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Determination of Leaf and Stomata Characteristics of Some Late Blooming Almond (*Amygladus communis* L.) Cultivars and Their Hybrids Grown in Semi-arid Climate Conditions

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Abstract: This study was carried out on some domestic and foreign late blooming varieties and their genotypes obtained as a result of hybridization in the almond collection parcel of Harran University Faculty of Agriculture in Sanliurfa/Turkiye in the summer period of 2020. In the research, 5 different cultivars and 6 hybrids were examined in the orchard. In the study, 198 leaves were taken from 3 trees of each variety and 9 of each tree from 2 directions. It was planned and carried out according to the split plots experimental design in random blocks. North and South directions were taken into account when taking leaf samples. According to the findings, when the leaf characteristics were examined in general, the highest values in terms of leaf width, leaf length, petiole length and leaf area were found in 'Genotype-7' hybrids among cultivars and hybrids. As a result of stoma analysis on the lower surface of the leaves, the highest overall average (196.47 units mm^{-2}) was determined in Genotype-3, while the lowest (127.10 units/mm^2) was found in 'Ferragnes' variety. As a result of the analysis, when all the average values were taken into account, it was determined that the leaf area was 16.74 cm^2, the average stomata density was 153.51/mm^2 and the leaf area was 256975.74/leaf stomata. It is thought that the study will be descriptive for almond varieties and hybrids grown in the same ecology. This study was carried out in order to determine the effects of the adaptation of the almond varieties that are intensively grown in the Southeastern Anatolia Region and that bloom late, and some hybrids that are likely to be grown in the region in the future, on the morphological characteristics.

Keywords: Almond, genotype, leaf, leaf area, stomata density

Yarı Kurak İklim Koşullarında Yetiştirilen Bazı Geç Çiçek Açan Badem (*Amygladus communis* L.) Çeşitleri ve Hibritlerinin Yaprak ve Stoma Özelliklerinin Belirlenmesi

Öz: Bu çalışma, Şanlıurfa/Türkiye'de Harran Üniversitesi Ziraat Fakültesi badem toplama parselinde melezleme sonucu elde edilen bazı yerli ve yabancı geç çiçek açan çeşitler ve bunların genotipleri üzerinde 2020 yılı yaz döneminde yapılmıştır. Bahçede çeşit ve 6 melez incelenmiştir. Çalışmada her çeşitten 3 ağaç ve her ağacın 2 yönünden 9'ar olmak üzere 198 yaprak alınmıştır. Yaprak örnekleri alınırken kuzey ve güney yönleri dikkate alınmıştır. Deneme tesadüf bloklarında bölünmüş parseller deneme desenine göre planlanmış ve yürütülmüştür. Elde edilen bulgulara gore yaprak özellikleri genel olarak incelendiğinde, çeşitler ve melezler arasında yaprak genişliği, yaprak uzunluğu, yaprak sapı uzunluğu ve yaprak alanı açısından en yüksek değerler 'Tip-7' melezlerinde bulunmuştur. Yaprakların alt yüzeyinde yapılan stoma analizi sonucunda en yüksek toplam ortalama (196.47 adetmm^{-2}) Tip-3'te, en düşük (127.10 adet/mm^2) ise 'Ferragnes' çeşidinde bulunmuştur. Analiz sonucunda tüm ortalama değerler dikkate alındığında yaprak alanı 16.74 cm^2, ortalama stoma yoğunluğu 153.51/mm^2 ve yaprak alanı 256975.74/yaprak stoma olarak belirlenmiştir. Çalışmanın aynı ekolojide yetiştirilen badem çeşitleri ve melezleri için tanımlayıcı olacağı düşünülmektedir. Güneydoğu Anadolu Bölgesinde yoğun olarak yetiştirilen ve geç çiçek açan badem çeşitleri ile bölgede gelecekte yetiştirilmesi olası bazı hibritlerinin bölgeye adaptasyonunun morfolojik özelliklere etkisini belirlemek amacıyla bu çalışma yürütülmüştür.

Anahtar Kelimeler: Badem, genotip, stoma yoğunluğu, yaprak, yaprak alanı

1. Introduction

Almond is a hard-shelled fruit variety belonging to the *Prunus* L. genus of the *Rosaceae* family. It was named *Amygdalus communis* L. by Linnaeus in 1753. Its homeland is Central and Western Asia. Almond has spread from this region to China, Iran, India, Syria and Mediterranean countries. Almond is a temperate climate fruit known for its ability to tolerate heat and drought. The ecological determinant of this fruit species is that it blooms early with minimal chilling requirement (Ak et al., 2002; Martinez-Gomez et al., 2003; Kuden et al., 2014; Ak et al., 2020).

Stomata play an important role in gas exchange and transpiration and in the establishment of the physiological balance in the plant. Stomata is important element that can reduce significant decreases in water potential, prevent embolism, and provide adaptation. It is important to determine the stomata parameters of plants grown in the same ecology (Kocacaliskan, 2008; Dickison, 2000; Escalona, 2013; Giday et al., 2013; Hopper et al., 2014; Boso et al., 2016; Sevik et al., 2017; Odabasioglu & Gursoz, 2019; Brodribb et al., 2020; McAdam et al., 2021; Lin et al, 2021).

According to the publications, the amount of stomata in the leaves varies according to the genotype, the shape of the leaves on the shoot and the location of the sample taken from the leaf (Rosselli, et al., 1989), the amount of stomata in the varieties with low sensitivity to cold is higher than the varieties with high sensitivity to cold, the amount of stomata in two different planes of the leaves (Çağlar & Tekin, 1999), there is a direct proportionality between the leaf size and the amount of stomata in the leaf (Demirkaya, 1999), small stoma sizes provide adaptation (Pinto et al., 2004) has been reported. Recent studies of trees suggest that the stomata regulate transpiration in a manner that optimizes the capacitive discharge of water from stem tissue, while at the same time avoiding excessive embolism (Ahmad & Prasad, 2012).

The aim of this study is to determine some leaf and stomata characteristics of some domestic and foreign late blooming cultivars and their genotypes. Thus, genotypes and their relationships with parents in terms of leaves and stomata were revealed. In this direction, it is aimed to determine the response of almond, which is an important fruit genotype, to leaf and stomata parameters in semi-arid conditions, depending on the varieties, with different statistical methods.

