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Full Length Article

Stomata Size and Frequency in some Walnut (*Juglans regia*) Cultivars

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ABSTRACT

This study compared the stomata frequency, stomata size, chlorophyll content and leaf characteristics (leaf length, width & leaf area) of 11 cultivars of walnuts. The stomata frequency, stomata size (stomata length and width) of all 11 cultivars were in the range 183-335 stomata/mm^2, 17.21-30.10 and 10.65-20.06 µm, respectively. The stomatal frequency of Chandler was the largest and the stomata length and width of Fernor were the largest among all the walnuts cultivars. Chlorophyll and leaf characteristic were measured in same leaf, which were used for measured stomata frequency and size previously. The total chlorophyll and leaf area in all cultivars were in the range 0.34-2.37 mg/g and 26.77-86.92 cm^2. The largest chlorophyll content was measured in Pedro, while the largest leaf area was measured in Howard. Remarkable positive correlation was found among leaf characteristics and stomata frequency in walnut cultivars.

Key Words: Stomata; Walnut; Leaf characteristics; Chlorophyll content

INTRODUCTION

Stomatal function is important to physiology and adaptability to varying environmental conditions and productivity of plants. Adaptability of the plants is closely associated with transpiration and photosynthesis process occurred in their leaves. The number and distribution of the stomata per unit leaf area have an important role in these processes by adjusting CO_2, O_2 and moisture exchange between the leaves and the atmosphere (Salisbury & Ross, 1992; Brownlee, 2001). Stomatal frequency differs greatly from species to species, ranging from 125 to over 1000 m^2 (Ryugo, 1988). In some resources, it has been known that stomata density changes with characteristics such as drought, net photosynthesis production (Bierhuizen, 1984), precipitation change (Mısırlı & Aksoy, 1994), vegetative developmental phases and grafts on different rootstocks (Çaglar & Tekin, 1999) and altitude (Çağlar *et al.*, 2004; Aslantaş & Karakurt, 2009). Stomata are more numerous over the lower epidermis (hipostomatic) than the upper epidermis (epistomatic) in leaves of most species, but leaves of some species have stomata both side (amfistomatic) (Kaçar, 1989). Stomata occur only on lower epidermis (hipostomatic) of walnut leaf. Stomatal structure varies greatly in concert with species, habitat and leaf architecture. Besides, it is established that stomatal density is an important factor in resistance against diseases (Yang *et al.*, 2004). It is reported that stomata density and conductance of lower leaves of tabacco is used as a selection marker for disease-resistance breeding (Yang *et al.*, 2004).

Many researchers reported that there are large heritable differences between species in stomatal size, frequency and morphology. for instance some vegetables (Yanmaz & Eriş, 1984), grapes (Eriş & Soylu, 1990), chestnut types (Şahin, 1989), apple rootstocks and cultivars (Pathak *et al.*, 1976; Gülen *et al.*, 2004; Aslantaş & Karakurt, 2009), walnut genotypes (Çağlar *et al.*, 2004). Pistachio rootstock and cultivars (Çağlar & Tekin, 1999; Özeker & Mısırlı, 2001).

Leaf morphological traits, including stomatal density and distribution and epidermal features may affect gas exchange quite remarkably and their relationships with key environmental factors such as light, water status, and CO_2 levels (Woodward, 1987; Nilson & Assmann, 2007). Leaf area measurement can be used for studies on cultural practices such as training, pruning, irrigations, fertilization etc. reliable leaf area measurement make it easy for researcher investigating the effect of light, photosynthesis, respiration plant water consumption and transpiration (Uzun, 1996). In walnut study, leaf characteristics and frequency of stomata and relations them could be used for long-termed adaptations study.

The aim of this study was to determine leaf and stomatal characteristics of walnut cultivars, to determine whether there are differences for these characteristic, and relationships among them.

MATERIALS AND METHODS

The study was carried out in the experimental area of Hakkari province, Turkey. The experimental area located at

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the Eastern Anatolia region of Turkey (37°N longitude, 044°E altitude, 1602 m above sea level). Eleven walnut cultivars (Chandler, Fernor, Franquette, Hartley, Howard, Midland, Pedro, Kaman-1, Maraş-12, Maraş-18 & Şebin) were used in this study. All walnut (*Juglans regia* L.) cultivars were grafted on *Juglans regia* rootstock with a trees spacing, 7.5x7.5 m and five-years old. Leaf samples were collected at 3rd quarter of August month at 2010 year. In this month, 4th leaf was sampled in the south side of tree. Two trees and two leaves in each walnut cultivars were used and they Were sampled from the same position. Stoma frequency was measured on the middle portion of the leaf by nail polish on lower surfaces (Brewer, 1992). The prepared slides were photographed using Nikon (Eccipse TE 300) microscope by a 10 ocular and a 20 objective. On the photographer, İmageJ (Ver 1.44I) analyzer program was used to determine stoma frequency, stoma length and width. In addition, Leaf samples were scanned by Samsung SCX-4200 and then leaf length, leaf width and leaf area were determined to by İmageJ 1.44I (www. rsbweb.nih.gov/ij) analyzer program over scanned leaf photography. For Chlorophyll a, chlorophyll b and total chlorophyll, 0.5 g fresh leaves were extracted in 80% acetone and were determined spectrophotometrically by Lichtentaler formula (Lichtentaler, 1994).

