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Variation of leaf morphological traits in natural populations of *Fagus orientalis* Lipsky in the Caspian forests of Northern Iran

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Abstract. Oriental beech (*Fagus orientalis* Lipsky) is a dominant tree species in the Caspian forests, where occupies approximately 18% of the forested area and produce more than 35% of the total wood stock volume in this region. However, little information is available about its variation along the Caspian forests of Northern Iran. This work studied the morphological variation of five native oriental beech populations growing in the western Caspian region in Guilan province (Astara, Asalem, Fuman, Chere, Shenrud). Eight leaf morphological traits, including leaf length, leaf width, petiole length, leaf area, leaf dry mass per unit leaf area, leaf thickness and leaf density were measured in 200 trees. The results showed that all measured leaf morphological traits were remarkably different among the populations, with the exception of distance between veins. A hierarchical classification of all populations led to the formation of three major groups: (i) Astara, (ii) Asalem, (iii) the rest of populations. Leaf morphological dissimilarities are possibly attributed to the genetic variations, developed as a result of adaptation to diverse environmental conditions. However, multisite common garden experiments would be needed in order to completely separate environmental and genetic factors explaining the observed level of natural variability.
Keywords Caspian forest, leaf area, leaf morphological traits, natural population, oriental beech.

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Introduction

Leaves are highly important organs of a tree, sensitive to growth conditions, especially during a leaf expansion phase (Masarovicova 1988, Bayramzadeh et al. 2008). Consequently, they can effectively adapt to the habitat from which the plants originate (Niinemets & Kull 1994, Niinemets 1995, Bussotti et al. 1995, Tognetti et al. 1995, García-Plazaola & Becerril 2000, Wittmann et al. 2001, Toan et al. 2010, Amjad Ali et al. 2011) by making appropriate changes in their morphology and anatomy (Castro-Diez et al. 1997, Gravano et al. 1999, Kull et al. 1999, Bussotti et al. 2000, Gratani et al. 2003). For example, *Fraxinus pennsylvanica*, *Quercus petraea* and *Fagus crenata*, the tree species distributed widely in North America, Italy and Japan respectively, exhibited morphological and physiological variations which could be related to their habitat (Abrams et al. 1990, Gratani et al. 2003, Bayramzadeh et al. 2008).

The analysis of morphological leaf traits provides deep insight into the taxonomy, genetics, biogeography and evolution, which are the parts of the broad classification of scientific areas related to an effective preservation of natural ecosystems (Main 1966). Therefore, such kinds of studies can be very useful in species with wide geographical ranges, for which the little information is available.

Up to now, examples of the morphological variation are remained undocumented for the Caspian forests of Northern Iran, where environmental and edaphic conditions differ noticeably. One of the tree species which occurs throughout the Caspian Sea provinces is oriental beech (*Fagus orientalis* Lipsky). It is a dominant tree species in the Caspian forests, which occupies approximately 18% of the forested areas in the region, mainly extending in a wide range of 700 to 2200 m above the Caspian Sea level and produce more than 35% of the total wood stock volume (Ahmadi et al. 2009).

In this study, five natural populations of oriental beech growing in the western Caspian forests were investigated to determine variations in leaf morphological traits. This study will make a suitable framework for any researcher, who would like to probe the genetic variation among natural populations of *Fagus orientalis* Lipsky in the Caspian forests of Iran.

Materials and methods

Study sites

The research was conducted in five natural populations of oriental beech, growing in the western Caspian region (Guilan province): Astara, Asalem, Fuman, Chere, and Shenrud (Figure 1). The populations were distant enough to present climatic as well as edaphic differences among them (Table 1). A seventeen-year meteorological data (1988-2005) extracted

Table 1 Locations, climatic and edaphic characteristics of five natural populations of *Fagus orientalis* in Guilan province.

