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The effect of different nursery conditions on some of the leaf and stomata characteristics in Chestnuts

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Summary

The aim of this study was to determine the effect of different nursery conditions on some leaf dimensions and stomatal characteristics in the chestnut. The study was carried out in the open field and under shaded (50 %) and unshaded greenhouse conditions. In this study, scions of the Marigoule and Unal cultivars were grafted on seedlings of the 554-14 genotype with the inverted radicle grafting method. Grafting was performed during the second week of May in the first year and during the last week of April in the second year. Significant differences in leaf dimensions and stomata density were found in different nursery conditions. The width and length of leaf lamina were higher in the shaded greenhouse ($P<0.01$). The ratio of the leaf lamina width/length ($P<0.01$) and stomata density ($P<0.05$) were higher in the open field condition. The highest correlation ($r = -0.847$) was determined between stomata length and stomata width/length ratio ($P<0.01$). These results indicate that the leaf lamina width and length increased in the shaded greenhouse unlike the stomata density which decreased slightly. However, stomata dimensions remained the same in all three nursery conditions.

Abbreviations: SEM: Standard Error Mean, NS: Non Significant

Introduction

Plants are able to modify their growth, development and physiology according to their environment. This ability of plants plays a key role in determining their tolerance to stresses and maintains efficient growth (Murchie and Horton, 1997; Walters et al., 2003). Stomatas are important organelles that affect the adaptation skills of plants (Salisbury and Ross, 1992; Brownlee, 2001) by directing transpiration and photosynthesis and by giving clues about suitable plant cultivation. They are epidermal valves that are essential for plant survival because they control the entry of carbon dioxide assimilated in photosynthesis and optimize water use efficiency (Schroeder et al., 2001; Bergmann and Sack, 2008). Therefore, stomata play a vital role in the ability of land plants to balance water loss with photosynthetic performance. In leaves, the pattern of stomatal distribution is highly variable between species but is regulated by a mechanism that maintains a minimum of one cell spacing between stomata (Casson and Gray, 2008).

Many factors influence stomatal behavior in the plants (Salisbury and Ross, 1992). Stomatal number, size and density show a variation that depends on the plant's species, cultivar, clones and cultivation conditions (Kliewer et al., 1985; Caglar and Tekin, 1999; Caglar et al., 2004). The number, index, and size of stomata are, also, affected by light intensity (Pazourek, 1970; Thomas et al., 2003) light color (Kim et al., 2004; Lee et al., 2007; Kilic et al., 2010). Indeed, an increase in light intensity increased the stoma number, index and size. While blue and red light increases the stomata size and decreased the stomata number, respectively, white light increased the stomata number and size (Lee et al., 2007). In addition the stomata density changes with characteristics such as dependence on drought, net photosynthesis production (Bierhuizen et al., 1984), vegetative development phases (Caglar and Tekin, 1999) and altitude (Caglar et al., 2004).

Leaves are the most important organs for photosynthesis and transpiration in plants, and the arrangement, size, shape and anatomy of them differ greatly in different environments (Neophytou et al., 2007). Leaf parameters such as size and shape are frequently recorded variables in plant research (Serdar and Kurt, 2011). The shape and structure of leaves varies considerably depending on climate, primarily due to the availability of light and the potential for water loss due to temperature and humidity.

Temperature, light intensity and relative humidity are the fundamental environmental factors in plant growing. These factors should be considered especially in micropropagation and the acclimatation of new cultivars to different ecologies. The morphological, physiological and anatomical characteristics of plants such as chestnut trees are largely influenced by developmental, environmental and cultivational factors (Pereira-Lorenzo et al., 1996; Ozturk and Serdar, 2011). The environmental conditions during and following grafting are one of the factors influencing the healing of the graft union and plant growth. The stomata density of fruit tree leaves can be related to the adaptation processes of any fruit tree. Thus, it is worthwhile to investigate the suitability of open, shaded and unshaded greenhouse conditions for grafted seedling production systems. Different studies have been carried out to investigate the effects of different nursery conditions on graft success and plant development in the chestnut (Anagnostakis, 2007; Ozturk et al., 2009; Zarafshar et al., 2010; Ozturk and Serdar, 2011). But, there have been no studies concerned with the effects of different nursery conditions on leaf dimensions and stomata characteristics.