Data were subject to analysis of variance and correlation by using SPSS, 17.0 (www. Spss.com) program. Means showing, statistically significant differences for stoma characteristics, leaf characteristics and chlorophyll contents were compared using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The average stomata frequency was 273.21/mm². This parameter ranged from a minimum 183/mm² for Şebin to a maximum 335/mm² for Chandler. The average stomata size was 22.51 µm in length and 15.38 µm in width. The highest stomata length and width were record at Fernor (30.10-20.06 µm) cultivars. The lower length and width were record Franguette (17.21-10.65 µm) cultivars (Table I).

There was a significant variation in leaf stomata frequency among in cultivars. Similarly, Çağlar *et al.* (2004), reported significant differences in stomatal frequency and size among genotypes of walnuts. Çağlar *et al.* (2004) record that number of stomata on the lower surface of leaf walnut varied between 217 and 120/mm², stoma length varied between 14-18 and 21-28 µm, which were higher with increasing altitudes. Besides, stomata frequency and size are significant both for plant genetics and ecology (Fregoni & Roversi, 1968).

İn this study, stomatal frequency of foreign cultivars (Chandler, Fernor, Franquette, Hartley, Howard, Midland & Pedro) was higher than domestic cultivars (Kaman-1, Maraş-12, Maraş-18 & Şebin). Leaf stomata frequency of the genotypes can be related to adaptation process of the trees. The stomatal frequency, stoma length and width in our result were higher than that reported by Çağlar *et al.* (2004). This could be due to that our experimental area was located at high altitude (1602 m above sea level) than others study experimental.

The some investigators have reported that there was a trend towards increasing stomatal density at higher elevations Çağlar *et al.* (2004) in walnut, Aslantaş and Karakurt (2009) in apple. In addition some literature shows that stomatal density is more in irrigation condition. Study carried out by Mısırlı and Aksoy (1994) showed increase in stomatal density with increase in rainfall years.

Table II illustrate that within the walnut cultivars, the mean chlorophyll (chlorophyll a & b) pigment levels ranged from a minimum of 0.34 mg/g for Fernor to a maximum of 2.37 mg/g for Pedro without any significant statically different among cultivars (P≤0.05). Chlorophyll a was determined predominant chlorophyll in total chlorophyll. The cholorophyll a pigment levels ranged from a minimum 0.28 mg/g for Fernor to a maximum of 195 mg/g for Pedro. A study carried out by Jyothi and Raijadhav (2004), show that, Cholorophyll a, b and stoma density decreased under water stress in Rangpur Lime.

Table I: Stomatal characteristic of walnuts cultivars

Cultivars	Stomatal frequency (mm²)	Variation range	Stomata length (µm)	Variation range	Stomata width (µm)	Variation range
Chandler	335±6.48a	315-347	24.02±0.21c	18.59-31.66	16.09±0.12bcd	12.39-24.43
Fernor	251±7.21d	237-360	30.10±0.84a	18.99-31.55	20.06±0.11a	18.01-21.77
Franquette	324±7.53abc	311-337	17.21±0.09e	11.63-18.22	10.65±0.07h	6.61-11.63
Hartley	329±9.53ab	292-375	20.90±0.29d	20.35-30.02	16.78±0.45bc	12.30-17.70
Howard	290±8.42c	269-322	25.74±0.29b	20.47-28.22	15.74±0.09cd	13.16-22.35
Kaman-1	201±10.9ef	287-223	21.29±0.66d	19.26-30.61	14.54±0.18ef	12.39-20.98
Maraş-12	230±9.26de	229-247	23.79±0.18c	21.86-27.87	16.98±0.35b	11.73-17.68
Maraş-18	250±9.56d	219-268	21.79±1.45d	14.92-18.91	14.48±0.93f	10.02-15.85
Midland	293±9.38bc	276-338	20.20±0.21d	19.28-25.55	13.13±0.15g	11.73-17.70
Pedro	314±2.40abc	311-319	21.79±0.03d	21.76-30.21	15.17±0.29def	12.72-17.02
Şebin	183±8.51f	167-246	20.78±0.05d	17.27-21.70	15.62±0.10de	11.43-18.45
Minimum	183.00		17.21		10.65	
Maximum	335.00		30.10		20.06	
Average	273.21		22.51		15.38	