Population	Longitude	Latitude	Yearly precipitation mean ± SD (mm)	Mean yearly temperature (°C)	Soil texture	pH mean ± SD	Organic matter mean ± SD (%)
Astara	48° 52’ E	38° 24’ N	1400 ± 64	15.1	Sandy loam	5.62 ± 0.12	5.53 ± 0.63
Asalem	48° 94’ E	37° 70’ N	1685 ± 124	16.3	Loam	5.47 ± 0.14	4.99 ± 0.82
Fuman	49° 18’ E	37° 13’ N	994 ± 49	15.9	Loam	5.17 ± 0.08	5.53 ± 0.56
Chere	50° 00’ E	37° 12’ N	1081 ± 73	16.4	Loam	5.72 ± 0.12	5.71 ± 0.85
Shenrud	49° 28’ E	36° 44’ N	1469 ± 71	16.7	Clay	7.72 ± 0.05	3.06 ± 0.54

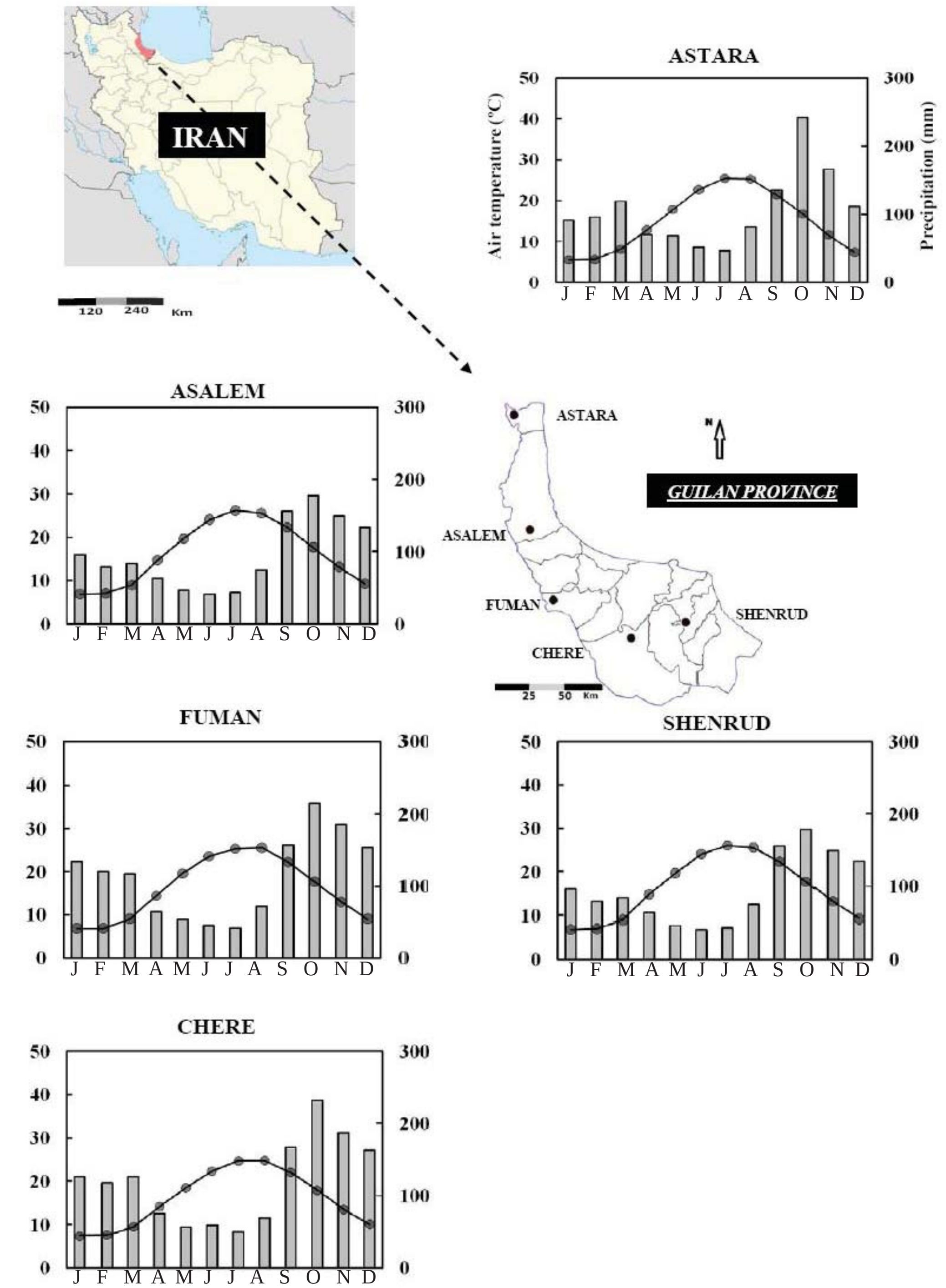

Figure 1 Localization of the five *Fagus orientalis* studied populations

from the records of the nearest meteorological stations for each location were related to the monthly means of precipitation and temperature (Fi-gure 1). To observe the dissimilarities of the edaphic factors, nine soil samples of 0-40 cm in depth were collected in each location. The samples were air dried and ground to enable passage through a 2 mm sieve. Soil analyses were carried out by the following methods: particle size distribution by the hydrometer method (Gee & Bauder 1986), organic matter (OM) content by the Walkley-Black procedure (Nelson & Sommers 1996), and pH values using a glass electrode in mixture of soil and deionized water (1:5, w/v).