The objective of this study was to determine the effects of open field, shaded and unshaded greenhouse conditions on the leaf and stomata characteristics of Marigoule and Unal chestnut cultivars.

Materials and methods

Plant Materials

This study was carried out in Samsun, Turkey. The Unal and Marigoule chestnut cultivars were used in the study. The scions of them were taken from an orchard in the province of Samsun, Turkey in February and were stored at 4 ± 0.5 °C until they were used for grafting. Two or three bud scions were used for inverted radicle grafting. Newly germinated seeds of chestnut genotype 554-14 (Soylu and Serdar, 2000) were used as rootstock. The inverted radicle grafts were carried out on 10th May in the first year and 29th April in the second year (Ozturk et al., 2009). Grafted seeds were planted in pots (30 x 40 cm) filled with a medium containing 3/4 soil + 1/4 ground pine needles and manure. The growing mediums were clay loam with 3.03 % and 1.62 % organic matter and pH 7.30 and pH 7.82 in the 2 experimental years, respectively.

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Study Design

The study was carried out in the open field and under shaded and unshaded greenhouse conditions. Shading was carried out with netting having a light transmission of 50 % and the samples were immediately covered with this netting after grafting. The experimental design was a randomized plot. Three replications and 20 grafts per replication were used. Leaf samples were taken from 10 plants per replication. The temperature, relative humidity and light intensity of the nursery conditions were measured using a datalogger (Kimo KT100, France). Data of nursery conditions measured during the experimental period are presented in Fig. 1-3.

Leaf and Stomata Measurement

In order to determine the stomata characteristics of the leaves, samples were taken from the middle part of shoots at the closing time of stomata, 09.00-11.00 a.m. on 25 and 26 September (SAHIN and SOYLU, 1991; SERDAR and KURT, 2011). Two leaves per plant and 20 leaves per replication were sampled. After leaf sampling, 6 leaf pieces containing the membrane of the stomatal surface were taken from each leaf by the systematic random sampling method (KORKMAZ et al., 2000). In this preparation, firstly nail polish was applied to the areas in between the lateral veins of the lower surface containing stomata (SAHIN and SOYLU, 1991). After drying out for approximately five minutes, leaf pieces (2.0-2.5 cm^2) containing the membrane of the stomatal surface was taken out with an adherent acetate sheet (BOZOGLU and KARAYEL, 2006). After taking the stomatal surface, the length and width of the leaf lamina were measured immediately. The stomata count and measurements of dimensions including the width and length were done in three regions having an average area of 180 mm^2 per sampling through 40 x 100 or 20 x 100 magnifying lenses. The length and width of leaf lamina were measured in five plants (approximately 50 leaves) and the width/length ratio of leaf lamina was calculated. All measurements were repeated in both years (2006 and 2007) in order to determine the effects of nursery conditions on the studied parameters.

Data Analyses

Some leaf and stomata characteristics of the cultivars, including the effects of nursery conditions, cultivars and years and their interactions, were analysed by a three factored ANOVA test. Therefore the data was analyzed in a randomized block design as a factorial arrangement (3 x 2 x 2) of treatments. For data on leaf characteristics individual leaves were considered to be the experimental unit (n = 60 per treatment). When the F test was significant, differences were determined by Duncan's multiple range tests. All analyses were performed using the SPSS statistical package. Results are presented as means and a pooled SEM (Standard Error Mean). Correlation analysis was applied to assess whether the characteristics of the leaves depend on the stomatal behavior in chestnuts as influenced by the nursery conditions.

