Effects of elevational gradient on leaf and stomatal morphology of Caucasian alder (*Alnus subcordata*) in the Hyrcanian forest, Iran

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Abstract

Akbarian, M.R., Tabari, M., Akbarinia, M., Zarafshar, M., Meave, J.A., Yousefzadeh, H., Sattarian, A. 2011. Effects of elevational gradient on leaf and stomatal morphology of Caucasian alder (*Alnus subcordata*) in the Hyrcanian forest, Iran. *Folia oecol*., 38: 1–7.

Alnus subcordata C.A.Mey (Betulaceae) is a commercial, fast growing species that is widely distributed in the Hyrcanian forest of northern Iran. In this study, the effect of altitude on the whole leaf and stomatal morphology of this species was analyzed. In eastern Mazandaran province, ten sites were selected along an elevational gradient. The altitudinal separation between sites was 100 m. At each site, leaves from six individual trees were collected for determination of leaf traits. Correlation analyses showed a significant, positive relationship of tooth number with altitude, whereas leaf blade and petiole length were significantly but inversely related to this factor. Similarly, stomatal length decreased with the increasing altitude. A principal component analysis (PCA) was performed to identify those traits causing the main differentiation between sites. Leaf width and length, together with petiole length, were best correlated with PC1 scores, whereas stomatal traits were best correlated with PC2 scores. The large plasticity of the studied leaf traits of *A. subcordata* was confirmed through a plasticity analysis (Pl = 0.56). The trait displaying the lowest plasticity was leaf base shape (Pl = 0.2). A high plasticity in leaf traits subjected to environmental fluctuations was also observed, especially for apex length, leaf length, petiole length, tooth number and size of stomata. These findings may explain the broad elevational distribution of *A. subcordata* in the Hyrcanian forest. It is concluded that leaf base shape is a valuable trait for the taxonomy in the genus *Alnus*.

Key words

Alnus, leaf traits, PCA, plasticity, stomata

Introduction

The Hyrcanian forest (northern Iran) is one of the most ancient and unique forest communities of the world: about 80 tree and 50 shrub species occur in its less than two million hectares (Mohajer, 2007). *Parrotia persica, Populus caspica, Pterocarya fraxinifolia, Quercus castaneifolia, Alnus glutinosa and A. subcordata* are well-known representatives of the unique flora that thrives in this forest. *Alnus subcordata* (Caucasian alder) is one of the commercial and fast growing species that is currently exploited in the Hyrcanian forest. In addition to a substantial timber production, alder is important because of its ability to fix nitrogen into the soil. For this reason, alder is utilised as an auxiliary species for reforestation, especially for providing adequate growing

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conditions for beech saplings (Fagus orientalis), and for soil improvement (Taleshi et al., 2009). Alder displays a very broad ecological range, as it occurs across many elevational floors from the coastal plain at sea level to an altitude of around 2,000 m. Its wide elevational range suggests that this species is finely adapted to a large variety of ecological conditions.

As altitude changes in mountain systems, ecological, edaphic and climatic conditions such as temperature, rainfall, solar radiation vary considerably (Korner, 2007), and trees often show a range of responses to them, particularly by modifying their morphological and physiological attributes (Turesson, 1992; Velázquez-Rosas et al., 2002). Such changes may be achieved in two ways: plasticity or genetic changes (Bruschi et al., 2003; Hovenden and Vander Schoor, 2006).

Along an elevational gradient both leaf morphology and foliar shapes can vary noticeably (Hovenden and Vander Schoor, 2006). For example, a large variation in leaf traits of *Nothofagus* trees such leaf size, thickness and total leaf area associated with elevation (Hovenden and Vander Schoor, 2004). Similarly, large variations in leaf characteristics of *Parrotia persica* were observed along an elevational gradient of the Hyrcanian forests (Yousefzadeh et al., 2010). Interestingly, leaf morphology and ecological gradient are sometimes poorly correlated (Kovacic and Nikolic, 2005). The study of plant responses to environmental changes along elevational gradients is particularly relevant in the case of those species like *A. subcordata* that have very broad ecological amplitudes, as it may shed light on the prevailing mechanisms of species coexistence and unique responses of species to the environment (Qiang et al., 2003; Holland and Richardson, 2009; Royer et al., 2006). Accordingly, the aim of this study was to examine the variability of leaf traits in *A. subcordata* along the broad elevational gradient through which it occurs, focusing on general leaf and stomatal morphology.