2. Materials and Methods

2.1. Materials

This study was carried out in Harran Plain, where is one of the main target sites of the Southeastern Anatolia Project (GAP) carried out in Southeast Turkey. Sanliurfa province is located in the Southeastern Anatolia Region of Turkey, on the Syrian border. The orchard from which the almond varieties were obtained had an elevation of 350 meters and located at 36° 88 latitude and 38° 92 '' longitude. Climate data from 1929 to 2021 are as follows. Highest temperature 46.8 ^{0}C (July); the lowest temperature was measured as -12.4^{0}C (February). The annual average precipitation in Sanliurfa is calculated as 462 mm. Annual average temperature is 18.6 ^{0}C, evaporation is 2048 mm, wind speed is 2.8 m/sec (Anonymous, 2022).

In this study, genotypes obtained by crossing 'Ferragnes', 'Lauranne', 'Gulcan-1' and 'Gulcan-2' with foreign origin varieties were used. Hybrid individuals were produced within the scope of TUBITAK TOVAG projects 108O388 and 113O963 (Acar et al. 2013, Acar et al. 2014). Six hybrid genotypes were used from some hybrid plants obtained from domestic and foreign almond varieties in this study area. These are: 'Genotype-3' ('Lauranne' x 'Nurlu'), 'Genotype-4' ('Gulcan-2' x 'Lauranne'), 'Genotype-6' ('Gulcan-1' x 'Guara'), 'Genotype-7' ('Gulcan-2' x 'Guara'), 'Genotype-8' ('Gulcan-2' x 'Moncayo'), 'Genotype-14' ('Gulcan-2''Penta').These cultivars and genotypes that aged 5 years were grafted onto 'GF-677' rootstock. Plants are irrigated by drip irrigation method according to normal needs.

2.2. Methods

In the study, a total of 198 leaves were taken, 3 trees from each variety and 9 from each tree from 2 directions. The north and south directions of the trees were taken into account. The upper and lower surfaces of the leaves were completely painted with transparent nail polish and left to dry. Tape was attached to the dried nail polish leaves and then removed and attached to the slides. Thus, stomata were examined under the microscope (Las Leica DM1000) with 10X (objective)-10X (ocular) magnification (field of view 0.319 mm^2) (Bekisli, 2014).

Leaf aspect ratio was determined by dividing the leaf width value by the leaf length value. A total of 18 leaves, selected from 2 different directions of the selected cultivars and hybrids, were collected and the leaf lengths were measured with a ruler without including the petiole in the laboratory (Oraguzie et al., 1998; Kotobuki, 1996).Leaves were scanned to a computer and the Image-J software was used for leaf surface measurements (Kaya et al., 2003).

The number of stomata displayed in the 0.776 mm² field of view of the slide molds photographed under the microscope was determined by adapting it to the 1 mm² field (Kara & Ozeker, 1999; Bekisli, 2014; Dikmetas, 2017).The length and width of 10 stomata in the photographs of the stoma patterns were measured in the MShot-1.3.10 computer program and measured in µm (Bekisli, 2014).The stomata aspect ratio was obtained by dividing the stomata width by the stomata length. The wet weights of the leaf samples were determined, and the turgor weight was found by keeping them in petri dishes containing 100 mL of water for 24 hours. Afterwards, the samples were dried in an oven at 65-

70ºC and their dry weights were determined. Sanchez et al. (2004) determined the RLWC.

The data obtained from the experiment were analyzed by using the Minitab statistical software, and the differences between the cultivars were determined by the LSD (P≤0.05).Principal component analysis (PCA) and clustering analysis (Dendogram) were performed using the PAST 4.03 software.

3. Results and Discussion

3.1. Analysis of leaf parameters

The findings of some leaf characteristics of 11 different almond cultivars used as material in this study are given in Tables 1, 2, 3,4,5 and 6.

The highest length value (11.83 cm) in the leaves in the north direction was found in Genotype-7, while the lowest average was determined as 8.41 cm in Genotype-3. Other genotypes and cultivars ranged between these two values. When the averages of the leaves in the south direction were examined, the highest value was found in Genotype-7 with 12.72 cm, while the lowest value was determined in Gulcan-2 variety (Table 1). The difference between the means was found to be statistically significant.

Table 1. Leaf lengths of different almond varieties and genotypes (cm)

Çizelge 1.*Farklı badem çeşitlerinin yaprak uzunlukları (cm)*

Varieties and Genotypes	Leaflengths (cm) North (Value±Standard Deviation)	South (Value±Standard Deviation)	Average
FERRAGNES	11.20 ±1.236 ab	10.49 ±0.325 b-e	10.84
GUARA	10.64 ±1.484b-e	11.00 ±0.618bc	10.82
GULCAN-1	9.03 ±1.777gh	11.27 ± 0.700 b	10.15
GULCAN-2	8.70 ±1.705gh	8.76 ±1.046h	8.73
LAURANNE	9.70 ±1.192 d-g	9.74 ±1.053 e-g	9.72
GENOTYPE-3	8.41 ±0.321h	9.28 ±0.569f-h	8.84
GENOTYPE -4	9.46 ±0.958f-h	9.39 ±1.218f-h	9.42
GENOTYPE -6	10.41 ±1.109b-f	9.77 ±0.876ef	10.09
GENOTYPE -7	11.83 ±0.936a	12.72 ±1.005a	12.27
GENOTYPE -8	10.91 ±0.896a-d	9.49 ±0.566f-g	10.20
GENOTYPE -14	11.42 ±0.528 ab	10.71 ±0.520 b-d	11.06
Average	10.15	10.24	10.19
LSD*	1.137	0.813	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05).

The highest width value (3.41 cm) in the leaves in the north direction was found in Ferragnes, while the lowest value was determined as 2.49 cm in Genotype-3. Other genotypes and cultivars ranged between these two values. When the averages of the leaves in the south direction were examined, the highest value was found in Ferragnes with 3.63 cm, while the lowest value was determined in the Genotype-8 (Table 2).