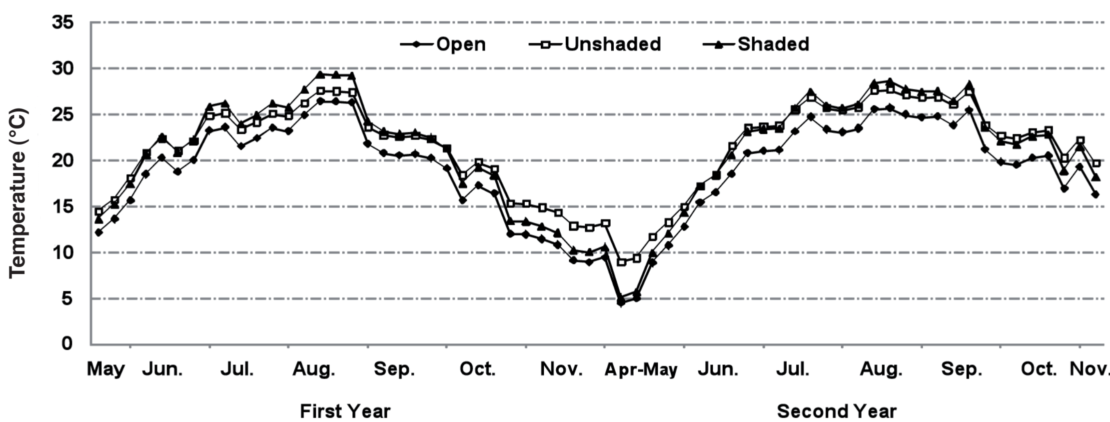

Fig. 1: The temperature data of nursery conditions with respect to years

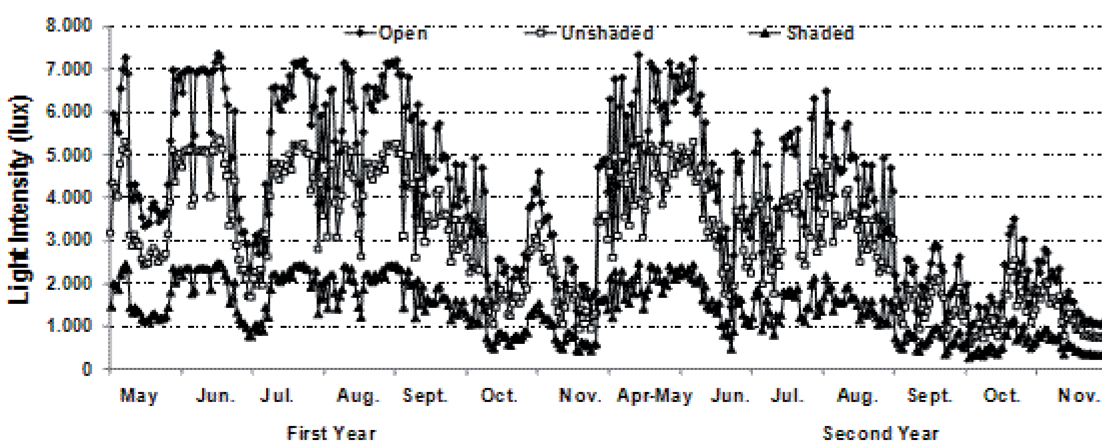

Fig. 2: The light intensity data of nursery conditions with respect to years

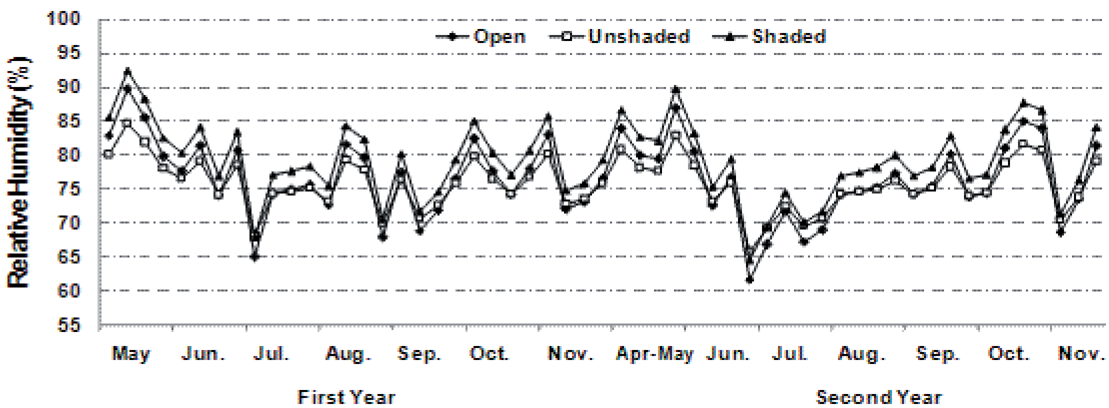

Fig. 3: The relative humidity data of nursery conditions with respect to years

Results and discussion

Leaf characteristics

Leaf dimensions and ratio of lamina width/lenght of chestnuts grown in the shaded and unshaded greenhouse and open field conditions are presented in Tab. 1. Nursery conditions affected the length, width, and width/length ratio of leaf lamina (P<0.01). Except for the width/length ratio of leaf lamina, the length and width was higher (P<0.01) in the shaded greenhouse. However, the width/length ratio of leaf lamina was higher (P<0.05) in the open field (Tab. 2). Lamina width and length were higher in the shaded greenhouse (4.6 and 17.8 cm respectively) (P<0.01) than both those grown in the unshaded greenhouse (3.4 and 13.6 cm respectively) and in the open field (3.5 and 12.9 cm respectively) (Tab. 2). The plants grown in the shaded greenhouse were nearly 75 % wider and had longer leaves than those grown in the other conditions. In this study, the shaded and unshaded greenhouse and open field conditions had a mean temperature of 21.1, 21.4 and 18.9 °C, a mean relative humidity of 79.2, 75.7 and 76.5 %, and a mean light intensity of 1392, 3007 and 4130 lux, respectively (Fig. 1-3).

These results indicate that leaf dimensions may be affected by higher temperatures (Fig. 1), a lower light intensity (Fig. 2) and a higher relative humidity (Fig. 3). These results confirm the findings of OZTURK and SERDAR (2011). As compared to shaded leaves, those growing in the sun usually have smaller leaf dimensions (leaf width, length and area) and a higher dry mass per leaf area unit (LICHTENTHALER et al., 1981). Leaf dimensions in the strawberry were higher in plants grown in the shade than those grown in unshaded and open field conditions (OZTURK and DEMIRSOY, 2004). Increases in the area and number of leaves are a common response of leaves that are adapted to shadier conditions and of species that are shade tolerant or intermediate with respect to shade tolerance (LAMBERS et al., 1998).