[a-g] Mean separation, within columns, by Duncan's multiple range test, 5%

Table II: Chlorophyll content of leaf in some the walnut cultivars (mg/g)

Cultivars	Chlorophyll a	Chlorophyll b	Chlorophyll a/b	Total chlorophyll
Chandler	1.27±0.61a	0.28±0.14a	4.54	1.54±0.75a
Fernor	0.28±0.16a	0.06±0.03a	4.67	0.34±0.19a
Franquette	1.57±0.69a	0.33±0.15a	4.76	1.90±0.84a
Hartley	1.06±0.17a	0.17±0.09a	6.24	1.23±0.26a
Howard	1.26±0.23a	0.26±0.23a	4.85	1.52±0.05a
Kaman-1	1.08±0.38a	0.21±0.06a	5.14	1.28±0.44a
Maraş-12	1.47±0.08a	0.29±0.01a	5.07	1.76±0.09a
Maraş-18	1.46±0.67a	0.29±0.19a	5.03	1.75±0.90a
Midland	0.79±0.08a	0.17±0.02a	4.65	0.96±0.11a
Pedro	1.95±0.68a	0.41±0.15a	4.76	2.37±0.84a
Şebin	1.27±0.13a	0.28±0.03a	4.54	1.55±0.16a
Minimum	0.28	0.06	4.54	0.34
Maximum	1.95	0.41	6.24	2.37
Total	1.22	0.25	4.54	1.47

[a]Mean separation, within columns, by Duncan's multiple range test, 5%

Table III: Leaf area of some the walnut cultivars

Cultivars	Leaf length (cm^2)	Leaf width (cm^2)	Leaf area (cm^2)
Chandler	11.83±0.61cde	6.11±0.33abc	52.59±5.20cde
Fernor	9.96±0.56def	3.89±0.24d	26.77±2.76e
Franquette	11.91±0.18cde	6.02±0.82abc	50.52±10.35cde
Hartley	12.80±0.94bc	6.32±0.12ab	56.02±5.85cd
Howard	15.20±0.56ac	7.83±0.89a	86.92±10.53a
Kaman-1	12.35±0.42cd	7.19±0.59a	57.83±9.37bcd
Maraş-12	13.96±1.47abc	7.86±0.75a	77.45±10.36abc
Maraş-18	9.45±0.17de	5.96±1.01abc	41.43±7.84de
Midland	9.75±1.06def	4.40±0.11cd	32.77±5.00de
Pedro	9.06±1.44e	4.50±0.21bcd	28.54±7.07de
Şebin	16.20±0.12a	7.77±0.14a	85.55±0.38ab
Minimum	9.06	3.89	26.77
Maximum	16.20	7.86	86.92
Total	11.95	6.07	53.02

[a-f]Mean separation, within columns, by Duncan's multiple range test, 5%

In the present study, leaf width, length and leaf area were affected statically significant by cultivars as could be seen in Table III. In walnut cultivars, Leaf length was determined to range between 9.06 cm^2 for Pedro cultivar and 16.20 cm^2 for Şebin cultivar. Leaf width varied between 3.89 cm^2 for Fernor cultivars and 7.86 cm^2 for Maraş-12 cultivars. The average leaf area was found 53.02 cm^2. This parameter ranged from a minimum of 26.77 cm^2 for Fernor cultivar to a maximum of 86.92 cm^2 for Howard cultivar. Generally the leaf area of foreign cultivars (Chandler, Fernor, Franquette, Hartley, Midland & Pedro) except Howard cultivars were found higher than domestic cultivars (Kaman-1, Maraş-12, Maraş-18 & Şebin). Previous studies carried out by Hokanson *et al.* (1993) in strawberry, Aslantaş and Karakurt (2009) in apple and Chandra (2004) in some alpine plants showed that leaf area decreased along with hoisted altitude.

There was a positive and significant correlation between stomata width and stomata length (P≤0.01) in this study (Table IV). As seen in some relationships resulted with higher correlation coefficients. No significant correlations were found between stoma frequency and all investigated characteristics except for leaf length and leaf