Table 2. Leaf widths of different almond varieties and genotypes (cm)

Çizelge2.*Farklı badem çeşitlerinin yaprak genişlikleri (cm)*

Varieties and Genotypes	Leafwidth (cm) North (Value±Standard Deviation)	South (Value±Standard Deviation)	Average
FERRAGNES	3.41 ± 0.337 a	3.63 ± 0.239 a	3.52
GUARA	3.08 ± 0.097 a-d	3.50 ± 0.304 ab	3.29
GULCAN-1	2.11 ± 0.496 g	2.64 ± 0.245 d-f	2.37
GULCAN-2	2.58 ± 0.465 f	2.70 ± 0.415 d-f	2.64
LAURANNE	2.54 ± 0.200 f	2.97 ± 0.320 d	2.75
GENOTYPE-3	2.49 ± 0.214 f	2.73 ± 0.217 d-f	2.98
GENOTYPE -4	2.58 ± 0.286 f	2.61 ± 0.252 ef	2.61
GENOTYPE -6	2.59 ± 0.226 f	2.63 ± 0.234 d-f	2.59
GENOTYPE -7	3.03 ± 0.234 b-e	3.50 ± 0.180 ab	2.61
GENOTYPE -8	3.14 ± 0.409 ab	2.57 ± 0.234 f	3.26
GENOTYPE -14	3.12 ± 0.204 a-c	2.84 ± 0.142 de	2.85
Average	2.79	2.94	2.86
LSD*	0.346	0.345	-

*: Classification is made according to LSD 5%. The difference between the averages bearing different on the same column is statistically significant. (P <0.05)

The highest value in the leaf stem length in the northern direction was determined in Genotype-14. The lowest value was determined in Gulcan-1 cultivar. In the south direction, the highest value was observed in Genotype-7 with 2.34 cm, and the lowest value was observed in Genotype-3 with 1.3 cm (Table 3).

Table 3. Petiole lengths of different almond cultivars and genotypes (cm)

Çizelge 3.*Farklı badem çeşitleri ve çeşitlerinin yaprak sapı uzunlukları (cm)*

Varieties and Genotypes	Petiole lenghts (cm) North (Value ±Standard Deviation)	South (Value ±Standard Deviation)	Average
FERRAGNES	1.80 ± 0.346d	1.43 ± 0.229cd	1.62
GUARA	1.66 ± 0.328ef	1.56 ± 0.142cd	1.61
GULCAN-1	1.30 ± 0.415i	1.66 ± 0.371cd	1.48
GULCAN-2	1.57 ± 0.418h	1.36 ± 0.490 de	1.47
LAURANNE	1.67 ± 0.578ef	1.61 ± 0.464cd	1.64
GENOTYPE-3	1.34 ± 0.278h	1.30 ± 0.254e	1.32
GENOTYPE -4	1.69 ± 0.297e	1.59 ± 0.483cd	1.64
GENOTYPE -6	1.91 ± 0.395c	1.69 ± 0.333c	1.80
GENOTYPE -7	2.09 ± 0.407b	2.34 ± 0.312a	2.22
GENOTYPE -8	1.50 ± 0.269 g	1.60 ± 0.187cd	1.55
GENOTYPE -14	2.40 ± 0.259a	2.18 ± 0.253ab	2.29
Average	1.72	1.67	1.70
LSD*	0.033	0.325	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

The averages of the ratio of leaf width to leaf length were found to be statistically significant between cultivars and genotypes. The highest value (0.305 cm) in the leaves in the north direction was found in Ferragnes, while the lowest average was determined as 0.233 cm in Gulcan-1. Other genotypes and cultivars ranged between these two values. When the averages of the leaves in the south direction were examined, the highest value was found in Ferragnes with 0.346 cm, while the lowest value was determined in Gulcan-1 cultivar (Table 4).

Table 4. The leaf aspect ratio of different almond varieties and genotypes
Çizelge 4.*Farklı badem çeşitlerinin yaprak en/boy oranı*

Varieties and Genotypes	The leaf aspect ratio North (Value±Standard Deviation)	South (Value±Standard Deviation)	Average
FERRAGNES	0.305 a	0.346 a	0.325
GUARA	0.289 a-c	0.318 ab	0.306
GULCAN-1	0.233 h	0.235 i	0.234
GULCAN-2	0.296 ab	0.308 bc	0.302
LAURANNE	0.262 d-g	0.304 bc	0.283
GENOTYPE-3	0.296 ab	0.295 b-d	0.295
GENOTYPE -4	0.273 b-f	0.278 c-f	0.275
GENOTYPE -6	0.249 e-g	0.270 d-h	0.259
GENOTYPE -7	0.256 e-g	0.275 d-g	0.265
GENOTYPE -8	0.288 a-d	0.270 d-h	0.279
GENOTYPE -14	0.273 b-f	0.266 d-h	0.270
Average	0.275	0.288	0.281
LSD*	0.026	0.031	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

When the averages of the leaf area were examined, the highest value (25.43 cm²) was found in Genotype-7 leaves in the north direction, while the lowest average was determined as 9.50 cm² in Gulcan-1. Other genotypes and cultivars ranged between these two values. When the averages of leaves in the south direction are examined, the highest value was determined as 20.70 cm² in Genotype-7, while the lowest value was determined in Genotype-4 hybrids (Table 5).

Table 5. Leaf area of different almond varieties and genotypes (cm²)
Çizelge 5.*Farklı badem çeşitlerinin yaprak alanları (cm²)*

Varieties and Genotypes	Leaf area (cm²) North (Value±Standard Deviation)	South (Value±Standard Deviation)	Average
FERRAGNES	20,60 ± 3,197bc	20,27 ± 2,467ab	20,33
GUARA	20,17 ± 2,709b-d	19,33 ± 3,37a-c	19,75
GULCAN-1	9,51 ± 3,701j	14,95 ± 3,437e-g	12,23
GULCAN-2	11,05 ± 4,231ij	13,93 ± 4,888e-g	12,49
LAURANNE	17,11 ± 3,029d-g	15,75 ± 4,113d-f	16,43
GENOTYPE-3	16,49 ± 2,566e-h	14,26 ± 2,178e-g	15,37
GENOTYPE -4	13,39 ± 2,083hi	11,46 ± 1,507g	12,42
GENOTYPE -6	16,17 ± 2,887e-h	14,26 ± 1,994e-g	15,22
GENOTYPE -7	25,43 ± 2,918a	20,70 ± 2,174a	23,07
GENOTYPE -8	17,79 ± 3,064cd	13,37 ± 1,999fg	15,58
GENOTYPE -14	23,14 ± 1,481ab	19,28 ± 2,955a-d	21,21
Average	17,34	16,14	16,74
LSD*	3,377	3,533	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

When the averages of there lative water content of the leaves were examined, the highest value (75.21%) was found in the leaves in the north direction, while the lowest average was found in Genotype-14 as 41.42%. Other genotypes and cultivars ranged between these two values. When the averages of leaves in the south direction were examined, the highest value was found in Genotype-8 with 81.88%, while the lowest value was determined in Genotype-14 hybrids (Table 6).

