JOURNAL OF FOREST SCIENCE, *63*, 2017 (6): 275–281

doi: 10.17221/138/2016-JFS

Variability in leaf and crown morphology correlated with light availability in five natural populations of *Quercus castaneifolia* C.A. Mey

Fariba BABAEI[1], *Seyed Gholamali JALALI*[1]*, *Hormoz SOHRABI*[1], *Anoshirvan SHIRVANY*[2]

[1]*Department of Forestry, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Nour, Iran*

[2]*Department of Forestry, Faculty of Natural Resources, University of Tehran, Karaj, Iran*

**Corresponding author: jalali_g@modares.ac.ir*

Abstract

Babaei F., Jalali S.G., Sohrabi H., Shirvany A. (2017): Variability in leaf and crown morphology correlated with light availability in five natural populations of *Quercus castaneifolia* C.A. Mey. J. For. Sci., 63: 275–281.

In this study, we investigate seedlings of *Quercus castaneifolia* C.A. Mey, from five different provenances for the research on leaf and crown morphological variations in relation to a light gradient under controlled conditions in a greenhouse. The results show that significant variations occurred in many parameters due to the effects of light availability. The seedling responses to low light include the proportional allocation of more biomass to leaves, leading to higher leaf mass, leaf area, crown area, specific leaf area and leaf area ratio, in contrast, the seedlings grown in high irradiance faced a high temperature resulting in higher transpiration. At this period, seedlings alter their leaf and crown size to prevent overheating. In this experiment, in spite of the same treatments in controlled conditions in a greenhouse, the seedlings from different provenances indicate different responses to light levels. It seems that the seedlings try to maximize their surface area for the intake of light as the most limiting resource in wet provenances. Such responses under the same treatment are adaptive strategies which allow oak seedlings to have the best function under stressed conditions. For *Q. castaneifolia* as a species with broad fundamental niches in Hyrcanian forests, these variations may be achieved by a combination of genotypic differentiation and phenotypic plasticity.

Keywords: plasticity; Hyrcanian forest; controlled conditions; leaf mass; leaf area; leaf area ratio

Leaves are important organs for photosynthesis and play an important role in survival and growth of a plant. Leaves react most sensitively to environmental factors such as soil moisture, nutrients and light (Roche et al. 2004; Sugiura, Tateno 2011); subsequently, the causal relationships between leaf traits and various environmental factors can be recognized. The leaf shape and structure are defined mainly in a brief period of primary morphogenesis based on the possible role of reaction-diffusion systems and can be altered by the allometric expansion (Franks, Britton 2000; Dengler, Kang 2001).

Phenotypic plasticity also occurs to produce a range of leaf traits that are environmentally affected. Many previous studies have revealed that leaf physiognomy is an excellent tool for ecological studies and geometric measurement of leaf morphology is easy and interpretable, then it should receive much attention in future.

Light is one of the ecological controllable factors in the forest that can strongly influence seedling growth and its future development (James, Bell 2000). Leaf properties often change in accordance with available light (Sugiura, Tateno 2011). In low irradiance, light is considered a limiting resource, so in order to enhance light capture, more biomass is allocated proportionally to leaves, leading to a higher leaf mass ratio, specific leaf area, leaf area ratio but less to roots. Wide crowns with a high leaf area are an effective strategy to reduce self-shading (Poorter 1999). In high irradiance, the radiation load is increased. Thus, a high temperature resulting in higher transpiration is a serious problem for the seedlings. At this period, they invest more biomass into root mass to facilitate water uptake and to alter their leaf size to prevent overheating. The requirements for optimal leaf functioning often change in accordance with irradiance. It is assumed that leaf traits with high plasticity to irradiance are more important for plant functioning in different light environments than traits with little or no plasticity (Rice, Bazzaz 1989; Rozendaal et al. 2006). Plasticity is usually defined as the differential response of a genotype to different environmental conditions (Rozendaal et al. 2006). The importance of plasticity also differs among leaf traits. It was found that physiological traits show higher plasticity than morphological traits (Valladares et al. 2000; Bloor, Grubb 2004; Rozendaal et al. 2006).

