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Stomatal Characteristics of *Fagus orientalis* Lipsky in Geographically Separated Locations 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 which occupies approximately 18% of the forested area in this region. However, little information is available about its variation along the Caspian forests of Northern Iran. This work studied the stomatal characteristics of five native oriental beech populations grown in the Western Caspian region in Guilan province. To determine the variations in stomatal characteristics, stomatal density and stomatal pore length were measured in this study. The results showed that the differences amongst natural populations of *Fagus orientalis* for measured traits were remarkable ($p<0.05$). The observed stomatal differences might be explained by the geographical variation (such as climate, latitude and altitude) among seed sources of *Fagus orientalis* as the stomata are one of the key regulation factors of the relation between plant and external environment.

Key words: Caspian forest, *Fagus orientalis*, geographical variation, stomatal density, stomatal pore length

INTRODUCTION

Vascular plants can alter much of their bodies in order to better adapt themselves to the environment (Castro-Diez *et al*., 1997; Gravano *et al*., 1999; Kull *et al*., 1999; Bussotti *et al*., 2000; Gratani *et al*., 2003). Thus, from an ecological viewpoint, the knowledge of the stomata architecture and function is a critical matter to understand the fitness of plants to their environment. In addition, many efforts have been done to give stomata a taxonomic significance (Metcalfe and Chalk, 1979; Evert and Eichhorn, 2006). Therefore, such kinds of studies can be very useful in species with geographical ranges for which the little information is available.

As yet, examples of the variations in stomatal characteristics remain undocumented for widely distributed trees in 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 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 and 2400 m above the Caspian Sea level and produce more than 35% of the total wood stock volume (Ahmadi *et al*., 2009).

The aim of the current research was to study the variability of the stomatal characteristics in five natural populations of oriental beech grown in the western Caspian forests. This study will make a suitable framework for researcher, who would like to perform taxonomy and/or probe the genetic variation among natural populations of *Fagus orientalis* L. in the Caspian forests of Iran.

MATERIALS AND METHODS

Study sites description: The study was conducted in five natural populations of oriental beech grown in the western Caspian region in Guilan province, called Astara, Asalem, Fuman, Chere and Shenrud. The populations were distant enough to show climatic as well as edaphic differences among them (Table 1). A seventeen-year meteorological data (1988-2005) which were extracted from the records of the nearest meteorological stations, for each location were related to the monthly means of precipitation and temperature.

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

Measurements of stomatal characteristics: For measurements of the Stomatal Density (SD, stomatal number mm^{-2}) and stomatal pore length (SPL μm), small squares (~5 mm^2) of the leaves were cut and placed in 5 mL of 30% H_2O_2 solution with 0.1 g of tetrasodium pyrophosphate and then were warmed at 35°C about one week . Cuticles were removed from the solution, washed, stained with 1% safranin and examined under a light microscope (Bayramzadeh *et al*., 2011; Hovenden and Brodribb, 2000).

Statistical analysis: Means of the studied traits of every population were compared using Fisher tests of ANOVA analysis. The statistical analysis of the data was carried out using the Stat Graphics Plus version 5.1 statistical package (Stat Point, Inc., Northern Virginia, USA).

RESULTS AND DISCUSSION

Differences amongst natural populations of *Fagus orientalis* for the measured traits were remarkable ($p<0.05$) (Fig. 1, 2). Chere with the smallest stomatal density (102±7.27 stomatal number mm^{-2}) noticeably differed from the others. Chere and Astara showed larger stomatal pore length (12.85±0.37, 12.55±0.71 μm, respectively) in compare with other locations. Stomatal density and stomatal pore length showed negative significant relation($r = -0.70$; $p<0.01$).

As among the environmental factors, precipitation is the most important factor affecting the stomatal characteristics (Aasamaa *et al*., 2001), the relationship between precipitation and stomatal characteristics were exmined in this study. Stomatal density and stomatal pore length didn't relate to the amount of precipitation in the studied locations ($r = 0.30$ and $r = -0.14$, respectively).