Table 6. Relative water content of leaves (%)
Çizelge 6.*Yaprakların oransal su içeriği (%)*

Varieties and Genotypes	Relative water content (%) North (Value±Standard Deviation)	South (Value±Standard Deviation)	Average
FERRAGNES	66,59 ± 14,546 a	78,00 ± 6,944 ab	72,29
GUARA	72,10 ± 15,752 a	72,83 ± 12,521 a-d	72,46
GULCAN-1	64,43 ± 21,282 a	61,11 ± 18,870 f	62,77
GULCAN-2	75,21 ± 16,280 a	66,12 ± 19,786 b-f	70,66
LAURANNE	67,59 ± 12,758 a	65,63 ± 16,651 b-f	66,61
GENOTYPE-3	63,08 ± 14,074 a	62,22 ± 13,611 ef	62,65
GENOTYPE -4	75,15 ± 10,028 a	67,90 ± 17,730 a-f	71,52
GENOTYPE -6	69,90 ± 16,898 a	72,61 ± 13,137 a-e	71,25
GENOTYPE -7	75,02 ± 9,011 a	77,85 ± 14,330 a-d	76,43
GENOTYPE -8	70,93 ± 15,267 a	81,88 ± 11,131 a	76,40
GENOTYPE -14	41,42 ± 12,211 b	49,60 ± 14,863 g	45,51
Average	67,40	68,70	68,05
LSD*	16,998	15,590	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

3.2. Analysis of stomata parameters

Stoma parameters are given in Tables 7,8,9 and 10. The highest amount of stomata at the tip of the leaves taken from the north was observed in Laurenne variety with 199, while the lowest value was determined in Genotype-4 with 109 units. The highest amount of stomata of the leaf taken from the middle was again in Lauranne variety, while the lowest number of stomata was seen in Ferragnes variety. In the leaves taken from the bottom part, the highest stomata amount was seen in Genotype-3, while the lowest stomata number was determined in Genotype-14 (Table 7).

While the highest number of stomata was seen in Laurenne variety with 216 pieces, the lowest value was determined in Guara variety with 106 pieces. In the leaves in the middle parts, the highest value was determined in Genotype-3, 213, and the lowest value was observed in the Ferragnes variety with 83. In the leaves taken from the bottom of the shoot, the highest value was seen in Genotype-3, while the lowest value was observed in Guara variety. These; leaf length (cm), petiole (pedicel) length (cm), leaf width (cm), leaf area, leaf width/leaf length ratio, leaf fresh weight, leaf dry weight, leaf relative moisture content, etc. features were examined (Table 7).

The highest value (34.46 µm) of stomata lengths at the tip of the leaves in the northern direction was found in Genotype-3, while the lowest average was determined as 26.7 µm in Genotype-14. Other genotypes and cultivars ranged between these two values. In cases of stomata width in the same direction, the highest value was found to be 22.26 µm in Genotype-6. The lowest value was found to be 12.47 µm in Genotype-14. Other cultivars and genotypes ranged between these two values (Table 8).

In the northern direction, the highest value (35.85

μm) of stomata lengths at the base of the existing leaves was found in Genotype-3, while the lowest average was determined as 28.08 μm in Genotype-14. Other cultivars and genotypes ranged between these two values. The highest stoma of the leaves in the northern direction was found at 22.65 μm Guara. The lowest value was determined in Genotype-14 with 14.06 μm (Table 8).

Table 7. Stomata density per unit area (pcs/mm^2)
Çizelge 7. *Birim alandaki stoma yoğunluğu (adet/ mm^2)*

Varieties and Genotypes	Stomata density per unit area(pcs/mm^2)								AVR.
	North (Value±Standard Deviation)				South (Value±Standard Deviation)				
	T**	M	B	Average	T	M	B	Average	
FERRAGNES	176,49	99,90	116,55	130,98 ± 40,282c-f	166,50	119,88	83,25	123,21 ± 41,724ef	127,10
GUARA	176,49	156,51	133,20	155,40 ±21,666a-d	106,56	123,21	93,24	107,67 ± 15,015 f	131,54
GULCAN-1	153,18	123,21	139,86	138,75 ± 15,015b-f	183,15	166,50	123,21	157,62 ± 30,94a-e	148,19
GULCAN-2	116,55	133,20	116,55	122,10 ± 9,612d-f	169,83	133,20	139,86	147,63 ± 19,512b-f	134,87
LAURANNE	199,80	189,81	143,19	177,60 ± 30,215ab	216,45	163,17	149,85	176,49 ± 35,241a-d	177,05
GENOTYPE-3	199,80	159,84	213,12	190,92 ± 27,727 a	193,14	199,80	213,12	202,02 a ± 10,173	196,47
GENOTYPE -4	109,89	149,85	129,87	129,87 ± 19,979 c-f	209,79	166,50	156,51	177,60 ± 28,321a-c	153,74
GENOTYPE -6	116,55	133,20	123,21	124,32 ± 8,380 d-f	139,86	123,21	163,17	142,08 ± 20,072b-f	133,20
GENOTYPE -7	153,18	173,16	169,83	165,39 ± 10,704a-c	159,84	149,85	99,90	136,53 ± 32,113b-f	150,96
GENOTYPE -8	189,81	136,53	133,20	153,18 ± 31,766 b-e	216,45	176,49	149,85	180,93 ± 33,521ab	167,06
GENOTYPE -14	109,89	116,55	93,24	106,56 ± 12,006f	116,55	83,25	139,86	180,93 ± 28,451ab	167,06
Average	176,49	142,86	137,53	152,290	180,82	145,85	137,53	113,220	153,51
LSD*	-	-	-	39,007	-	-	-	47,980	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05) **T: Tip of leaf M: Middle of leaf B: Bottom of leaf