In this study, we chose leaves from *Quercus castaneifolia* C. A. Mey, the chestnut oak from five different provenances for the research of leaf morphological variations to a light gradient. Chestnut oak is one of the most valuable species in Hyrcanian forests (Sabeti 1994). These forests with a very unique climatic variation are a green belt stretching over the northern range of Alborz Mountain to the coast of the Caspian Sea (Rouhi-Moghaddam et al. 2008). It is characterized by various ecological conditions from 213 to 2,200 mm precipitation, zero to 5,671 m elevation. Precipitation decreases climatically zonewise regularly from west to east (Domoers et al. 1998). This species is a light demanding deciduous tree, distributed either in pure or mixed populations with hornbeam from the coastal plains to the highlands along the southern shore of the Caspian Sea from west to east (Sabeti 1994).

In this study, we want to answer these questions: how do chestnut oak leaves respond to a light gradient? Can seed provenance affect leaf light responses? The experiment was conducted in controlled conditions in a greenhouse.

MATERIAL AND METHODS

Descriptions of collected provenances. To conduct this study, five provenances of chestnut oak were collected in the form of seeds along the precipitation gradient from west to east of the Hyrcanian forests (from 2,045 mm in the west to 488 mm in the east). The general characteristics of the studied sites are shown in Table 1. This experiment was conducted in a greenhouse at the Faculty of Natural Resources and Marine Sciences of Tarbiat Modares University, situated within the Hyrcanian forests (36°34'54''N, 52°2'32''E, mean sea level: –22 m) in Mazandaran province (North of Iran).

Light regime treatment. To study the leaf morphological response of chestnut oak seedlings to a light regime, seven different irradiances, i.e. 10, 20, 30, 50, 60, 70 and 100%, were considered. The inner space of the greenhouse was divided into six parts (as shade houses). Each part was approximately 3.5 m long by 1.5 m wide and 2.2 m high. Then, the six specified irradiances were provided by covering the walls of the shade houses using layers of neutral plastic, polyethylene, which transfers 90% of full light, and covering the roofs with an increasing number of layers of neutral shade. Each extra layer intercepted 10% of the incoming light. Seedlings grown in the open area received a 100% irradiance level. Each irradiance level contained 150 oak seedlings from five provenances (30 seedlings were placed randomly in the treatment for each provenance), plus 40 cm buffer at either of the northern and southern end to avoid edge effects. Photosynthetically active radiation measurements were done in the shade houses based on comparisons of treatment vs. open sky instantaneous read-

Table 1. Characteristics of studied ecotypes (Pilembera – western provenance, Kelardasht, Lajim – central provenances, Kordkûy, Loveh – eastern provenances)

Province	Provenances	Altitude (m a.s.l)	Coordinates	Temperature* (°C)	Rainfall* (mm)
Guilan	Pilembera	650	37°34'25''N 49°01'40''E	15.1	2,045
Mazandaran	Kelardasht	1,000	36°35'52''N 51°05'30''E	16.4	1,293
	Lajim	800	36°18'22''N 53°05'48''E	18.0	703
Golestan	Kordkûy	800	36°43'27''N 54°07'21''E	17.8	601
	Loveh	800	37°21'11''N 55°39'44''E	17.8	488

*annual mean

ings performed at noon at seedling height with an "SA" quantum sensor (LI-COR Biosciences, USA) (Bloor, Grubb 2004) (Table 2).

Growth treatment. Chestnut oak seeds were collected from about 10 to 15 mature healthy trees with a diameter range of 40–50 cm, located in five provenances. Seeds were sown in plastic pots (15 × 10 cm) filled with one-third of forest top soil and two-thirds of river sand. The oak forest region near the study site was a source of the forest soil. The forest soil was used to provide a substrate with natural composition of macro- and micronutrients and the river sand provided a texture with adequate drainage, which allowed for daily watering of the seedlings and facilitated the harvest of the whole root system, including fine roots. After germination in spring, in late March to early April, seedlings were positioned at a 10% irradiance level. Moving the seedlings to higher irradiance levels was carried out gradually to avoid bleaching or wilting in response to the transfer, and they were left to grow, and were watered twice weekly.