Material and methods

The study was conducted in the eastern portion of the Hyrcanian forest (Mazandran province, Iran). Ten sites were systematically selected along the established transect to represent the altitudinal variation. The elevational difference between adjacent sites was 100 m (Table 1, Fig. 1). In October 2009, we selected six individual mature trees per each site. Several terminal leaves were collected from the outer light exposed part of the crown of each tree and then mixed in order to sample five leaves randomly. Only leaves lacking signs of abnormal growth, mechanical damage, or pathogen or insect infestation were used. The parameters measured included seven macro-morphological traits: lamina length (LL), lamina width (LW), lamina width at the first decile of leaf length from the base (LW 0.1), lamina width at the ninth decile of leaf length from the base (LW 0.9), petiole length (PL), apex length (AL), number of teeth (counted in a 2 cm section of the leaf margin) (Fig. 2). In addition, we calculated four ratios: leaf length/leaf width (LL/LW), leaf length/petiole length (LL/LP), leaf base shape character (LW 0.1/LW) and leaf apex shape character (LW 0.9/LW). These ratios, which represent independent shape variables, have been used extensively in leaf morphometrics analyses. Stomatal characteristics were determined on two leaves per each tree. The leaf was boiled for 15–20 minutes. Very thick epiderm layers from leaf were sampled by cutter. Stomatal characteristics were studied on 10 stomata per each leaf by light microscopy. Finally, the following variables related to leaf stomata were measured: stomata length (SL), stomata width (SW), stomata area (SA), and stomata density (SD).

Table 1. Geographical location and elevation of the sampling sites of leaves of *Alnus subcordata* in the Hyrcanian forest (northern Iran)

Site	Elevation [meter]	Longitude	Latitude
1	250	54°16′22″ E	36°45′07″ N
2	350	53°26′53″ E	36°41′24″ N
3	450	54°23′06″ E	36°44′44″ N
4	550	53°36′50″ E	36°40′50″ N
5	650	54°16′28″ E	36°44′29″ N
6	750	53°36′43″ E	36°40′21″ N
7	850	54°22′60″ E	36°47′30″ N
8	950	53°36′45″ E	36°40′07″ N
9	1,050	54°23′35″ E	36°47′04″ N
10	1,150	54°36′49″ E	36°39′56″ N

Variation in leaf parameters was examined using principal component analysis (PCA). Generally, PCA helps to describe the total variation in a sample in a few dimensions (Rholf, 1971). Eigenvectors were calculated to assess the contribution of each variable to site separation related to leaf traits (Zarafshar et al., 2010).

Total within-population plasticity (Pl) was calculated for each trait using the smallest and the greatest mean values, as follows:

$$Pl = 1 - (x/X)$$

where x is the smallest value and X is the largest value measured for any given leaf trait (Ashton et al., 1998; Bruschi et al., 2003).

Results

Table 2 summarises the results of PCA, including the eigenvalues, the proportion of the total variance explained