Table 8. Stomata length (μm)
Çizelge 8. *Stoma boyu (μm)*

Varieties and Genotypes	Stomata lenght (μm)								AVR.
	North (Value±Standard Deviation)				South (Value±Standard Deviation)				
	T	M	B	AVR.	T	M	B	AVR.	
FERRAGNES	33,60± 1,541 a-c	31,76 ± 1,884 c-g	32,38 ± 4,958 a-g	32,58	33,60 ± 2,184 b	34,74 ± 2,327 ab	34,57 ± 2,682 b-d	34,30	33,44
GUARA	30,36± 4,164 d-g	30,92± 4,656 c-g	34,39 ± 4,173 a-d	31,89	27,85 ± 4,122 fg	32,99 ± 7,03 a-f	33,97 ± 2,409 b-d	31,60	31,74
GULCAN-1	26,72 ± 3,874 h	30,03 ± 2,959 e-g	35,5 ± 1,006 ab	30,75	27,45 ± 2,245 fg	29,25 ± 4,709 e-j	38,05 ± 3,007 a	31,58	31,16
GULCAN-2	32,03 ± 3,995 a-e	36,72 ± 1,663 a	35,85 ± 1,434 a	34,87	19,28 ± 3,311 h	34,81 ± 3,573 a	33,82 ± 2,524 bc	29,30	32,08
LAURANNE	30,29 ± 3,133 d-g	32,26 ± 3,701 c-f	31,30 ± 3,065 c-g	31,28	27,67 ± 4,239 fg	32,70 ± 3,702 a-h	34,56 ± 2,847 b-d	31,64	31,46
GENOTYPE-3	34,46 ± 2,803 a	33,59 ± 3,123 a-d	29,70 ± 1,877 gh	32,58	33,30 ± 3,311 b-d	32,34 ± 3,037 a-d	32,56 ± 1,862 cd	32,73	32,65
GENOTYPE -4	29,84 ± 2,658 e-h	28,88 ± 3,114 g	34,80 ± 3,712 a-c	31,17	28,88 ± 2,341 f	25,31 ± 3,277 k	35,32 ± 4,933 a-d	29,84	30,50
GENOTYPE -6	34,39 ± 3,078 ab	35,86 ± 1,872 ab	30,96 ± 2,871d-h	33,74	36,84 ± 3,814 a	33,17 ± 2,859 a-e	35,97 ± 1,983 a-c	35,33	34,53
GENOTYPE -7	31,36 ± 2,788 c-f	33,78 ± 3,244 a-c	33,53 ± 4,551 a-f	32,89	32,63 ± 3,411 b-e	34,29 ± 2,428 a-c	33,86 ± 6,94 b-d	33,59	33,24
GENOTYPE -8	32,70 ± 1,88a-d	33,19 ± 2,983 b-e	33,61 ± 1,521 a-e	33,17	33,53 ± 2,371 b-d	32,50 ± 2,65 a-i	37,11 ± 2,335 ab	34,38	33,77
GENOTYPE -14	26,70 ± 4,631 h	32,65 ± 3,618 b-f	28,08 ± 4,529 h	29,14	27,62 ± 2,139 fg	33,84 ± 1,645 a-d	32,37 ± 1,575 d	31,28	30,21
Average	31,13	32,69	32,74	32,19	28,97	32,36	34,74	32,02	32,10
LSD*									

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

Table 9. Stomata width (μm)
Çizelge 9. *Stoma eni (μm)*

Varieties and Genotypes	Stomata width (μm)								AVR.
	North (Value±Standard Deviation)				North (Value±Standard Deviation)				
	T	M	B	Avr.	T	M	B	Avr.	
FERRAGNES	19,03 ± 3,697 b-e	17,23 ± 1,126 d-h	18,92 ± 4,799 b-e	18,39	33,60 ± 1,761 b	19,09 ± 1,454 a-e	18,61 ± 2,421 c-g	23,77	21,08
GUARA	17,36 ± 3,129 c-g	19,05 ± 2,385 cd	22,65 ± 2,566 a	19,69	27,84 ± 4,38 f	18,47 ± 3,535 a-f	20,29 ± 1,609 b-e	22,20	20,94
GULCAN-1	14,59 ± 2,694 hi	14,83 ± 1,751 i	18,64 ± 2,969 b-g	16,02	27,44 ± 2,648 f	16,91 ± 3,968 b-i	25,36 ± 1,356 a	23,24	19,63
GULCAN-2	19,12 ± 2,424 b-d	22,76 ± 2,533 ab	18,34 ± 1,575 b-h	20,07	29,27 ± 2,379 f	21,07 ± 2,525 a	20,09 ± 1,849 b-e	23,48	21,77
LAURANNE	18,02 ± 2,602 c-f	18,11 ± 3,782 c-f	17,25 ± 2,185 c-i	17,79	27,67 ± 2,726 f	19,12 ± 3,288 a-d	20,71 ± 3,778 bc	22,50	20,14
GENOTYPE-3	12,47 ± 1,828 i	15,15 ± 2,135 hi	14,06 ± 1,723 k	18,91	27,62 ± 2,379 f	14,17 ± 2,133 hi	17,43 ± 1,533 g	23,41	21,16
GENOTYPE -4	20,68 ± 2,038ab	18,86 ± 3,107 c-e	17,20 ± 2,773 c-j	17,45	33,30 ± 2,137 b-d	18,42 ± 2,551 a-g	18,51 ± 2,714 c-g	20,77	19,11
GENOTYPE -6	16,64 ± 2,445 f-h	16,20 ± 1,591 f-i	19,50 ± 1,647 bc	21,45	28,88 ± 2,934 f	13,88 ± 2,227 hj	19,56 ± 1,855 c-g	26,33	23,89
GENOTYPE -7	22,26 ± 2,284 a	22,85 ± 1,701 a	19,24 ± 2,832 b-d	19,68	36,83 ± 2,222 a	20,12 ± 1,387 ab	22,03 ± 3,613 b	23,95	21,81
GENOTYPE -8	19,12 ± 6,088 b-d	19,93 ± 1,325 c	20,00 ± 2,308b	18,59	32,63 ± 1,035 b-e	19,84 ± 1,559 a-c	19,38 ± 1,825 c-g	24,18	21,38
GENOTYPE -14	19,20 ± 1,396 bc	17,8 ± 1,951 c-g	18,77 ± 2,648 b-f	13,89	33,53 ± 1,722 bc	16,98 ± 0,796 a-h	22,03 ± 2,703 b	19,74	16,81
Average	18,04	18,43	18,60	18,36	30,78	18,01	20,36	23,05	20,70
LSD(%5)*	2,244	2,605	2,331	-	2,285	3,588	2,214	-	