Sampling was carried out once the seedlings had been growing for nine months since germination (21 to 23 November). The crown length at two perpendicular directions and the height of the insertion point of the lowest leaf were measured. The leaf size (area, perimeter, length, width, length to width ratio) in three seedlings was determined for all leaves along the stem with a CI-202 portable laser leaf area meter (CID, Inc., USA). After sampling, the seedling leaves were removed and oven-dried at 70°C for at least 48 h for estimating the leaf dry weight (Poorter 1999). Sampling was carried out from three randomly selected individuals of every provenance from every shade house. Detailed

Table 2. Rank order of the treatment based on comparisons of treatment vs. open sky instantaneous readings made at noon at seedling height with a quantum sensor

Irradiance treatment (%)	PAR (% of full daylight ± SE)
100	100.00* ± 0.00
70	70.40 ± 2.29
60	57.19 ± 2.21
50	49.12 ± 2.39
30	31.17 ± 3.42
20	19.62 ± 3.23
10	11.02 ± 0.56

PAR – photosynthetically active radiation, SE – standard error, *mean of 5 replicates ± SE

information on morphological parameter measurements and definitions is shown in Table 3.

Statistical analysis. Two-way ANOVA was used to test the differences between the gradients of light and provenances in morphological parameters, using light treatment and provenances as fixed factors. All dependent variables were transformed to natural logarithms before analyses (Poorter 1999). Plasticity was calculated as the absolute difference between the maximum trait value at one of the light levels and the minimum trait value at the other light level divided by the maximum value and multiplied by 100 (Valladares et al. 2000; Rozendaal et al. 2006). Total plasticity per provenance was expressed as the averaged plasticity of all leaf traits. Plasticity (Pl) was calculated by Eq. 1:

$$Pl = \frac{\text{max trait value} - \text{min trait value}}{\text{max trait value}} \times 100 \quad (1)$$

All statistical analyses were performed by the SPSS software package (Version 17.0, 2008).

Table 3. Leaf morphological parameters and their definitions

Variable	Definition
LDM (g)	leaf dry mass
SLA ($m^2 \cdot kg^{-1}$)	specific leaf area = ratio of leaf area to leaf dry mass
LAR ($m^2 \cdot kg^{-1}$)	leaf area ratio = total leaf area/total seedling dry mass
LN	leaf number
RCRD (%)	relative crown depth = 100 × (stem length – crown height)/stem length
CA (m^2)	crown area = π × 0.25 × average crown width × crown length
LA (cm^2)	leaf area
LL (cm)	leaf length
LW(cm)	leaf width
LP(cm)	leaf perimeter
LE	leaf elongation = ratio of leaf length to leaf width
CL (cm)	crown length
CD (cm)	crown depth
TLA (m^2)	total leaf area = total leaf area per plant

RESULTS

The results of the two-way ANOVA are given in Table 4 showing the effects of different gradients of light availability and provenances on leaf morphological parameters. There was a significant variation between provenances and light levels at the end of the experiment. Most parameters are influenced by light and provenances but, as indicated by the high *F*-values, the light levels were the most important determinant of variation in leaf morphology. All parameters were significantly influenced by light levels except leaf number (LN) and crown depth (CD).

Graphs are shown of the corrected values for those variables largely influenced by light (Fig. 1). Most leaf morphological parameters were negatively correlated with light except relative crown depth (RCRD), other parameters such as leaf perimeter (LP), leaf area (LA), leaf area ratio (LAR), leaf length (LL), leaf width (LW), crown length (CL) and total leaf area (TLA) decreased with light in a linear way, in contrast RCRD increased with light. Leaf dry mass (LDM), specific leaf area (SLA), crown area (CA), leaf elongation (LE) responses to light were logarithmic and LP showed a polynomial response to light.

In wet provenances the variations in LDM, LA, CA, RCRD, and CL were strongly correlated with light, but in dry provenances this relation gradually disappeared (refer to the coefficient of determination R^2 in Fig. 1). In contrast, the relationship between light and LW was weak in wet provenances and became gradually stronger in dry ones.

