Phenotypic variation in chestnut (*Castanea sativa* Mill.) natural populations in Hyrcanian forest (north of Iran), revealed by leaf morphometrics

Mehrdad Zarafshar[1], Moslem Akbarinia[*2], Piero Bruschi[3], Sieid Mohsen Hosseiny[4], Hamed Yousefzadeh[5], Mehdi Taieby[6], Ali Sattarian[7]

[1, 2, 4, 5]Department of Forestry, Natural Resource Faculty, Tarbiat Modaress University, Noor, Mazandaran, Iran
[3]Laboratory of Applied and Forest Botany, Department of Plant Biology, University of Florence, Italy
[6]Department of Forestry, Gorgan University, Gorgan, Golestan, Iran
[7]Department of Forestry, Gonbad Kavoos University, Gonbad Kavoos, Iran

Abstract

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Sweet chestnut (*Castanea sativa* Mill.) is marginally distributed – as a rare species, in Hyrcanian forest, located in the north of Iran. In Iran, this species is economically important for timber and nut production, therefore its conservation is very necessary. However, no information exists on its variability in the Iranian chestnut populations. The aim of this study is to survey the variability in leaf morphology of three chestnut natural populations. Twenty trees per a population and forty leaves per a tree were sampled, data of nine characteristics (lamina length, lamina width, petiole length, distance from leaf base to the leaf maximum width, tooth width, tooth length, tooth distance, vein (count variable), teeth (counted variable) and four characteristic ratios (leaf length/leaf width, leaf length/petiole length, leaf length/distance from leaf base to the widest point, distance from leaf base to the leaf widest point/petiole length), were recorded. Principal components analysis (PCA) was used to separate inter-relationships into statistically independent basic components. Most of the variation (85%) was explained by the first four components, and leaf size emerged as the most important variable in the corresponding eigenvectors. We used one-way ANOVA on the scores of the factors extracted in the PCA. These analyses revealed significant between-population differences with regard to most of factors. The results of discriminant analysis showed a high percentage of correctly classified cases in all actual populations (in total 93%). The patterns of leaf plasticity exhibited low values for all parameters. We concluded that leaf parameters are suitable variables for detecting levels of phenotypic variability among chestnut natural populations. The high diversity observed in the populations is very important for the conservation of the species genetic resources.

Key words

Chestnut, Iran, morphological traits, plasticity, variation

Introduction

The genus *Castanea* (Fagaceae) consists of seven species widely distributed across temperate zones of the Northern Hemisphere (Rutter et al., 1990). Among these species, European or sweet chestnut (*Castanea sativa* Mill.) is indigenous to the Caucasus Mountains (Ertan, 2007) and distributed in Southern Europe and

* Corresponding author address: Tarbiat Modaress University, Noor, Mazandaran, Iran, Box 14155-4838;
Tel.: +98-122-6253103, +98-122-6253101; Fax: +98-122-6253499; E-mail: Akbarim@modares.ac.ir

throughout the Mediterranean regions (Aravaopoulos et al., 2001).

Castanea sativa has been long recognized as a multi-purpose species (Aravanopoulos, 2005), because it is widely cultivated for timber and nut production, and because it represents an integral part of economy in many areas, particularly in rural regions (Diamandis and Perlerou, 1996).

In Iran, this species, marginally distributed in Western Hyrcanian forest (in the north of Iran – Gilan province), was first studied by Jazireie (1961). Although *Castanea sativa* has been called a rare species in Iran, and the natural stands of this species are protected stands, the number of trees per hectare is decreasing continuously. Seed collection by indigenous people, grazing and diseases are the main factors affecting chestnut distribution in Iran. Rampant seed scavenging by humans has nearly eliminated the ability of chestnut stands to regenerate naturally.

Because excessive utilization is one of the main destroying factors in *Castanea sativa* natural populations, sound management of natural stands is necessary (Aravanopoulos et al., 2001). Evaluation of genetic diversity and population structure of natural Chestnut stands is crucial for good management strategies and conservation strategy and sustainable utilization of this natural resource (Lang and Hang, 1999). When selecting gene conservation strategies, the magnitude and structure of genetic variation in natural populations must be known.

Morphological traits, especially ”easy to use” and unambiguous traits (Cousen, 1963; Olsson, 1975; Kremer et al., 2002), have often served as tools for studying genetic diversity (Neophytou et al., 2007) because they sometimes differentiate faster than isoenzymes or selective neutral DNA markers (Ishida et al., 2003). Generally, population relationships and diversity via the study of morphological traits form an important component in the study of species (Aravanopoulos, 2005).

Traditionally, leaf morphological traits have been employed by scientists for studying phenotypic diversity. This has been widely accepted, because leaves are the most important organs for photosynthesis and transpiration in plants, and arrangement, size, shape and anatomy of leaves differ greatly in different environments (Bruschi et al., 2003), and are easy to measure (Neophytou et al., 2007).

To our knowledge, no such study has been pursued on *Castanea sativa* natural populations in Hyrcanian forest. Porbabaei (2008) has surveyed ecological aspects in the Iranian Chestnut natural stands only. The use of multivariate statistics, as an addition to the classical univariate approach, provides an opportunity to reveal the importance of the morphometric traits in phenotypic diversity studies (Aravanopoulos, 2005). In our study, we analyzed leaf morphological traits by means of multivariate analysis, in order to evaluate phenotypic variation and likely kin groups in a natural stand of chestnut.