The cultivar had a significant effect on the leaf dimensions (P<0.01) and also the leaf lamina width and length changed considerably between the two years (P< 0.01). The leaf lamina width and leaf lamina width/length ratio of the Marigoule cultivar were wider (P<0.01) and higher (P<0.01) than that of the Unal. The lamina length of the Unal cv. was longer (P<0.01) than that of the Marigoule cv. (Tab. 1). It was observed that year had a significant effect on the dimensions of the leaf, except for the lamina width/length ratio. The lamina width and length in the second year was higher than that in the first year (P<0.01). On the contrary, the highest lamina width/length ratio was obtained from the plants grown in the open field in the first year (0.29).

The interaction between nursery condition and cultivar had a significant effect on the lamina width and length (P<0.05). Significant effects of nursery condition and year interactions on the leaf width (P<0.05) and leaf length (P<0.01) and leaf width/length ratio (P< 0.01) were observed (Tab. 1). The highest lamina width and length was obtained from the plants grown in the shaded greenhouse in the second year (4.8 and 18.2 cm, respectively). The interaction between year and cultivar had no significant effect on the leaf dimensions and also there were significant effects of nursery condition x cultivar x year interaction on the leaf lamina width (P<0.05) (Tab. 1).

Although the ratios obtained from leaf dimensions may be more stable, leaf dimensions can be a bit changeable according to environmental conditions and cultural practices (SERDAR and KURT, 2011). The same researchers reported that leaf dimensions (width and length of the leaf lamina, leaf length and leaf area) depend on genotypes rather than year and year x genotype in the chestnut.