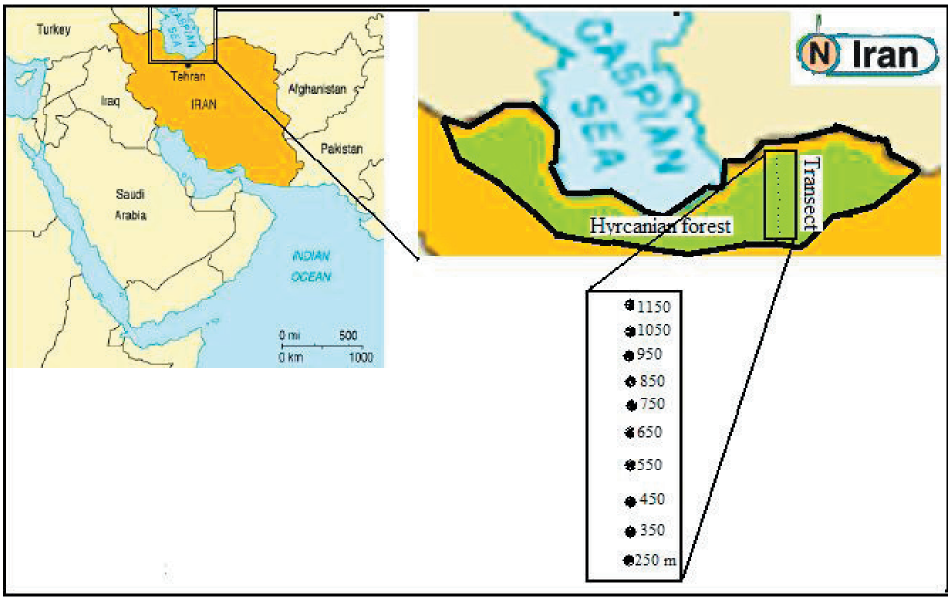

Fig. 1. Location of the sampling sites in the Hyrcanian forest (northern Iran).

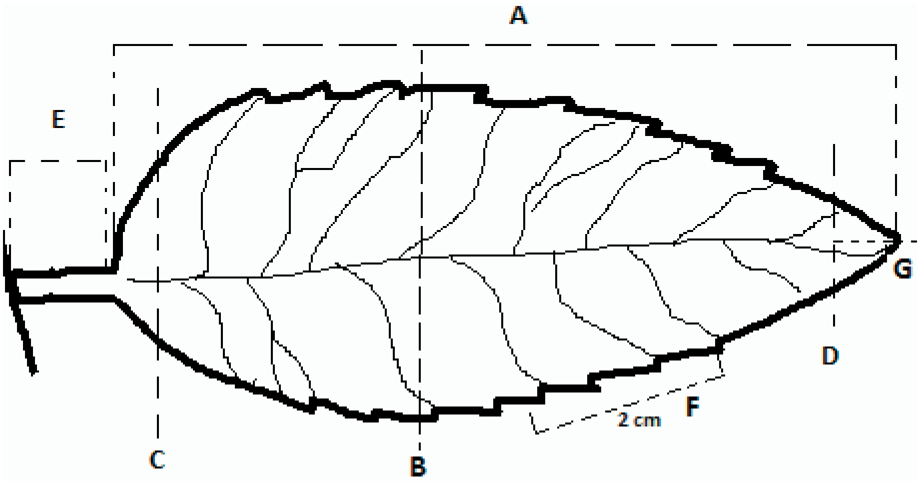

Fig. 2. Macro-morphological leaf traits used as input for the principal component analysis. A: lamina length (LL); B: lamina width (LW); C: lamina width at the first decile of leaf length (LW 0.1); D: lamina width at the ninth decile of leaf length (LW 0.9); E: petiole length (PL); F: number of teeth (counted in a 2 cm section of the leaf margin) (NT); G: apex length (AL).

by each component, and the cumulative proportion of this variance explained by the first components. The first four principal components accounted for 78% of the total variance of all traits, whereas the other components explained a small percentage of total variation (22%). Together, PC1 and PC2 accounted for 55% of the total variance (31% and 24.0%, respectively), indicating a high degree of correlation among the analysed parameters. The individual percentages of explained variance for PC3 and PC4 were 15% and 8%, respectively. Leaf size parameters such as lamina length, lamina width, lamina width at the ninth length decile, and petiole length were positively correlated with PC1, while stomatal length was negatively related to this component's scores. Leaf shape parameters were not significantly correlated with PC1. In turn, PC2 was positively related to all stomata parameters but stomata density. Lamina width at the first decile, leaf apex shape and leaf shape parameters showed strong correlations with PC3. PC4 was significantly, negatively related with tooth number and traits reflecting leaf base shape. Results of the correlation analyses between leaf traits and ecological (elevation) and geographical (longitude, latitude) factors are presented in Table 3. Tooth number was significantly and positively related to elevation, whereas lamina length and petiole length showed significant, negative correlations with this factor. This analysis showed that elevation is not the only factor capable of causing significant variation in leaf traits, as stomatal length was significantly and negatively related to latitude. The dissection of the shared variance components (elevation, tree, and error) based on the axes derived from the PCA showed that PC1 accounted for 49% of total variance related to elevation, for 19% of variance related to within-site tree diversity, and for 33% of total variance related to errors such as within-canopy leaf variability. In the second and third axis less than 20% of the total variance related to altitude was accounted for (Table 4).