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

While the highest value (36.84 μm) of stomata lengths at the tip of the leaves in the south direction was found in Genotype-6, the lowest average was determined as 9.28 μm in Gulcan-2. Other genotypes and cultivars ranged between these two values. In cases of stomata width in the same direction, the highest value was found in Genotype-6, 36.83 μm. The lowest value was found to be 27.62 μm in Genotype-14. Other cultivars and genotypes ranged between these two values (Table 8).

Table 10. Stomata aspect ratio
Çizelge 10. *Stoma en/boy oranı*

Varieties and Genotypes	Stomata aspect ratio								
	North				South				AVR.
	T	M	B	Avr.	T	M	B	Ort.	
FERRAGNES	0,60 a	0,56 b	0,57 c	0,58	0,60 a	0,69 a	0,63 a	0,64	0,61
GUARA	0,57 b	0,55 b	0,89 a	0,67	0,54 b	0,56 b	0,53 b	0,54	0,61
GULCAN-1	0,49 c	0,52 c	0,52 c	0,51	0,59 a	0,57 b	0,66 a	0,61	0,56
GULCAN-2	0,59 a	0,62 a	0,51 d	0,57	0,63 a	0,58 b	0,59 b	0,60	0,59
LAURANNE	0,54 b	0,62 a	0,46 e	0,54	0,53 b	0,39 c	0,49 c	0,47	0,50
GENOTYPE-3	0,60 a	0,56 b	0,57 c	0,58	0,62 a	0,57 b	0,56 b	0,58	0,58
GENOTYPE -4	0,62 a	0,56 b	0,55 c	0,58	0,59 a	0,54 b	0,55 b	0,56	0,57
GENOTYPE -6	0,64 a	0,63 a	0,62 b	0,63	0,60 a	0,60 b	0,61 a	0,60	0,62
GENOTYPE -7	0,60 a	0,57 b	0,57 c	0,58	0,61 a	0,59 b	0,60 b	0,59	0,59
GENOTYPE -8	0,63 a	0,56 b	0,64 a	0,61	0,68 a	0,58 b	0,62 a	0,62	0,62
GENOTYPE -14	0,49 c	0,42 c	0,44 e	0,45	0,46 b	0,43 c	0,53 b	0,47	0,46
Average	0,58	3,77	0,58	1,64	0,59	0,55	0,58	0,57	1,11
LSD(%5)*	0,071	0,054	0,081	-	0,109	0,083	0,064	-	-

*: The difference between the averages with different letters on the same column is statistically significant. (P <0.05)

While the highest value (38.05 µm) of stomata lengths at the bottom of the leaves in the south direction was found in Gulcan-1, the lowest average was determined as 32.37 µm in Genotype-14. Again, in cases of stomata width in the same direction, the highest value was 25.36 µm in Gulcan 1. The lowest value was found to be 17.42 µm in Genotype-14. Other cultivars and genotypes ranged between these two values (Table 8). It was determined that the difference between the averages of the leaves taken from both the north and south directions in terms of stoma length and width was statistically significant.

The highest value in stomata width was detected in Genotype 6 (22.86 µm), and the lowest value was detected in Gulcan-1 variety with 14.83 µm. Other cultivars and genotypes ranged between these two values (Table 9).

The highest value (0.647 µm) of stomata aspect ratios at the tip of the leaves in the north direction was found in Genotype-6, while the lowest value was 0.495 µm in Genotype-14. In the stomata width/length conditions in the middle part in the same direction, the highest value was found in Genotype-6 as 0.635 µm. The lowest value was found to be 0.425 µm in Genotype-14. According to the width/length data at the bottom of the same direction, the highest value was found in Guara with 0.893 µm, while the smallest value was found in Lauranne with 0.491 µm. Other cultivars and genotypes ranged between these two values (Table 10).

The highest value for the stomata aspect ratio (0.684 µm) at the tip of the leaves of the shoots taken from the south was found in Genotype-8, while the lowest average was determined as 0.464 µm in Genotype-14. Again, the highest value was found in Ferragnes at 0.696 µm in stoma width/length conditions in the middle part in the same direction. The lowest value was found to be 0.392 µm in Genotype-14. According to the stomata width/length data at the bottom of the leaves of the same direction, the highest value was found in Gulcan-1 with 0.667 µm, while the smallest value was found in Lauranne with 0.495 µm. Other cultivars and genotypes ranged between these two values (Table 10).

3.3. Correlation of leaf and stomata parameters

When the number of stomata in the leaf of the cultivars and genotypes was examined, the highest stomata value was determined as 354334,26 in Genotype-14. The lowest stoma value was determined as 168452,63 in Gulcan-2 (Table 11).

Table 11. The number of stomata in the leaf of different almond cultivars and genotypes (pieces)
Çizelge 11.*Farklı badem çeşitleri ve çeşitlerinin yapraktaki stoma sayıları (adet)*

Varieties and Genotypes	Stomata Density in the Leaf		
	Leaf Area (mm^2)	Stomata Density (pieces/mm^2)	Total Stomata Density (pieces/leaf)
FERRAGNES	2033	127,10	258394,30
GUARA	1975	131,54	259791,50
GULCAN-1	1223	148,19	181236,37
GULCAN-2	1249	134,87	168452,63
LAURANNE	1643	177,05	290893,15
GENOTYPE-3	1537	196,47	301974,39
GENOTYPE -4	1242	153,74	190945,08
GENOTYPE -6	1522	133,20	202730,40
GENOTYPE -7	2307	150,96	348264,72
GENOTYPE -8	1558	167,06	260279,48
GENOTYPE -14	2121	167,06	354334,26
Average	1674	153,51	256975,74

As seen in Table 12, there is a significant positive correlation between leaf width and leaf area, genotypes and leaf length ($p \leq 0.01$). There is also a significant positive correlation between leaf area and leaf width ($p \leq 0.01$). There is a significant correlation between stoma width and stoma length ($p \leq 0.01$).In addition to stomata closure, which reduces transpiration rate per unit leaf area, branch reduction appears to be an effective mechanism that confers plants the ability to maintain water balance by reducing the total transpiration area of the canopy and hence total plant water losses (Rood et al., 2000; Fischer & Polle, 2010; Ahmad & Prasad, 2012).