Stomata Characteristics

Stomata are necessary for gas exchange, and have an important role in environmental stress, ploidy levels and resistance to drought, disease and insects (MEHRI et al., 2009). The leaf stomata frequency of the genotypes can be related to the process of adaptation of the trees. The nursery conditions used in the present study affected the stomata density (P<0.05), but didn't affect the width and length and width/length ratio of stomata (Tab. 1). Stomata density of chestnuts grown in the open and unshaded greenhouse conditions was higher (P<0.05) than that of those grown in shaded greenhouse conditions, 312.7, 292.6 and 273.4 per mm^2 respectively (Tab. 2). RYUGO (1988) reported that stomatal frequency varied from 125 to over 1000 per mm^2. Moreover, light intensity has a strong effect on stomata density and dimensions (THOMAS et al., 2003). As compared to leaves in the shaded regions, leaves exposed to the sun usually have a greater stomata density (LICHTENTHALER et al., 1981). And also, Stomata density reduced with different shading levels in banana leaves (ISRAELI et al., 1995). In the present study, except for the stomata density, the width and length and width/length ratio of stomata were not affected by the nursery conditions. An increase in light intensity results in an increase in the stomata density, index and size (THOMAS et al., 2003). Also, different colors of light affect the stomata number and size (KIM et al., 2004; KILIC et al., 2010). LEE et al. (2007) reported that white light increased the stomata number and size while blue and red light increased the stomata size and decreased the stomata number. In our study, shading decreased the stomata number, but didn't affect the stomata dimensions. Thus, stomatal differentiation is seen to be very sensitive to a change in the supply of light. This result may be due to the dark green shading cover that was used in the study and may

Tab. 1: The width and length and width/length of leaf lamina and the number, width and length and width/length of stomata in chestnuts grown in the shaded and unshaded greenhouse and open field condition with respect to years and cultivars

Nursery Conditions	Cultivars	Leaf Lamina			Stomata			
		Width	Length	Width/Length	Number	Width	Length	Width/Length
		First Year (2006)						
Shaded	Mariquel	5.44	16.31	0.33	417.96	17.00	21.67	0.78
	Unal	3.39	18.59	0.18	272.37	19.17	24.17	0.79
Unshaded	Mariquel	3.73	11.88	0.31	390.01	17.17	22.67	0.76
	Unal	2.34	12.57	0.18	326.70	18.33	23.92	0.77
Open	Mariquel	3.71	10.70	0.34	419.10	17.17	22.33	0.77
	Unal	2.48	10.74	0.23	313.15	18.83	23.67	0.80
		Second Year (2007)						
Shaded	Mariquel	5.61	16.68	0.33	222.60	18.17	23.33	0.78
	Unal	4.03	19.44	0.20	181.39	18.00	25.00	0.72
Unshaded	Mariquel	4.73	14.63	0.32	263.38	18.67	24.67	0.76
	Unal	2.96	15.26	0.19	190.94	18.67	24.67	0.76
Open	Mariquel	4.71	14.31	0.33	294.18	17.83	23.50	0.76
	Unal	3.26	15.73	0.20	225.17	19.22	25.44	0.76
Pooled SEM		0.032	0.152	0.002	1.366	0.12	0.16	0.04
Main effect of								
Nursery Conditions		**	**	**	*	NS	NS	NS
Cultivar		**	**	**	**	**	**	NS
Year		**	**	N.S	**	NS	**	*
Nursery Conditions x Cultivar		*	*	N.S	N.S.	NS	NS	NS
Nursery Conditions x Year		*	**	**	N.S.	NS	NS	NS
Cultivar x Year		N.S	N.S	N.S	N.S.	NS	NS	NS
Nursery Conditions x Cultivar x Year		*	N.S	N.S	N.S.	*	NS	NS

Pooled SEM. pooled standard error of the mean. NS. $P > 0.05$; *. $P < 0.05$; **. $P < 0.01$.

Tab. 2: The width and length and width/length ratio of leaf lamina and the number and width and length and width/length ratio of stomata in chestnuts grown in the shaded and unshaded greenhouse and open field condition

Nursery Conditions	Leaf Lamina			Stomata			
	Width (cm)	Length (cm)	Width / Length	Number	Width (µ)	Length (µ)	Width / Length
Shaded	4.62±0.99 a	17.80±1.60 a	0.26±0.07 b	273.36±2.24 b	18.08±0.99	23.54±1.6	0.76±0.02
Unshaded	3.44±0.95 b	13.58±1.74 b	0.25±0.06 b	292.61±1.89 ab	18.21±0.98	23.98±1.1	0.75±0.02
Open	3.54±0.85 b	12.87±2.38 b	0.27±0.06 a	312.72±1.92 a	18.26±1.02	23.74±1.4	0.77±0.02
Significance	**	**	**	*	NS	NS	NS

a.b Means with different letters in the same column were significantly different (**$P < 0.01$. *$P<0.05$).

