental conditions, climatic factors such as temperature and precipitation are the parameters that affect plant growth the most (Ertugrul *et al.* 2021; Sevik *et al.* 2021). Since the change in elevation directly affects these parameters, its effect on plant growth is important.

CONCLUSIONS

1. Differences in environmental factors were not found to greatly affect stoma density and size, indicating that these anatomical features are more genetic in nature. Nevertheless, it is considered useful to investigate the agronomic and physiological features as well as anatomical features in adaptation studies on different varieties under different conditions and in research on the subject of plant densities, fertilization, and irrigation.
2. Seed orchards are high-cost facilities that ensure continuously and abundantly high-quality seed production. Regarding the establishment of seed orchards with the purpose of growing chestnuts efficiently and abundantly and provision of genetic diversity, it will be possible to ensure the efficiency of seed orchards to be established with graft items to be obtained from the 2nd (200 to 400 m) and 3rd (400 to 600 m) altitude zones.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

DECLARATION

Not applicable

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