Table 12. Pearson correlation between genotypes, leaf and stomata sizes, RWC and stomata density
Çizelge 12.*Genotipler, yaprak ve stoma boyutları, YOSK ve stoma yoğunluğu arasındaki Pearson korelasyonu*

Leaf and stomata features	Genotypes	Leaf length	Leaf width	Leaf area	RWC	Stomata density	Stomata lenght
Leaf length	0.393**						
Leaf width	0.530**	0.654**					
Leaf area	0.574**	0.697**	0.696**				
RWC	-0.098	0.091	0.153	-0.012			
Stomata density	-0.413**	-0.222*	-0.323**	-0.425**	0.132		
Stomata lenght	-0.062	-0.034	-0.003	0.049	0.209*	0.014.	
Stomata width	-0.200*	-0.121	-0.035	-0.037	0.382**	0.081	0.782**

*: p≤0.05, **: p≤0.01

3.4. Cluster and principal component analyses

In our study, a dendogram tree was formed as a result of 11 different parameters examined in 11 different almond variety, and as a result, 4 different groups were formed in the dendogram graph (Figure 1). According to the dendogram, 'Lauranne' and 'Genotype-3' are in the first group. The second group includes 'Guara', 'Genotype-8' and 'Ferragnes'. The third group includes 'Genotype-7' and 'Genotype-14'. 'Gulcan-1', 'Genotype-4', 'Genotype-6' and 'Gulcan-2' are more similar to each other than the aforementioned features.

As seen in Figure 2, the 11 almond varieties used in the study, which have similar characteristics, are located in the different place. The main logic of these groupings is shown in Figure 2. The arrows in the graph show the values of the different parameters examined, that is, these values increase as you move towards the arrow direction.

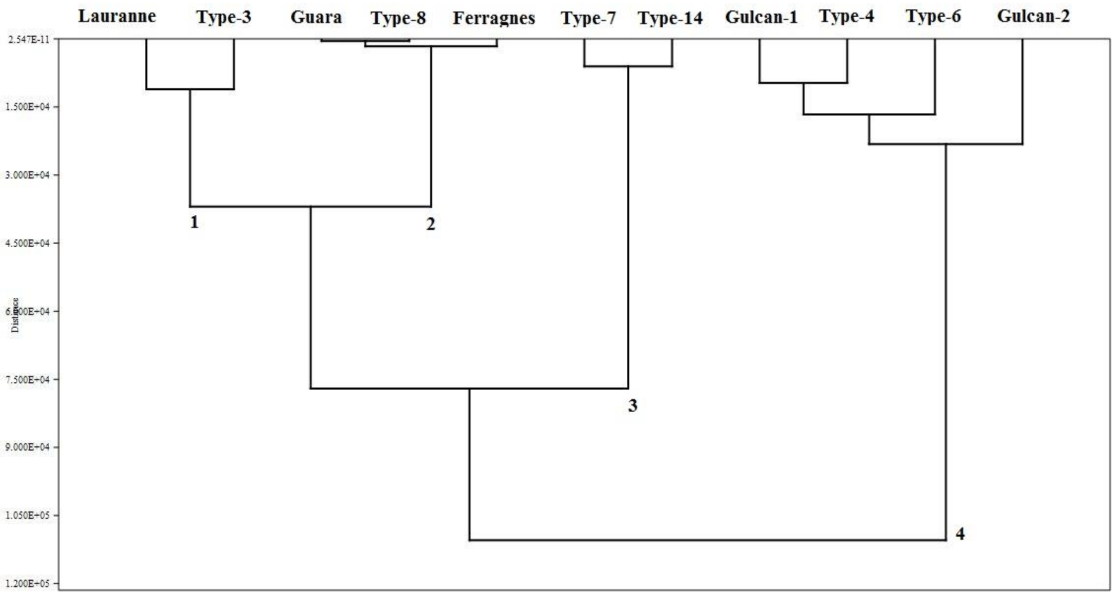

Figure 1. Comparative dendogram plot of some almond varieties
Şekil 1.*Bazı badem çeşitlerinin karşılaştırmalı dendogram grafiği*

Ordination of the 10 parameters given by PCA (Figure 2) indicates that the almond genotypes produced by PAST classification are markedly distinguishable and show a clear pattern of segregation on the ordination planes. The eigenvalues for the first two PCA axes are 0.175 and 0.590, respectively. The high eigenvalue for PCA axis 1 indicates that it explains the major variation in genotypes composition of almond groups. In this context, Figure 2 shows the clusters resulting from the classification of species and varieties. According to Figure 1 and Figure 2, it is revealed that the classification technique divides almond varieties into four different classes.

Under mild to moderate water deficits, stomata closure is one of the earliest plant responses, concomitant with the reduced water potential and turgor associated with even a small decrease in relative water content (Franco et al., 2006; Ahmad and Prasad, 2012). In this context, it is important that stoma measurements and physiological parameters to be made support each other. Changes in stomata morphology resulted in increased leaf water potential.