Leaf collection and measurement

Forty fully expanded leaves (sunned leaves from the middle part of tree crown) were collected in summer 2010 from 40 healthy trees (in total 1600 leaves from each of the study locations), that were 30-50 cm in diameter at breast height. The trees grown in 1000-1300 m a.s.l. were selected randomly from north-facing slope of the locations.

Leaf length (*LL*, mm), leaf width (*LW*, mm), leaf area (*LA*, cm^2), and distance between veins (*DBV*, mm) were determined on fresh ones by an image analysis software, ImageJ (National Institutes of Health, Maryland, USA). A digital caliper with an accuracy of 0.01 mm was used to measure petiole length (*PL*, mm)(Figure 2). Leaves were oven-dried at 80º C for 72 hours and weighed for the calculation of leaf dry mass per unit leaf area (*LMA*, g cm^{-2}). Leaf density (*LD*, gr cm^{-3}) was obtained according to Witkowski and Lamont (1991) as the ratio of *LMA* to leaf thickness (*LT*, mm). *LT* was also measured on leaf cross sections.

Statistical analysis

Means of the morphological traits of every population were compared using Fisher tests of ANOVA analysis at 5% and 1% levels of significance. A Principal Component Analysis (PCA) was conducted on the individual-tree mean for each trait. An acceptable principal component (PC) solution was determined based on visual examination of the screen plot and the Kaiser criterion (all eigen values grea-

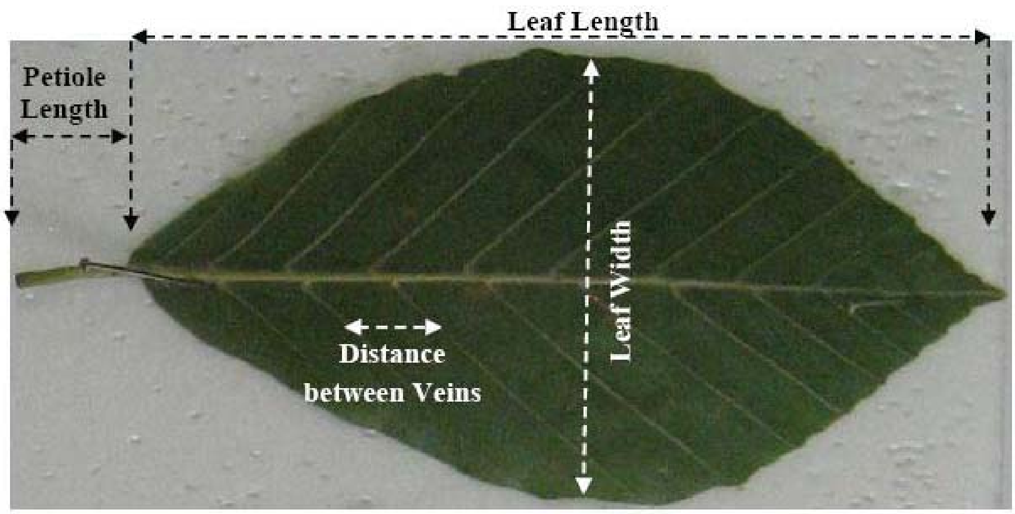

Figure 2 Diagram of oriental beech leaf illustrating measurements of leaf length (*LL*), petiole length (*PL*), leaf width (*LW*) and distance between veins (*DBV*)

ter than 1). Component scores and PC loadings were determined after Varimax axis rotation to maximize the variance of the squared loadings (Johnson & Wichern 1992). As a final point, a hierarchical cluster analysis was performed and a dissimilarity matrix was computed using the Ward (1963) method.

The statistical analysis of the data was carried out using the SPSS version 16.0 and the Stat Graphics Plus version 5.1 statistical packages.