Table 2. Correlation coefficients between leaf traits and four principal components analysis and proportion of variability by the first four components

		Factor 1	Factor 2	Factor 3	Factor 4
Leaf traits	LL [cm]	**0.33**	0.29	0.04	0.07
	LW [cm]	**0.35**	0.22	0.23	0.00
	LW 0.1 [cm]	0.10	–0.33	**0.57**	0.12
	LW 0.9 [cm]	**0.34**	0.22	0.25	–0.15
	PL [cm]	**0.35**	0.24	–0.01	–0.10
	NT	–0.10	–0.22	–0.09	**–0.54**
	AL [cm]	0.21	0.20	–0.25	–0.28
Leaf shape	LL/LW	– 0.05	0.16	**–0.39**	–0.16
	LW 0.1/ LW	– 0.07	0.00	0.07	**–0.68**
	LW 0.9/ LW	– 0.17	–0.23	**0.42**	0.07
	LL/PL	– 0.22	–0.07	0.03	0.07
Stomata trait	SL	**– 0.33**	**0.29**	0.12	–0.05
	SW	**– 0.24**	**0.37**	0.14	–0.05
	SA	**– 0.29**	**0.36**	0.14	–0.06
	SD	0.14	–0.28	0.23	–0.20
Variance	Eigen value	4.89	3.82	2.42	1.28
	Explained variance	30.58	23.90	15.17	8.05
	Cumulative variance [%]	30.58	54.48	69.66	77.71

Bold values indicate high contribution of the trait in the explained variance.

Table 3. Correlation between biometrical traits with ecological factors of 10 sites

		Altitude	Latitude	Longitude
Leaf traits	LL [cm]	–0.32*	0.19	–0.15
	LW [cm]	–0.22	0.18	–0.18
	LW 0.1 [cm]	–0.06	0.12	–0.002
	LW 0.9 [cm]	–0.17	0.14	–0.16
	PL [cm]	–0.29*	0.16	–0.09
	NT	0.35**	–0.04	0.04
	AW [cm]	–0.06	–0.02	–0.07
Leaf shape	LL/LW	–0.01	–0.02	0.12
	LW 0.1/ LW	–0.1	–0.14	–0.01
	LW 0.9/ LW	–0.14	–0.08	0.08
	LL/PL	0.09	–0.06	0.06
Stomata trait	SL	0.15	–0.28**	0.12
	SW	–0.04	–0.04	–0.12
	SA	–0.04	–0.17	–0.01
	SD	0.12	0.13	–0.01

*Selected Spearman R values significant at $p < 0.005$. **Selected Spearman R values significant at $p < 0.001$.

Table 4. Partitioning of variation by hierarchical component in all leaf traits

Axes	Variance [%]		
	Elevation	Tree	Error
Axes 1 [PCA1]	48.58	18.69	32.73
Axes 2 [PCA2]	17.26	46.84	35.90
Axes 3 [PCA3]	19.09	29.01	51.89
Axes 4 [PCA4]	8.91	70.81	20.28
Axes 5 [PCA5]	7.10	27.51	56.30

The plasticity values calculated for the studied leaf traits were generally high (mean Pl = 0.56). The largest plasticity corresponded to apex length (Pl = 1) and petiole length (Pl = 0.8), while the minimum was observed for leaf base shape (Pl = 0.2). Along the elevational gradient, the studied stomata parameters displayed low plasticity value (Fig. 3).