also be a consequence of less light intensity in shaded conditions than in the other nursery conditions (Fig 2). Different light conditions decreased the stomata number in the lower surface of leaves of tomato seedlings, compared to the control and transparent cover (KILIC et al., 2010). THOMAS et al. (2003) noted that in tobacco, the shading of mature leaves while exposing developing leaves to high light intensity resulted in a 12.7 % decrease in the stomatal index of the developing leaves compared with controls exposed only to high light intensity. PAZOUREK (1970) stated that the stomatal density decreases with lower light intensities and the largest reaction to the light intensity appeared in leaves with a higher stomata density. Also, ILGIN and CAGLAR, (2009) stated that plants grow in environments with a higher temperature may adapt to these conditions by reducing the number of stomata, or stomatal density on each leaf surface.

In the present study, we tried to determine by how much the nursery conditions affect the leaf dimensions and stomata characteristics in the chestnut (Tab. 1) and presented the mean data of temperature, light intensity and relative humidity of these nursery conditions (Fig. 1-3). But, we could not determine how responsible temperature, light intensity or relative humidity is for these effects.

Tab. 3: Correlations amongst the leaf and stomata characteristics

	Leaf Width	Leaf Length	Leaf Width/Length	Stomata Number	Stomata Width	Stomata Length
Leaf Length	0.394(*)					
Leaf Width/Length	0.738(**)	-0.308				
Stomata Number	0.040	-0.582(**)	0.473(**)			
Stomata Width	-0.534(**)	-0.229	-0.443(**)	-0.220		
Stomata Width	-0.733(**)	0.035	-0.806(**)	-0.473(**)	0.587(**)	
Stomata Width/Length	0.557(**)	-0.200	0.717(**)	0.432(**)	-0.078	-0.847(**)

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

The cultivar had a significant effect on the number and width and length of the stomata (P<0.01) but not on the stomata width/length ratio, and also there were significant effects of the year upon the stomata density and length (P<0.01) and stomata width/length ratio (P<0.05). The stomata density of the Marigoule cv. was higher (P< 0.01) than that of the Unal cv., 334.51 and 251.61, respectively. The stomata width and stomata width/length ratio of the Unal cultivar was wider (P<0.01) and higher (P<0.01) respectively than that of the Marigoule (Tab. 1). Stomata size and density show variation according to the plant's species, cultivar, clones and cultivation conditions (CAGLAR and TEKIN, 1999; CAGLAR et al., 2004).
The stomata density and stomata width/length in the first year was higher than that in the second year, (P<0.05; P<0.01) respectively. SERDAR and KURT (2011) reported that stomata width and length and the stomata width/length ratio change with respect to year and genotypes. Stomata density changed in relation to the year and cultivars in the grape (KARA and OZSEKER, 1999). In this study, except for the interaction of nursery condition x cultivar x year, there were no significant effects of the interaction of nursery condition x cultivar, nursery condition x year, cultivar x year on stomata characteristics in the chestnut. Also there were significant effects of nursery condition x cultivar x year interaction on stomata width (P<0.05) in the chestnut. The Unal cultivar grown in open conditions in the second year had a higher stomata width (P<0.05) than other nursery conditions.
A linear correlation has been found amongst the lamina width and lamina width/length ratio (r=0.738) and lamina width/length and stomata width/length (r=0.717) in the present study. The highest correlation (r=- 0.847, P<0.01) was found between mean stomata length and stomata width/length ratio (Tab. 3) respectively. SAHIN and SOYLU, (1991) cited that a significant linear correlation (r=0.758**) was found between the mean length and mean width of stomata of the cultivars in the chestnut.
The mean length and width of lamina in chestnut genotypes varied from 17.7 to 21.6 cm and from 4.10 to 6.77 cm, respectively (SERDAR and KURT, 2011). These researchers reported that the mean length and width of stomata in the studied chestnut genotypes varied from 23.3 to 25.9 µm and 17.1 to 18.1 µm, respectively. In the present study, corresponding values for the grafted headings of Unal and Marigoule cultivars grown under different nursery conditions were 24.5-18.7 µm and 23.0- 17.7 µm, respectively. These values indicate that the chestnut cultivars used in the current study acclimated to different nursery conditions well.