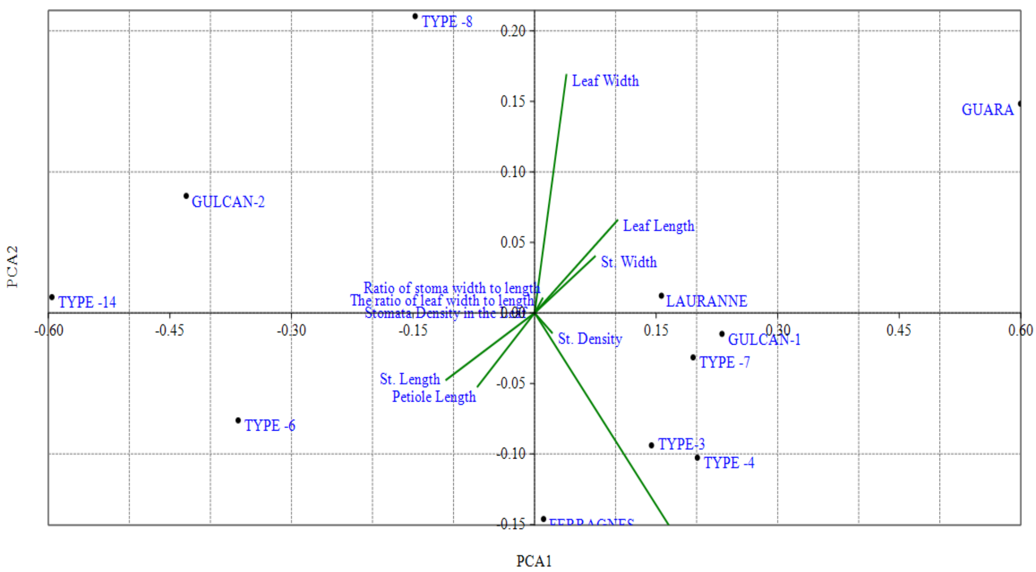

Figure 2. PCA plot of all measured variables for leaf parameters of some almond varieties
Şekil 2.*Bazı badem çeşitlerinin yaprak parametreleri için ölçülen tüm değişkenlerin PCA grafiği*

It was also found that a decrease in stomata conductivity was associated with an increase in leaf water potential in flooded bitter melon (Liao and Lin 1994).According to the PCA plot, 11 cultivars were found to be compatible with the relevant literature. It is recommended that the studies to be carried out in this direction should be supported by the aforementioned analyzes. On the other hand, Ashraf and Arfan (2005) did not find a significant relationship between stomata structure and water potential. However, according to Figure 2, there was a significant correlation between stomata density and RWC in 'Gulcan-1', 'Ferragnes', Genotype-3', 'Genotype-4' and 'Genotype-7' almond cultivars.

The study area has features reflecting the continental climate where semi-arid conditions are experienced. Sanliurfa has continental climate characteristics. Summers are very dry and hot, winters are rainy and relatively mild. The number of days with snow and frost is very few. For this reason, the difficulties of semi-arid climate are encountered in the cultivation of many fruit species. In this direction, it is important for a correct fruit growing to determine some differences of plant varieties and to determine the relationship between them. Photosynthesis rate, relative water content, leaf water content and stomata morphology of leaves change in regions where semi-arid conditions are experienced, such as in Sanliurfa. Ultimately, these conditions destabilize the membrane structure and permeability, protein structure and function, leading to cell death.

Almond is an important fruit species in regions with semi-arid climatic conditions. Leaf characteristics of different almond genotypes were found to be different due to the genotype effect and it was determined that stomata density could be affected by environmental conditions. A negative relationship between stoma frequency and stoma size was reported in the literature, and similar findings were obtained in the study. In Sanliurfa ecology, almonds see very high temperatures especially in June, July and August. For this reason, varieties or varieties/rootstock combinations that are resistant to heat and drought should be determined and grown accordingly. For this reason, in this study, newly hybridized cultivars were selected in the region and some leaves (leaf width, leaf length, petiole length, leaf area, leaf width-leaf length ratio) and stoma (number of stomata per unit leaf surface area) were selected under the conditions in the Harran University Almond Selection Plot.

Statistically significant differences were found between almond hybrids and cultivars in terms of all the characteristics examined. However, positive linear relationships were found between some leaf and stomata characteristics.

When the leaf characteristics were examined in general, the leaves of the ‘Genotype-7’ hybrid stood out among the cultivars and hybrids. In terms of leaf width, leaf length, petiole length and leaf area, the highest values were determined in ‘Genotype-7’ hybrids. When the petiole length was examined, it was determined that the longest petiole was in the leaves of the ‘Genotype-14’ hybrid.

4. Conclusion

The density and size of stomata in fruit trees vary according to species and variety, as well as the number and sizes of stomata per unit surface area with the effect of environmental and internal factors. In fruit trees, stomata sizes decrease significantly with the increase in the number of stomata. As the stoma size decreases, water loss also decreases. As a result, stoma density; It has been stated that it varies according to the genotype of plant grown, the ecology of the cultivation, the cultural processes applied, the maturity level of the leaves, the location and location of the leaves.

Positive linear relationships were determined between leaf area and the other factors (leaf length, leaf width and pedicel length). A positive linear relationship was determined between stomata length and stomata width of almond cultivar and hybrid genotypes used in the study. However, the number of stomata in the leaf; differed independently of stomata width, stomata length and leaf characteristics. Accordingly, the number of stomata in unit leaf surface area, stomata width and stomata length can be a defining factor for almond cultivars and hybrids in a given ecology. Investigations on different directions of almond leaves showed that stomata characteristics could change depending on the direction. Findings supporting this view were found in almost all of the investigated almond genotypes. These plants, which have late flowering and self-fertility characteristics, are extremely important for semi-arid conditions.

It was concluded that the closure of stomata causes an increase in leaf water potential. It can be hypothesized that stomata closure slows down the transpiration rate and thus prevents leaf dehydration. It is recommended to evaluate the varieties that can tolerate these problems in regions with low precipitation.

In this study, cultivars and genotypes were grown under the same conditions and the cultivars were compared with each other. Leaf size is an indication that it should be grown in moist and irrigated areas. Plants with small leaf sizes should be preferred in arid areas. The obtained findings will shed light on future studies by evaluating the performance of cultivars and hybrids under regional conditions. It is important to continue the studies on the formation of these plants from late blooming and self-fertile individuals. Since it is a species that can be caught in late spring frosts during flowering and small fruiting periods, these studies should continue.

Different results have been obtained in studies on stomata morphology, leaf characteristics and the relationship between water content and arid conditions. This shows that it is necessary to carry out different studies on the subject. The data obtained in determining the morphological and physiological characteristics of the leaves are the values reached under field conditions. In this respect, it is important to make pomological analyzes of the varieties in the same ecology and to evaluate the plants in question as genetic material.

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