Discussion

Environmental heterogeneity is a major cause of variation in leaf morphology (Geeske et al., 1994; Velázquez-Rosas et al., 2002; Mcpherson et al., 2004), and the effects of altitude on leaf morphology and physiology are important for a plant development. In this study, clear changes in leaf morphology were associated with elevational gradient in the Hyrcanian forest in Iran, as seen by the large proportion (approximately 49%) of total variance of leaf morphology that was explained by the altitudinal component of the environment.

Our results are in line with other's findings in various environments, such as significant reductions of lamina length with altitude (Velázquez-Rosas et al., 2002; Hovenden and Vander Schoor, 2004). We also confirmed the tendency for leaves to bear a larger number of teeth at higher altitudes, reported earlier by Royer and Wilf (2006). They suggested that a larger number of teeth along the border of the lamina provide protection for the leaf against high wind speeds and help ensure a better access to water for the plant. Similarly, our results confirm the long-held idea that tooth number is negatively associated with mean annual temperature (Greenwood et al., 2004; Royer et al., 2005; Traiser et al., 2005), an environmental factor that decreases with increasing elevation. Indeed, for a plant growing in a high elevation environment, having more teeth may represent a general adaptive advantage, albeit complex to dissect, as this trait has also been suggested to be mechanism resulting in increased photosynthesis, evaporation, respiration within the primary tooth in the growing season (Baker-Brosh and Peet, 1997; Royer and Wilf, 2006). Considering the close association between leaf morphology and environmental factors (Bruschi et al., 2003), *A. subcordata* displays multiple changes that confirm the response of this species to the elevational gradient, but the different traits sometimes display opposite behaviours. For example, there was a negative correlation between altitude and both petiole length and lamina length, while tooth number responded positively

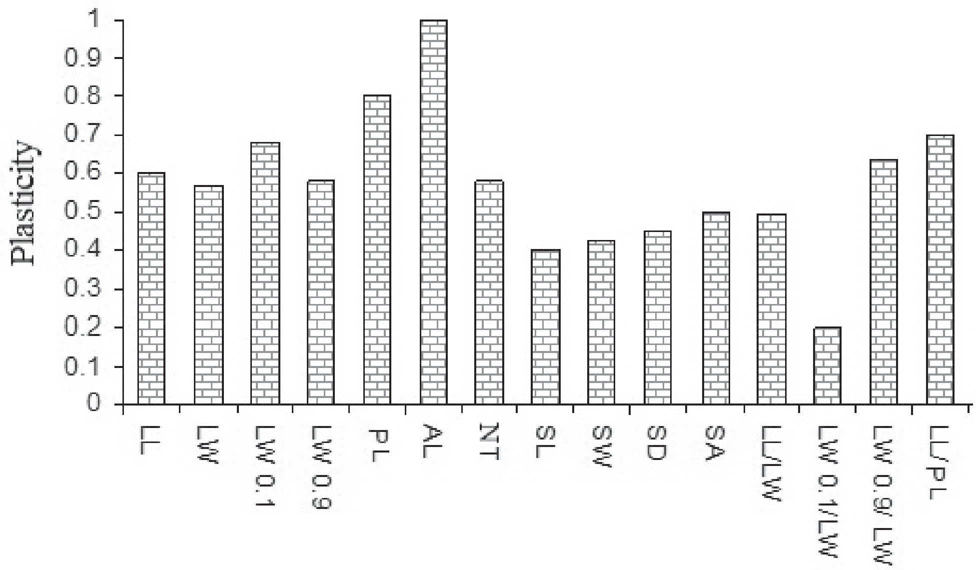

Fig. 3. Comparison of the plasticity calculated for the 15 analysed leaf traits. LL, lamina length; LW, lamina width; LW 0.1, lamina width at the first decile of leaf length; LW 0.9, lamina width at the ninth decile of leaf length; PL, petiole length; AL, apex length; NT, number of teeth; SL, stomata length; SW, stomata width; SD, stomata density; SA, stomata area.