Conclusion

In the present study, the results indicate that nursery conditions did affect the some of the leaf dimensions and stomata characteristics in the chestnut. Shaded greenhouse conditions resulted in a higher lamina width and length but it did not affect either the ratio of leaf lamina width/length or the stomata dimensions. In this event, the role of temperature, light intensity and relative humidity was probably significant. However, we did not determine which factors are more influential which could be the focus of a future study.

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References

ANAGNOSTAKIS, S.L., 2007: Effect of shade on growth of seedling American chestnut trees. Northern J. Apply Forestry 24(4), 317-318.
BERGMANN, D.C., SACK, F.D., 2008: Stomatal Development. Annual Rev. Plant Biol. 58, 163-81.
BIERHUIZEN, J.F., BIERHUIZEN, J.M., MARTAKIS, G.F.P., 1984: The effect of light and CO_2 on photosynthesis of various pot plants. Gartenbauswissenschaft 49, 215-257.
BOZOGLU, H., KARAYEL, R., 2006: Investigation of stomata densities in Pea (*Pisum sativum* L.) lines/cultivars. Online Journal Biological Science 6, 45-50.
BROWNLEE, C., 2001: The long and short of stomatal density signal. Trend in Plant Sci. 6, 441-442.
CAGLAR, S., SUTYEMEZ, M., BEYAZIT, S., 2004: Stomatal density in some selected walnut (*Juglans regia*) types. Akdeniz University Journal Faculty Agriculture 17, 169-174.
CAGLAR, S., TEKIN, H., 1999: The stomata density of pistachio cultivars on different pistacia rootstocks. Turkish J. Agric. Forest. 23(5), 1029-1032.
CASSON, S., GRAY, J.E., 2008: Influence of environmental factors on stomatal development. New Phytologist 178, 9-23.
ILGIN, M., CAGLAR, S., 2009: Comparison of leaf stomatal features in some local and foreign apricot (*Prunus armeniaca* L.) genotypes. Afr. J. Biotech. 8(6), 1074-1077.
ISRAELI, Y., PLAUT, Z., SCHWARTZ, A., 1995. Effect of shade on banana morphology, growth and production. Sci. Hortic. 62, 45-56.
KARA, S., OZSEKER, E., 1999: Farklı anaçlar üzerinde aşılı yuvarlak çekirdeksiz üzüm çeşidinin yaprak özellikleri ve stoma dağılımı üzerinde bir araştırma. Anadolu 9, 76-85.
KILIC, S., KARATAS, A., CAVUSOGLU, K., UNLU, H., OZDAMAR, H.U., PADEM, H., 2010: Effects of different light treatments on the stomata movements of tomato (*Lycopersicon Esculentum* Mill. cv. Joker) seedlings. J. Ani. Vet. Adv. 9(1), 131-135.
KIM, S.J., HAHN, E.J., HEO, J.W., PAEK, K.Y., 2004: Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysantemum plantlets in vitro. Scientia Hort. 101, 143-151.
KLIEWER, W.M.J.M., KOBRIGER, R.H., LIRA, S.T., LAGIER, S.T., COLLALTO, G., 1985: Performance of grapivines under wind and water stress condi-

tions. Proc. Intl. Symp. Cool Climate Viticulture and Enology, 198-216.

KORKMAZ, A., CIFTCI, N., BOSNAK, M., AGAR, E., 2000: A simplified application of systematic field sampling and low-cost video recording setup for viewing disector pairs exemplified in the rat cochlear nucleus. J. Micros. 200(3), 269-276.

LAMBERS, H., CHAPIN, F.S., PONS, T.L., 1998: Physiological, biochemical, and anatomical differences between sun and shade leaves. In: Plant Physiological Ecology, 26-36. Springer-Verlag, New York.

LEE, S.H., TEWARI, R.K., HAHN, E.J., PAEK, K.Y., 2007: Photon flux density and light quality induce changes in growth, stomatal development, photosynthesis and transpiration of *Withania somnifera* (L.) Dunal. Plantlets. Plant Cell Tiss. Org. Cult. 90, 141-151.