Fig. 1: The relationships of leaf width and Leaf area

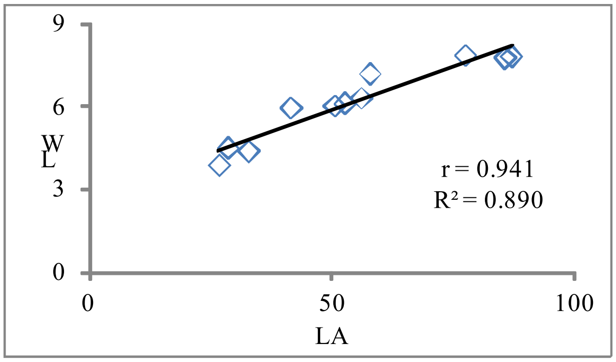

Fig. 2: The relationships of leaf length and Leaf area

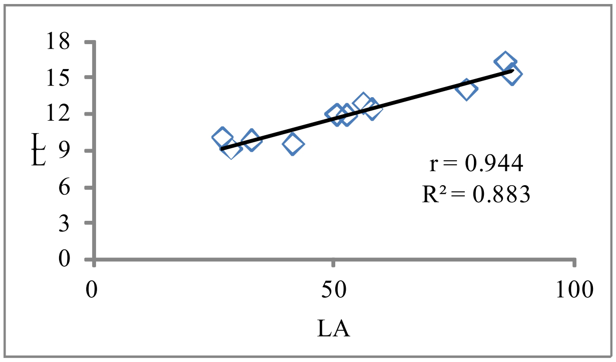

Fig. 3: The relationships of leaf width and Leaf length

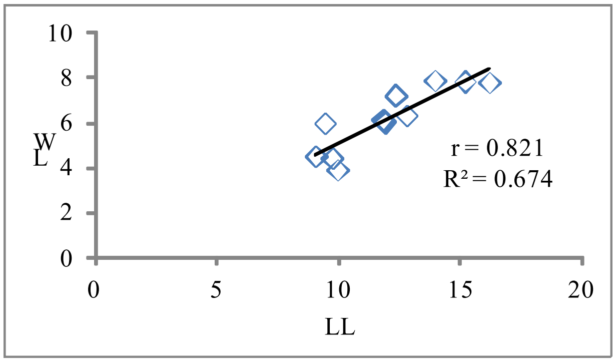

Fig. 4: The relationships of stomatal width and length

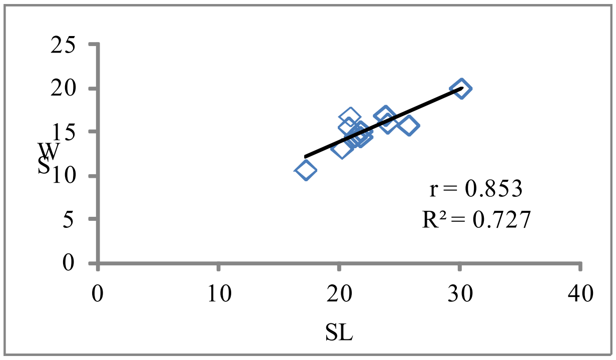

Table IV: Correlations among chlorophyll, leaf and stomatal characteristics in walnut cultivars

	SL	SW	SF	Chl. a	Chl. b	Total Chl.	LW	LL
SW	0.853**							
SF	-0,134	-0.212						
Chl *a*	-0.33	-0.292	0.130					
Chl *b*	-0.313	-0.298	0.069	0.982**				
Tot. chl	-0.331	-0.293	0.120	0.999**	0.988**			
LW	-0.252	-0.217	-0.311	0.134	0.119	0.131		
LL	-0.132	-0.057	-0.388*	-0.028	-0.019	-0.026	0.821**	
LA	-0.098	-0.48	-0.370*	0.085	0.092	0.086	0.941**	0.944**

**Correlation coefficient (r) significant at theP< 0.01 level, *r significant at theP< 0.05 level

SL: Stomata length, SF: Stomatal frequency, SW: Stomatal width, Chl. A: Chlorophyll A, Chl. B: Chlorophyll B, Tot. Chl: Total Chlorophyll, LW: Leaf width cm^2, LL: Leaf length cm^2, LA: Leaf area (cm^2)

area. Negative relationships were found among Stomatal frequency, leaf area and leaf length (P≤0.05). Our results for stomatal number and the leaf width were in conformity to some previous studies in citrus and grass species (Özeker & Mısırlı, 1999; Xu & Zhou, 2008). Özeker and Mısırlı (1999) reported negative correlation between the stomatal number and the leaf width in pistacia spp. Xu and Zhou (2008) showed a significant negative correlation between specific leaf area and stomatal density.

According to Loranger and Shipley (2010), there is probably a general link between stomatal density and morphological and physiological leaf traits at both the interspecific and intraspecific levels and Stomatal density can also be linked to leaf thickness and chlorophyll concentration, since thicker leaf with lower cholorophyll content tend to have higher stomatal density. In addition, leaf stomatal conductance is closely associated with leaf age, decreasing more in older leaves compared with young leaves under a given stress (Yang *et al*., 1995).

CONCLUSION

The result of this study provided evidence that there are cultivar differences with respect to stomatal and leaf characteristic. Therefore, this information is important in the development of adaptation studies and determination of genotypes. Investigation of relationships among leaf and stomatal characteristics, vegetative growth, altitude, transpiration rate and net photosynthesis production may contribute to the long-termed walnut adaptation studies.

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