Results

Morphological traits

Differences amongst natural populations of *Fagus orientalis* for all measured traits were remarkable, with the exception of *DBV* (Figure 3). Asalem had the largest leaf length (11.54 cm), *LW* (6.88 cm) and *LA* (43.81 cm^2), while Shenrud showed the smallest *LW* and *LA* (5.38 cm and 34.36 cm^2, respectively), but the smallest *LL* (10.08 cm) belonged to Astara (Figure 3 a, b, c). This result can be explained by the stronger relationship between *LA* and *LW* (r = 0.797), than between LA and *LL* (r = 0.387)(Table 2).

Astara and Fuman showed the highest and lowest values for *LT*, that were 0.013 mm and 0.007 mm, respectively (Figure 3f).

Asalem and Chere had the maximum and minimum values for *LMA* of 0.097 and 0.070 g cm^{-2}, respectively (Figure 3g). Shenrud (11.99 g cm^{-3}) and Astara (6.86 g cm^{-3}) showed minimum and maximum values for *LD* (Figure 3h).

Correlations between morphological traits

Table 2 shows the correlations between morphological traits. As shown in this table, leaf area was positively correlated with leaf length and leaf width ($p < 0.05$). However the relationship of leaf area with leaf width (r = 0.797) was stronger than with leaf length (r = 0.387)(Table 2).

There was a high positive correlation between dry mass of the leaves, leaf area, leaf width, leaf length and petiole length ($p < 0.05$). However, the correlation between dry mass of leaves and their thickness was not significant. The finding relationship between dry mass and leaf thickness for *Fagus orientalis* is in agreement with the findings of Teklehaimanot et al. (1998), who reported lack of significant relationship between dry mass and leaf thickness in *Parkia biglobosa*.

There was a very strong correlation between

Table 2 Pearson coefficient (r) of correlation between pairs of leaf morphological traits (n = 40). (*LW* - leaf width, *LL* - leaf length, *LA* - leaf area, *PL* - petiole length, *DM* - dry mass), *LT* - leaf thickness, *DBV* - distance between veins, *LMA* - leaf dry mass per unit leaf area, *LD* - leaf density)

	LW	*LL*	*LA*	*PL*	*DM*	*LT*	*DBV*	*LMA*	*LD*
LW	1.000								
LL	0.702**	1.000							
LA	0.797**	0.387*	1.000						
PL	-0.061	-0.544**	0.366*	1.000					
DM	0.862**	0.347*	0.851**	0.445**	1.000				
LT	-0.237	-0.802**	0.092	0.896**	0.255	1.000			
DBV	0.772**	0.564**	0.611**	0.285	0.863**	0.022	1.000		
LMA	0.339*	0.030	-0.006	0.263	0.521**	0.340*	0.655**	1.000	
LD	0.315	0.772**	-0.158	-0.949*	-0.187	-0.950**	0.026	-0.110	1.000

Note: ** - significant at $p < 0.01$ level, * - significant at $p < 0.05$.