LICHTENTHALER, H.K., BUSCHMANN, C., DOLL, M., FIETZ, H.J., BACH, T., KOZEL, U., MEIER, D., RAHMSDORF, U., 1981: Photosynthetic activity, chloroplast ultra-structure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. Photosyn. Res. 2, 115-141.

MEHRI, N., FOTOVAT, R., SABA, J., JABBARI, F., 2009: Variation of stomata dimensions and densities in tolerant and susceptible wheat cultivars under drought stress. J. Food Agric. Env. 7, 167-170.

MURCHIE, E.H., HORTON, P., 1997: Acclimation of photosynthesis to irradiance and spectral quality in British plant species: Chlorophyll content, photosynthetic capacity and habitat reference. Plant Cell. Environ. 20, 438-448.

NEOPHYTOU, C.H., PALLI, G., ARAVANOPOULOS, F.A., 2007: Morphological differentiation and hybridization between *Quercus alnifolia* Poech and *Quercus coccifera* L. (*Fagaceae*) in Cyprus. Silvae Genetica 56(6), 271-277.

OZTURK, A., DEMIRSOY, L., 2004: The effects of different shading treatments on yield and growth in Camarosa strawberry variety. Bahce 33(1-2), 39-49.

OZTURK, A., SERDAR, U., BALCI, G., 2009: The influence of different nursery conditions on graft success and plant survival using the inverted radicle grafting method on the chestnut. Acta Hort. 815, 193-197.

OZTURK, A., SERDAR, U., 2011: Effects of different nursery conditions on the plant development and some leaf characteristics in Chestnuts (*Castanea sativa* Mill.), Aust. J. Crop Sci. 5(10), 1218-1223.

PAZOUREK, J., 1970: The effect of light intensity on stomatal frequency in leaves of *Iris hollandica* hort., vat. Wedgwood. Biol. Plant. (Praha), 12(3), 208-215.

PEREIRA-LORENZO, S., FERNANDEZ-LOPEZ, J., MORENO-GONZALES, J., 1996: Variability and grouping of Northwestern Spanish chestnut cultivars. I. Morphological traits. J. Amer. Soc. Hort. Sci. 121, 183-189.

RYUGO, K., 1988: *Fruit Culture*: Its Science and Art, 344. John Wiley & Sons Inc.

SAHIN, T., SOYLU, A., 1991: Stomatal frequency of some chestnut cultivars grown in Marmara Region of Turkey, XIII. Int. Hort. Cong. 27 August - 1 September, Firenze, Italy, Abstract, Poster Number 3003.

SALISBURY, F.B., ROSS, C.W., 1992: Plant Physiology. 4th edition, 682. Wadsworth Publication Comp. California.

SCHROEDER, J.I., ALLEN, G.J., HUGOUVIEUX, V., KWAK, J.M., WANER, D., 2001: Guard cell signal transduction. Ann. Rev. Plant Physiol. Plant Mol. Biol. 52, 627-58.

SERDAR, U., KURT, N., 2011: Some leaf characteristics are better morphometric discriminators for chestnut genotypes. J. Agric. Sci. Tech. 13, 885-894.

SOYLU, A., SERDAR, U., 2000: Rootstock selection on chestnut (*Castanea sativa* Mill.) in the Middle of Black Sea Region in Turkey. Acta Hort. 538, 483-488.

THOMAS, P.W., WOODWARD, F.I., QUICK, P.W., 2003: Systemic irradiance signalling in tobacco. New Phyto. 161, 193-198.

WALTERS, R.G., STEPHARD, F., ROGERS, J.J.M., ROLFE, S.A., HORTON, P., 2003: Identification of mutants of Arabidopsis defective in acclimation of photosynthesis to the light environment. Plant Physiol. 131, 472-481.

ZARAFSHAR, M., AKBARINIA, M., BRUSCHI, R., HOSSEINY, S.M., YOUSEFZADEH, H., TAIEBY, M., SATTARIAN, A., 2010: Phenotypic variation in chestnut (*Castanea sativa* Mill.) natural populations in Hyrcanian forest (north of Iran), revealed by leaf morphometrics. Folia Oecologica 37(1), 113-121.

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