As Table 5 indicates, CD (Pl: 57.56%), SLA (Pl: 52.76%), TLA (Pl: 52.06%) and LAR (Pl: 52%) showed the highest plasticity and the lowest plasticity belonged to LE (Pl: 16.8%), LL (Pl: 29.53%) and LW (Pl: 30.96%).

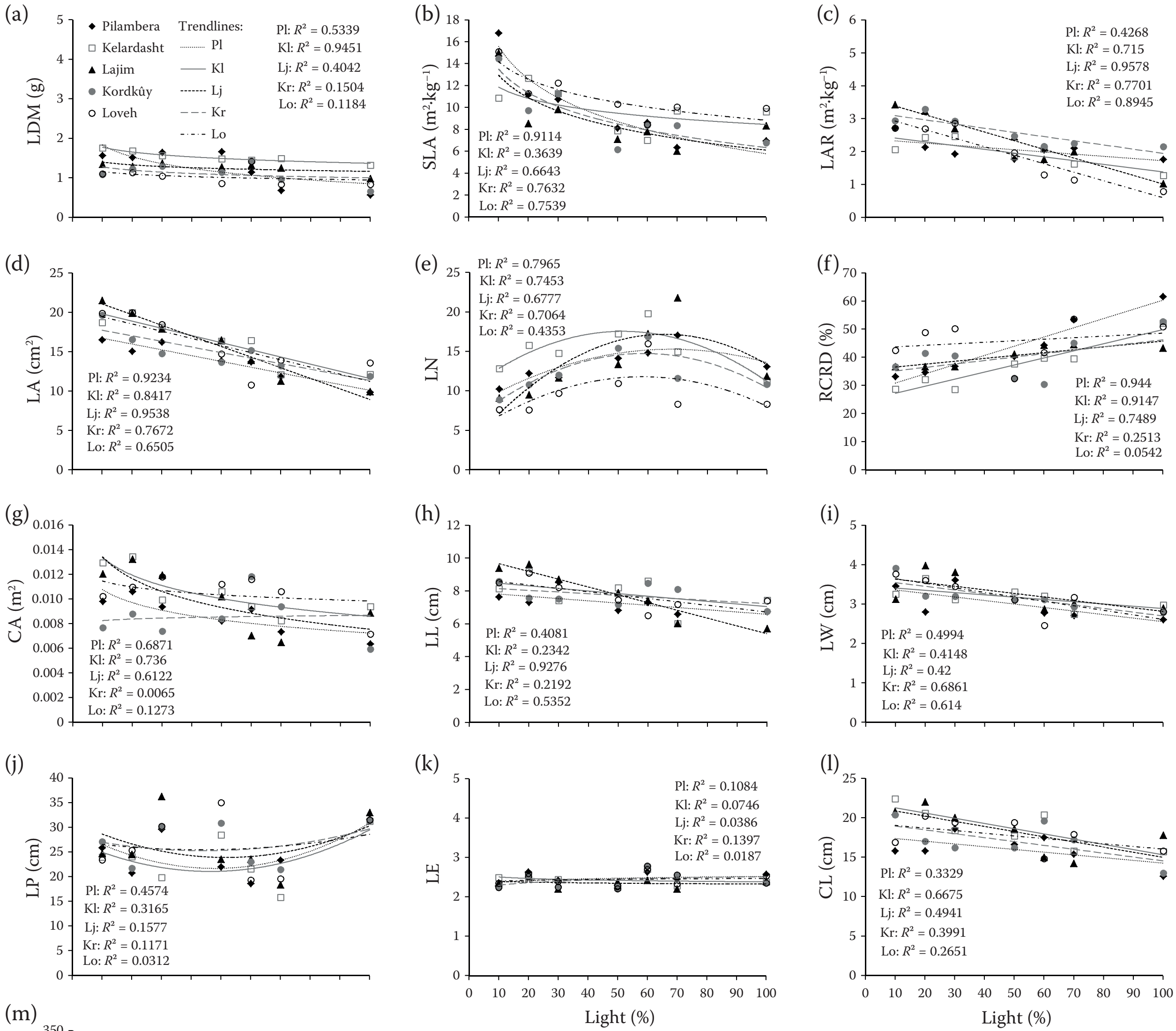
DISCUSSION

As the results show, most of the investigated variables are strongly influenced by the light environment (Table 4), indicating the importance of the irradiance effects on variables related to the variation in leaf characteristics. Light is highly variable in the forest understorey (Bloor, Grubb 2004; Rozendaal et al. 2006) and the ability to respond to different light levels may be critical to seedling growth success. From many studies, leaves are very sensitive to changes in light levels and as responding to it (Poorter 1999; James, Bell 2000; Bloor, Grubb 2004; Rozendaal et al. 2006; Xu et al. 2008; Kelly et al. 2009; Delagrange 2011). Seedling responses to low light include the proportional allocation of more biomass to leaves, leading to higher LDM, LA, SLA and LAR. By increasing the area of a given unit of leaf biomass, the interception of light is increased under low-light conditions. Wide crowns with a large leaf area are also an

Table 4. Two-way ANOVA with light (*n* = 7) and provenances (*n* = 5) as fixed factors

Variable	Light		Provenances		Light × provenances	
	F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
LDM (g)	**3.356**	***	2.32	NS	0.885	NS
SLA ($m^2 \cdot kg^{-1}$)	**5.01**	***	0.92	NS	0.694	NS
LAR ($m^2 \cdot kg^{-1}$)	**2.909**	**	1.037	NS	0.67	NS
LN	2.742	*	**3.628**	*	1.294	NS
RCRD (%)	**3.951**	***	2.159	NS	1.114	NS
CA (m^2)	**4.619**	***	4.429	***	2.061	***
LA (cm^2)	**31.39**	***	7.05	***	4.11	***
LL (cm)	**26.29**	***	4.57	***	7.6	***
LW (cm)	**28.85**	***	3.79	*	4.72	***