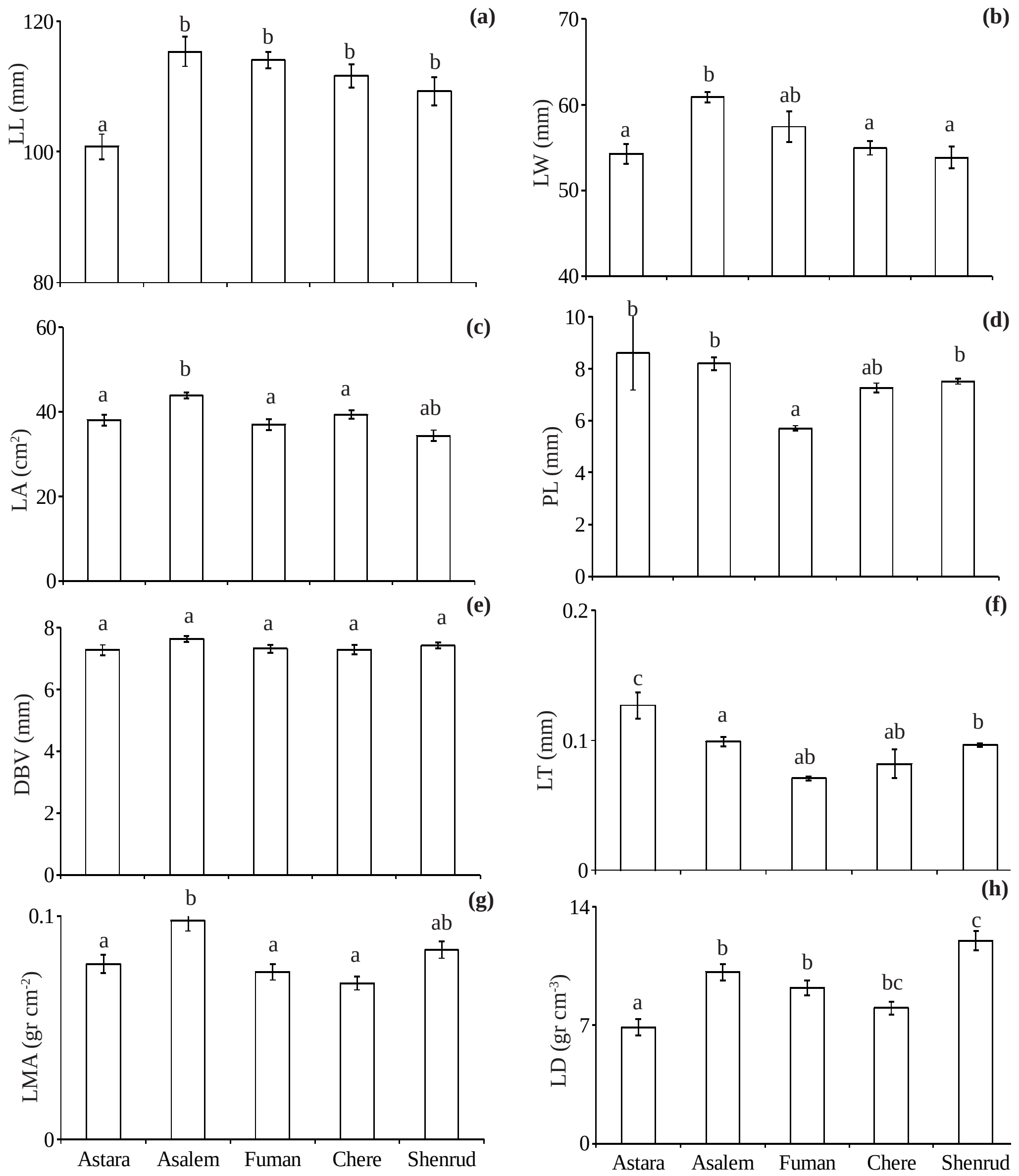

Figure 3 Different foliar traits in the five populations of *Fagus orientalis*: leaf length - *LL*, leaf width - *LW*, leaf area - *LA*, petiole length - PL, distance between veins - *DBV*, leaf thickness - *LT*, leaf dry mass per area - *LMA*, and leaf density - *LD*. Means with the same letters are not significantly different (ANOVA, $P < 0.05$).

LT and *LD* (r = -0.95). However, the linkage between *LT* and *LMA* as well as the correlation between *LD* and *LMA* were fairly weak (Pearson coefficient = 0.34 and -0.11, respectively). The different kinds of relationship among *LMA*, *LD* and *LT* were reported by different authors (Körner & Diemer 1987, Dijkstra 1990, Witkowski & Lamont 1991, Choong et al. 1992) suggesting that the above mentioned connections among *LMA*, *LD* and *LT* are possible.

Soil characteristics

Some of the studied edaphic factors also varied obviously among the five natural populations of *Fagus orientalis*. Soil texture was sandy loam for Astara, clay for Shenrud, and loam for the others. Soil pH and percentage of the organic matter in Shenrud were noticeably different from the others (7.72 and 3.06%, respectively).

Multivariate analysis

Principal component analysis reduced 9 input variables to 3 PCs explaining 97.04% of the total variance in our dataset. The first principal component, PCA1, explained 44.24% of the total variance, while PCA2 and PCA3 explained 40.65% and 12.15%, respectively (Table 3). Communality values (a measure of how well the input variables are explained by the three resulting PCs) were greater than 0.90 for all variables. The relationship of the original variables with PCs is indicated by PC loadings, which are akin to correlation coefficients between original characters and the PC (Table 3).

Scores in PC1 were positively related to PL and *LT* as well as negatively related to *LD* and *LL*. Scores in PC2 were positively related to *LW*, *LA*, *DM* and *DBV*. Scores in PC3 were positively related to *LMA*.

Finally, all morphological traits were used in a hierarchical cluster analysis (Figure 4). The resulting dendrogram allowed distinguishing three groups for this species in the studied area: (i) Astara, (ii) Asalem, and (iii) the rest of populations.