to the increasing altitude. Interestingly, in *A. subcordata* leaf shape parameters remained unaffected by the increasing elevation (as shown by the low plasticity value – approximately 0.2 – for the leaf base shape), in contrast to findings of other authors (Hovenden and Vander Schoor, 2004). Leaf base shape has often been used as an identifying character in the taxonomy of *Alnus* (Sabeti, 1965). Since our results show that this trait is little affected by the complex changes of environmental factors associated with elevational gradient, we conclude that it may be extensively utilised by botanists for the diagnosis of several species within the genus. Conversely, stomatal traits tended to be positively associated with altitude, for example stomatal length. Although stomatal morphology is mainly affected by leaf developmental stage (Čaňová et al., 2008) and leaf development is initially driven by gene expression (Li et al., 2010), we found some evidences for *A. subcordata* being forced to undergo altitudinal changes in stomatal traits, perhaps as a need to adapt to decreasing CO_2 concentrations, decreased temperature and modified light conditions in higher sites (Qieng et al., 2003). This possibility is also confirmed by the strong correlation between stomatal size traits and PC2.

We concluded that the phenotypic variation of *A. subcordata* leaves, especially that of lamina and petiole lengths, number of teeth and stomatal size, represent an integrative adaptive response to altitude-related varying environmental conditions.

Considering the large leaf variability displayed by the broadly distributed *A. subcordata* in the Hyrcanian forest, the potential diagnostic value of some traits for the taxonomy of the genus became evident.

Acknowledgement

We would like to appreciate Prof Robert Oz Augé (University of Tennessee, USA), Dr Sanja Kovacic (University of Zagreb, Croatia) and Mrs Aida Cune Sanchez (PhD student, Southampton University) for their valuable comments.

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Vplyv výškového gradientu na morfológiu listov a prieduchov *Alnus subcordata* v hyrkánskych lesoch Iránu

Súhrn

Alnus subcordata C.A.Mey (Betulaceae) je komerčnou rýchlorastúcou drevinou so značným prirodzeným rozšírením v oblasti hyrkánskych lesov v severnom Iráne. V tejto štúdii sme skúmali vplyv nadmorskej výšky na celkovú morfológiu listov a prieduchov tejto dreviny. Vo východnej provincii Mazandaran sme pozdĺž výškového gradientu vybrali desať výskumných plôch s výškovým rozdielom 100 m medzi jednotlivými plochami. Na každej ploche bolo vyselektovaných 6 dospelých stromov, z ktorých sa odoberali listy na určenie listových parametrov. Bola zistená významná pozitívna korelácia medzi počtom listových zubov a nadmorskou výškou, zatiaľ čo veľkosť listovej čepele a dĺžka listovej stopky negatívne korelovali s týmto faktorom. Podobne sa aj dĺžka prieduchov skracovala so stúpajúcou nadmorskou výškou. Za účelom identifikácie faktorov zodpovedných za diferenciáciu medzi výskumnými plochami bola uskutočnená analýza základných komponentov. Šírka a dĺžka listovej čepele spolu s dĺžkou listovej stopky najlepšie korelovali s hodnotami prvého základného komponentu, zatiaľ čo parametre prieduchov najlepšie korelovali s hodnotami druhého základného komponentu. Hodnotením plasticity sa zistila vysoká priemerná hodnota pre listové parametre (Pl = 0,56). Najnižšia plasticita bola zaznamenaná pre tvar listovej bázy (Pl = 0,20). Vysoká plasticita listových parametrov vystavených environmentálnym fluktuáciám bola pozorovaná pre dĺžku listového vrcholu, dĺžku listovej čepele, dĺžku listovej stopky, počet listových zubov a veľkosť prieduchov. Tieto zistenia môžu vysvetľovať rozsiahle výškové rozšírenie *A. subcordata* v hyrkánskych lesoch. Zo štúdie vyvodzujeme, že tvar listovej bázy je užitočným znakom pre taxonomické účely v rode *Alnus*.

Received September 14, 2010
Accepted January 26, 2011