LP (cm)	**106.04**	***	5.68	***	16.11	***
LE	**19.65**	***	1.66	NS	2.34	***
CL (cm)	**7.466**	***	4.943	***	2.488	***
CD (cm)	10.018	***	**13.142**	***	1.15	NS
TLA (m^2)	**9.057**	***	4.488	**	0.832	NS

LDM – leaf dry mass, SLA – specific leaf area, LAR – leaf area ratio, LN – leaf number, RCRD – relative crown depth, CA – crown area, LA – leaf area, LL – leaf length, LW – leaf width, LP – leaf perimeter, LE – leaf elongation, CL – crown length, CD – crown depth, TLA – total leaf area, *F* values of factors having the largest effect on leaf variables are in bold, *P* – significance, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS (not significant) = $P > 0.05$



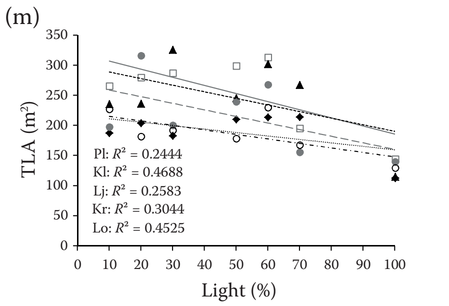


Fig. 1. Morphological responses: leaf dry mass – LDM (a), specific leaf area – SLA (b), leaf area ratio – LAR (c), leaf area – LA (d), leaf number – LN (e), relative crown depth – RCRD (f), crown area – CA (g), leaf length – LL (h), leaf width – LW (i), leaf perimeter – LP (j), leaf elongation – LE (k), crown length – CL (l), total leaf area – TLA (m) to light for five provenances, back transformed logarithms are shown. Graphs are shown for those variables largely influenced by irradiance at the final harvest

Pl – Pilembera, Kl – Kelardasht, Lj – Lajim, Kr – Kordkûy, Lo – Loveh

effective strategy to reduce self-shading. Seedlings grown in high irradiance faced a high temperature resulting in higher transpiration which is a serious problem for their survival. At this period, seedlings alter their leaf size and RCRD to prevent overheating leading to a decrease in LA, TLA, LL, LW and LN. Leaf perimeter increased in high light to exchange water vapour. These results are in line with Poorter (1999) and Fila and Sartorato (2011) studies.