Discussion

We found a high level of morphological variability among populations of *Fagus orientalis* in Guilan province (Figure 3). Principal component analysis also indicate that there are several multivariate directions of variation in the morphological traits among the investigated populations, which are represented by single PCs. Variation in all input variables was adequately explained by 3 principal components (accumulated variables, PCs). Nevertheless, these axes of variation were independent of each other (because of the nature of PCA), there are relationships among the majority of

Table 3 Principal components (PC) solution of 9 variables measured in leaves from different populations of *Fagus orientalis*. Data are PC loadings and communalities determined after Varimax axis rotation. The PC loadings < 0.5 are not shown. *LW* - leaf width, *LL* - leaf length, *LA* - leaf area, *PL* - petiole length, *DM* - dry mass, *LT* - leaf thickness, *DBV* - distance between veins, *LMA* - leaf dry mass per unit leaf area, *LD* - leaf density.

Variable	PC1	PC2	PC3	Communality
lw	-	0.89	-	0.94
ll	-0.79	-	-	0.95
la	-	0.96	-	0.99
pl	0.93	-	-	0.95
dm	-	0.90	-	0.98
Lt	0.97	-	-	0.98
dbv	-	0.76	-	0.92
lma	-	-	0.97	0.99
ld	-0.99	-	-	0.99
Explained variance (%)	44.24	40.65	12.15	-

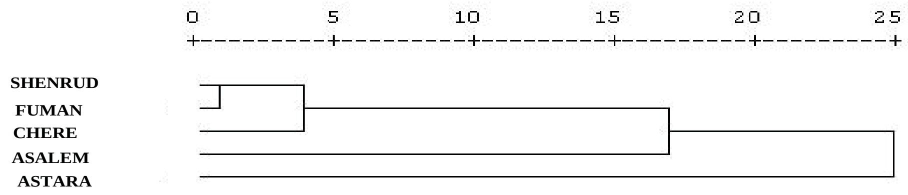

Figure 4 Dendrogram of the five populations of *Fagus orientalis* based on leaf morphological traits, made using hierarchical clustering method (Ward 1963)

the studied variables (Table 2), and these almost represent that similar environmental or genetic factors control the studied leaf traits in *Fagus orientalis* .

The observed foliar differences may be attributed to different genetic architectures developed as a result of adaptation to diverse environmental conditions exiting in distributional area of *Fagus orientalis*.

Our sampling covered a narrow latitudinal (36° 44' - 38° 24' N) and longitudinal (48° 52' E - 50° 0' E) ranges (Table 1), and the mean annual temperature didn't differ noticeably among the studied locations. Therefore, it can be said that the dissimilarities in leaves morphological traits of *Fagus orientalis* were unrelated to the latitude, longitude and mean annual temperature of the studied locations, but the chosen populations cover a rainfall gradient from 994 mm per year in Fuman, to 1685 mm per year in Asalem (Table 1).

Additionally, distribution of the precipitations in the studied locations (e.g., seasonal precipitation, January-March) differ obviously (Figure 1). Therefore, it seems that the dissimilarities in the amount and distribution of the precipitation in the studied locations are likely to influence transportable water by plants, which has to be transferred from stem to leaves and from leaves to atmosphere in the growing season, and induce variations in leaf characteris-tics (Koyama et al. 2004, Bayramzadeh et al. 2011).

Some of the studied edaphic factors also varied obviously among the populations. The difference of the studied edaphic conditions, however, is not in agreement with Figure 4 showing the three groups for *Fagus orientalis* in studied areas, and we suggest that the morphological leaf traits of *Fagus orientalis* are not affected significantly by the studied edaphic factors. However, it is supposed that combination of edafic factors with the precipitation might influence the foliar traits since the water-release characteristics of the soil are an important component effecting water availability (Warren et al. 2005).

In our study, we found a high level of morphological variability among populations of *Fagus orientalis* in Guilan province. However, multisite common garden experiments would be needed in order to completely separate environmental and genetic factors explaining the observed level of natural variability.

Conclusions

Morphological leaf traits noticeably differ among the five natural populations of *Fagus orientalis* throughout Guilan province in the western Caspian region of northern Iran. The observed foliar differences may be attributed to different genetic architectures developed as a result of adaptation to diverse environmental condition exiting in distributional area of *Fagus orientalis* and it seems that precipitation is the most important factor in this regard. Cluster analysis differentiated the 5 provenances in three groups: (i) Astara, (ii) Asalem, and (iii) the rest of populations.

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