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

V. Bayramzadeh

**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 grown 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, 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.

**Introduction**

Leaves are highly important organs of a tree, which are very sensitive to growth conditions, especially during a leaf expansion phase (Marsarovicova 1988, Bayramzadeh et al. 2008). Consequently, they can effectively adapt to the habitat from which the plants originate (Ni-

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 occurs throughout the Caspian Sea provinces is the 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 grown in the western Caspian forests were investigated to determine variations in leaf morphological traits. This study will make a suitable framework for 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 description**

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 from the records of the nearest meteorological stations for each location were related to the monthly means of precipitation and temperature (Figure 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:

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

**Figure 1** Localization of *Fagus orientalis* populations

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, cm²), and distance between veins (DBV) were determined on fresh ones by the 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). Leaves were oven-dried at 80°C for 72 hours and weighed for the calculation of leaf dry mass per unit leaf area (LMA, gr 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% level of significance. 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. 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 scree plot and the Kaiser criterion (all eigen values greater 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.

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 cm²). However, Shenrud showed the smallest LW, and LA (5.38 cm and 34.36 cm², respectively). Unlike, 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

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

= 0.80), rather than *LL* (*r* = 0.39)(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
gr cm$^{-2}$, respectively (Figure 3g). Shenrud (11.99 gr cm$^{-3}$) and Astara (6.86 gr cm$^{-3}$) showed minimum and maximum values for LD (Figure 3h).

Correlations between morphological traits. Table 2 shows the correlation 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 ($r = 0.80$) was stronger than leaf length ($r = 0.39$) (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), reported lack of significant relationship between dry mass and leaf thickness in Parkia biglobosa.

There was a highly correlated relationship between 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) suggest 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 3.05%, respectively).

**Multivariate analysis**

Principal component analysis reduced the 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). Communalities 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,

<table>
<thead>
<tr>
<th></th>
<th>LW</th>
<th>LL</th>
<th>LA</th>
<th>PL</th>
<th>DM</th>
<th>LT</th>
<th>DBV</th>
<th>LMA</th>
<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LW</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>0.702**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>0.797**</td>
<td>0.387*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>-0.061</td>
<td>-0.544**</td>
<td>0.366*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>0.862**</td>
<td>0.347*</td>
<td>0.851**</td>
<td>0.445**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>-0.237</td>
<td>-0.802**</td>
<td>0.092</td>
<td>0.896**</td>
<td>0.255</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBV</td>
<td>0.772**</td>
<td>0.564**</td>
<td>0.611**</td>
<td>0.285</td>
<td>0.863**</td>
<td>0.022</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMA</td>
<td>0.339*</td>
<td>0.030</td>
<td>-0.006</td>
<td>0.263</td>
<td>0.521**</td>
<td>0.340*</td>
<td>0.655**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>LD</td>
<td>0.315</td>
<td>0.772**</td>
<td>-0.158</td>
<td>-0.949*</td>
<td>-0.187</td>
<td>-0.950**</td>
<td>0.026</td>
<td>-0.110</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: ** - significant at $p < 0.01$ level, * - significant at $p < 0.05$
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 LL, 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 area studied: (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 as well indicated that there were several multivariate directions of variation in the morphological traits among the populations investigated, 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 the studied variables (Table 2), and these al-

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**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.  

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>Communality</th>
</tr>
</thead>
<tbody>
<tr>
<td>LW</td>
<td>-</td>
<td>0.89</td>
<td>-</td>
<td>0.94</td>
</tr>
<tr>
<td>LL</td>
<td>-0.79</td>
<td>-</td>
<td>-</td>
<td>0.95</td>
</tr>
<tr>
<td>LA</td>
<td>-</td>
<td>0.96</td>
<td>-</td>
<td>0.99</td>
</tr>
<tr>
<td>PL</td>
<td>0.93</td>
<td>-</td>
<td>-</td>
<td>0.95</td>
</tr>
<tr>
<td>DM</td>
<td>-</td>
<td>0.90</td>
<td>-</td>
<td>0.98</td>
</tr>
<tr>
<td>LT</td>
<td>0.97</td>
<td>-</td>
<td>-</td>
<td>0.98</td>
</tr>
<tr>
<td>DBV</td>
<td>-</td>
<td>0.76</td>
<td>-</td>
<td>0.92</td>
</tr>
<tr>
<td>LMA</td>
<td>-</td>
<td>-</td>
<td>0.97</td>
<td>0.99</td>
</tr>
<tr>
<td>LD</td>
<td>-0.99</td>
<td>-</td>
<td>-</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Explained variance (%) 44.24 40.65 12.15 -

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**Figure 4**  Dendrogram of the five populations of *Fagus orientalis* based on leaf morphological traits, made using hierarchical clustering method (Ward 1963)
most 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 (from 36° 44’ N to 38° 24’ N) and longitudinal (from 48° 52’ E to 50° 0’ E) ranges (Table 1). As well, 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. Unlike the chosen populations cover a rainfall gradient of 1685 mm per year in Asalem to 994 mm per year in Fuman (Table 1). As Asalem and Astara, which have the higher rainfalls than the other locations (Table 1), differed significantly from the other locations in the studied leaf morphological traits (Figure 4), leaf morphological dissimilarities is possibly attributed to the genetic variations caused by the rainfall of the locations.

Some of the studied edaphic factors also varied obviously among the populations. The difference of the studied edaphic conditions, however, was not in agreement with Fig. 4 showing the three groups for *Fagus orientalis* in studied areas. The authors concluded that the morphological leaf traits of *Fagus orientalis* were not affected significantly with the studied edaphic factors in this research.

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

1. Morphological leaf traits noticeably differed among the five natural populations of *Fagus orientalis* throughout Guilan province in the western Caspian region of northern Iran.
2. The observed foliar differences may be attributed to different genetic architectures developed as a result of adaptation to diverse rainfall exiting in distributional area of *Fagus orientalis*.
3. Cluster analysis differentiated the 5 provenances in three groups: (i) Astara, (ii) Asalem, and (iii) the rest of populations.

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### References