Although all seedlings were exposed to the same treatments in controlled conditions in a greenhouse, as presented by R^2, the variations in LDM, LA, CA, RCRD, and CL were strongly correlated with light in wet provenances compared with the seedlings of the dry ones. In contrast, the relationship between light and LW was weak in wet provenances and became gradually stronger in the dry ones. Changes in leaf and crown size as major factors dictating the light interception could be helpful for understanding seedling strategies in natural ecosystems. In general, plants growing in low-light environments have higher LA, LDM, CA and CL and lower RCRD than those growing in high-light environments regardless of functional groups. This issue could be explained by a

Table 5. Plasticity of estimated parameters in five different provenances

Provenance	LDM (g)	SLA ($m^2 \cdot kg^{-1}$)	LAR ($m^2 \cdot kg^{-1}$)	LN	RCRD (%)	CA (m^2)	LA (m^2)	LL (cm)	LW (cm)	LP (cm)	LE	CL (cm)	CD (cm)	TLA (m^2)
Pilembera	65.70	62.24	35.01	44.07	44.29	43.16	38.91	22.96	27.83	34.52	15.34	32.26	66.52	44.81
Kelardasht	24.57	44.62	48.06	44.23	52.86	35.22	39.86	34.33	24.80	49.32	17.23	29.91	65.72	53.96
Lajim	26.66	59.71	70.09	57.60	27.46	50.59	60.01	40.57	39.02	44.52	14.04	35.45	46.34	61.95
Kordkûy	53.61	53.14	34.43	49.24	44.57	41.84	41.52	21.41	28.57	31.78	19.35	36.27	56.44	55.85
Loveh	26.66	44.09	72.42	25.55	50.14	37.94	50.25	28.39	34.59	38.72	18.05	26.73	52.78	43.71
Plasticity mean	39.44	52.76	52.00	44.14	43.86	41.75	46.11	29.53	30.96	39.77	16.80	32.13	57.56	52.06

LDM – leaf dry mass, SLA – specific leaf area, LAR – leaf area ratio, LN – leaf number, RCRD – relative crown depth, CA – crown area, LA – leaf area, LL – leaf length, LW – leaf width, LP – leaf perimeter, LE – leaf elongation, CL – crown length, CD – crown depth, TLA – total leaf area

balanced growth hypothesis which predicts that, under a given regime of stresses, plants maximize their surface area for the intake of the most limiting resource (Markesteijn, Poorter 2009) such as water in dry provenances and irradiance in wet provenances. Adaptive strategies allow chestnut oak seedlings to have the best function under stressed conditions. For chestnut oak as a species with broad fundamental niches in Hyrcanian forests, variations in adaptive characteristics may be achieved by a combination of genotypic differentiation and phenotypic plasticity.

Plasticity was high (between 16.8 and 57.56%) for all morphological traits, and CD, SLA, TLA and LAR had the highest plasticity among all traits (Table 5). It was suggested that the leaf traits showing high plasticity in response to irradiance are more important to plant functioning in different light environments than the traits that show low or no plasticity (Rice, Bazzaz 1989; Rozendaal et al. 2006). In our experiment, CD and LE indicated the highest and the lowest plasticity, respectively. This suggests that CD, SLA, TLA and LAR with high plasticity are most important for light acclimation compared to LE. Poorter (1999) and Bloor and Grubb (2004) reported high plasticity in leaf traits in response to irradiance in their studies on morphological plasticity and on growth responses to an irradiance gradient, in shade-tolerant tropical rainforest tree seedlings exposed to light changes, and in 15 rain forest tree species, respectively. Similar results have been reported in other studies (James, Bell 2000; Rozendaal et al. 2006; Davi et al. 2008; Xu et al. 2008; Kelly et al. 2009; Delagrange 2011). SLA is one of the main leaf traits that characterize the species adaptation to environmental conditions (Davi et al. 2008). Similar trends have been found for leaf morphological responses to irradiance in similar experiments (Bloor, Grubb 2004; Rozendaal et al. 2006; Fila, Sartorato 2011).