As the stomata are one of the key regulation factors of the relation between plant and external environment (Malone *et al*., 1993; Bruschi *et al*., 2000; Van Hoof *et al*., 2006), the observed

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

Population	Longitude	Latitude	Yearly precipitation mm±(SD)	Mean yearly temperature (°C)	Soil texture	pH ±SD	Organic matter% ±(SD)
Astara	48°52' E	38°24' N	1400±(64)	15.1	Sany 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°0' 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)

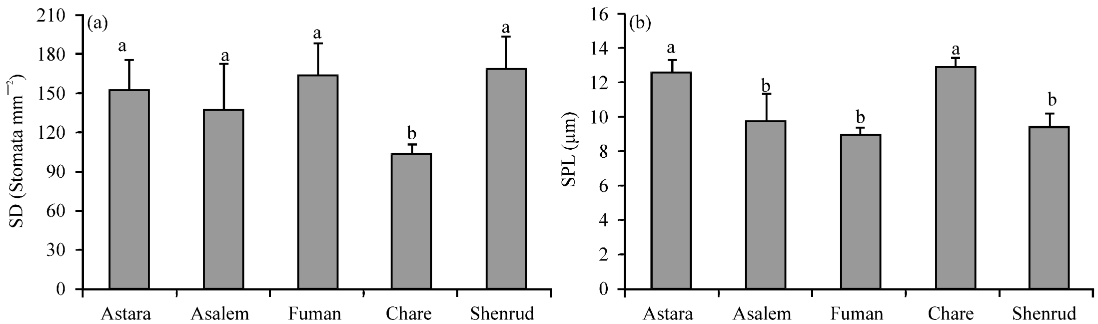

Fig. 1(a-b): Stomatal charactristics in the five natural populations of *Fagus orientalis*. Stomatal density (SD, stomata mm^{-2}), stomatal pore length (SPL, µm). Means with the same letters are not significantly different ANOVA, P<0.05

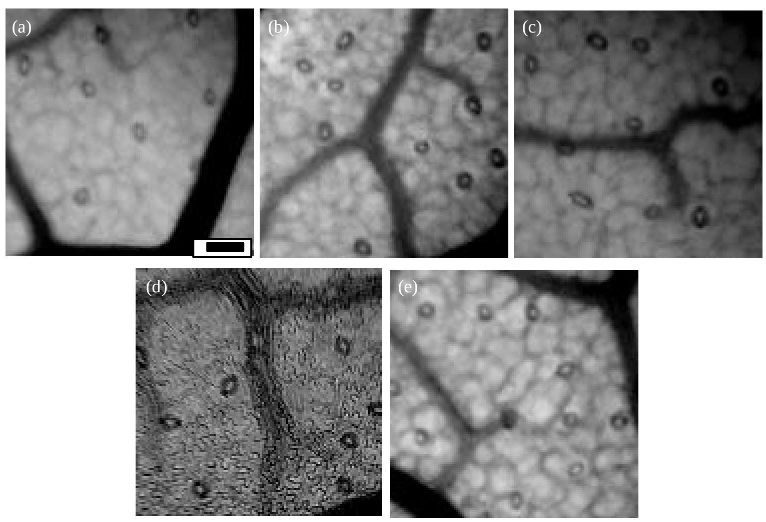

Fig. 2(a-e): Representative of the stomata in (a) Astara, (b) Asalem, (c) Fuman, (d) Chere and (e) Shenroud. Scalbar = 100 µm

stomatal differences among five natural populations of *Fagus orientalis* might be explained by the geographical variation (such as climate, latitude and altitude).

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 stomatal characteristics of the natural populations of *Fagus orientalis* were unrelated to the latitude, longitude and mean annual temperature of the studied locations. Unlike the chosen populations cover a precipitation gradient of 1685 mm per year in Asalem to 994 mm per year in Fuman (Table 1). Therefore, it can be suggested that the dissimilarities in the amount of the precipitation in the studied locations are likely to induce transportable water by plants which has to be transferred from stem to leaves and from leaves to atmosphere by stomata in the growing season and make variations in stomatal characteristics (Bayramzadeh *et al.*, 2011). This suggestion can be explained by the fact that, the plant must maintain movement of water from the soil to the leaf and stomatal responses to environmental change are a major feature of this maintenance (Raven, 2002).

However, our results showed that the stomatal traits were not related to the precipitation of the studied locations. This result can be related to the fact that means annual precipitation and other long-term measures (e.g., November-March precipitation) can be poor indicators of water availability. Evaporative demand, the amount of competing (transpiring) vegetation, the water-release characteristics of the soil and soil depth are important components of water availability (Warren *et al.*, 2005).

Therefore, it can be said that possibly the variations caused by the precipitation of the locations, however, multisite common garden experiments would be needed in order to completely separate environmental and genetic factors explaining the observed level of natural variability. Also further experiment on leaf anatomical traits such as determining of epidermis, cuticle, palisade and spongy thickness is needed in *Fagus orientalis*, since leaf anatomical traits are good indicators of habitat quality and they manifest variability in relation to climatic conditions (Batos *et al.*, 2010).

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