CONCLUSIONS

This paper has presented the data on the response of morphological characters of leaves and crowns of *Q. castaneifolia* C.A. Mey seedlings originating from five provenances to different light availability. Our experiment revealed that in spite of the same treatments in controlled conditions in a greenhouse, the seedlings from different provenances indicate different morphological responses to light levels. It seems that phenotypic plasticity helps this species to adapt itself and grow under different ecological conditions. Future work could investigate light availability and other environmental factors in species with broad fundamental niches. This would allow the managers to plan applications of nature-based management of forests.

Acknowledgment

We are grateful to S. Azizi, and S. Gharemahmodli, for their help with harvesting and supporting. B. Naseri is acknowledged for his help in collecting seeds.

References

Bloor J.M.G., Grubb P.J. (2004): Morphological plasticity of shade-tolerant tropical rainforest tree seedlings exposed to light changes. Functional Ecology, 18: 337–348.

Davi H., Barbaroux C., Dufrêne E., Françoisa C., Montpied P., Bréda N. (2008): Modelling leaf mass per area in forest canopy as affected by prevailing radiation conditions. Ecological Modelling, 211: 339–349.

Delagrange S. (2011): Light- and seasonal-induced plasticity in leaf morphology, N partitioning and photosynthetic capacity of two temperate deciduous species. Environmental and Experimental Botany, 70: 1–10.

Dengler N., Kang J. (2001): Vascular patterning and leaf shape. Current Opinion in Plant Biology, 4: 50–56.

Domoers M., Kaviani M., Schaefer D. (1998): An analysis of regional and intra-annual precipitation variability over Iran using multivariate statistical methods. Theoretical and Applied Climatology, 61: 151–159.

Fila G., Sartorato I. (2011): Using leaf mass per area as predictor of light interception and absorption in crop/weed monoculture or mixed stands. Agricultural and Forest Meteorology, 151: 575–584.

Franks N.R., Britton N.F. (2000): The possible role of reaction-diffusion in leaf shape. Proceedings of the Royal Society B: Biological Sciences, 267: 1295–1300.

James S.A., Bell D.T. (2000): Influence of light availability on leaf structure and growth of two *Eucalyptus globulus* ssp. *globulus* provenances. Tree Physiology, 20: 1007–1018.

Kelly J., Jose S., Nichols J.D., Bristow M. (2009): Growth and physiological response of six Australian rainforest tree species to a light gradient. Forest Ecology and Management, 257: 287–293.

Markesteijn L., Poorter L. (2009): Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought and shade tolerance. Journal of Ecology, 97: 311–325.

Poorter L. (1999): Growth response of 15 rain-forest tree species to a light gradient: The relative importance of morphological and physiological traits. Functional Ecology, 13: 396–410.

Rice S.A., Bazzaz F.A. (1989): Growth consequences of plasticity of plant traits in response to light conditions. Oecologia, 78: 508–512.

Roche P., Diaz-Burlinson N., Gachet S. (2004): Congruency analysis of species ranking based on leaf traits: Which traits are the more reliable? Plant Ecology, 174: 37–48.

Rouhi-Moghaddam E., Hosseini S.M., Ebrahimi E., Tabari M., Rahmani A. (2008): Comparision of growth, nutrition and soil properties of pure stand of *Quercus castaneifolia* and mixed with *Zelkova carpinifolia* in the Hyrcanian forests of Iran. Forest Ecology and Management, 255: 1149–1160.

Rozendaal D.M.A., Hurtado V.H., Poorter L. (2006): Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. Functional Ecology, 20: 207–216.

Sabeti H. (1994): Forests, Trees and Shrubs of Iran. Yazd, Yazd University Press: 886.

Sugiura D., Tateno M. (2011): Optimal leaf-to-root ratio and leaf nitrogen content determined by light and nitrogen availabilities. PLoS ONE, 6: e22236.

Valladares F., Martinez-Ferri E., Balaguer L., Perez-Corona E., Manrique E. (2000): Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: A conservative resource-use strategy? New Phytologist, 148: 79–91.

Xu F., Guo W., Xu W., Wang R. (2008): Habitat effects on leaf morphological plasticity in *Quercus acutissima*. Acta Biologica Cracoviensia Series Botanica, 50: 19–26.

Received for publication December 15, 2016
Accepted after corrections May 2, 2017