Material and methods

Three main natural populations of *Castanea sativa* in Hyrcanian forest (in the north of Iran – Gilan pro-vince) including Siamazgi valley, Vissrod valley and Ghalerod-khan valley were sampled (Fig. 1, Table 1). We first selected 20 mature trees per ech population. In order to minimize the possibility of intraspecific crossing, the trees were chosen to be at least 50 m apart. Forty leaves were collected from each of 20 trees per population. The leaves were sampled from the four sides of tree (10 leaves from each side) at 2.0 meters above the ground. The selected leaves did not show signs of abnormal growth, mechanical damage, pathogen presence or insect infestation. The parameters measured included nine morphological characters: lamina length (LL), lamina width (LW), petiole length (PL), distance from leaf base to the leaf maximum width (BW), leaf tooth width (LTW), leaf tooth length (LTL), tooth distance (TD), vein (count variable), teeth (count variable – Fig. 2) and four characters ratios: leaf length/ leaf width (LL/LW), leaf length/ petiole length (LL/LP), leaf length/distance from leaf base to the widest point (LL/BW), distance from leaf base to the leaf widest point/petiole length (BW/PL). These ratios, which form independent shape variables, have been used extensively in leaf morphometrics (Dickinson et al., 1987).

To correct for allometric effects, we calculated a measure of leaf overall size as the root square of the product (total leaf length × blade width) (Blue and Jensen, 1988). This new variable was regressed against leaf parameters, and the residuals used as input in the successive analyses. Thus, for each character *i* and OTU *j* (Operational Taxonomic Units) size-adjusted variables *Yij* were determined according to the formula: *Yij* adj. = *Yij* – *Yij*-cap, where *Yij*-cap was the expected value of *Yij* given the size of OTU *j*. Descriptive statistics were computed for three populations tested. Assumptions of normality were checked with Shapiro-Wilk's test. Normality of distribution of the characters was assessed for all variables. Principal components analysis (PCA) with standardized varimax rotation was used to separate interrelationships into statistically independent basic components. In PCA, eigenvectors were calculated to determine the contribution of each variable to the separation of the populations. An analysis of variance (one-way anova) was performed in order to test the main effects of populations on the scores of the first three factors extracted in the PCA.

In addition, we performed a discriminant analysis on the total data set to identify the multivariate relationships among the morphometric traits, and their changes between the populations. The scatter plot of the discriminant scores corresponding to each case of each population in the multivariate space, as defined by the first two discriminant functions, was provided for visualization of multivariate phenotypic variations. The statistical program SPSS (version 11.5 and 16) was used for all the analyses.

The total within-population plasticity (Pl) was calculated for each parameter using the smallest and greatest mean values Pl = 1 – (x/X), where x is the smallest value and X is the largest value for any given leaf measure (Ashton et al., 1998; Bruschi et al., 2003).

Results

Means, standard deviation and coefficient of variation (CV) for fourteen leaf parameters in chestnut trees of three natural populations are showed in Table 2. PCA was applied on all 14 morphometric traits. The eigenvalues, proportion of variance and cumulative proportion of the principal components are presented in Table 3. The first four principal components accounted for 85% of the total variance of all traits whereas the other components comprised a small percentage of total variation

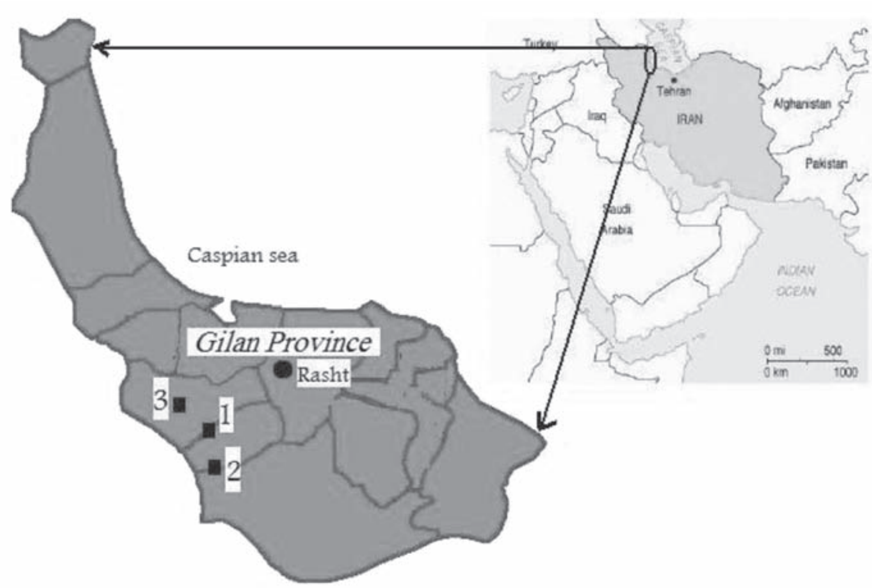

Fig. 1. Location of sampling areas in Hyrcanian forest (in the north of Iran). (1) Siamazgi Population; (2) Visrod population; (3) Ghalerodkhan Population. Distance between 1 and 2: 12 km. Distance between 1 and 3: 10 km. Distance between 2 and 3: 25 km

Table 1. Characteristics of *Castanea sativa* populations studied

Population	Longitude	Latitude	Altitude [m]	Soil type
Siamazgi	49°18'N	37°4'E	200–400	Forest Brown Soils/Low Ph
Visrod	49°15'N	37°15'E	200–500	Forest Brown Soils/ Low Ph
Ghalerodkhan	49°14'N	37°5'E	350–500	Forest Brown Soils/ Low Ph

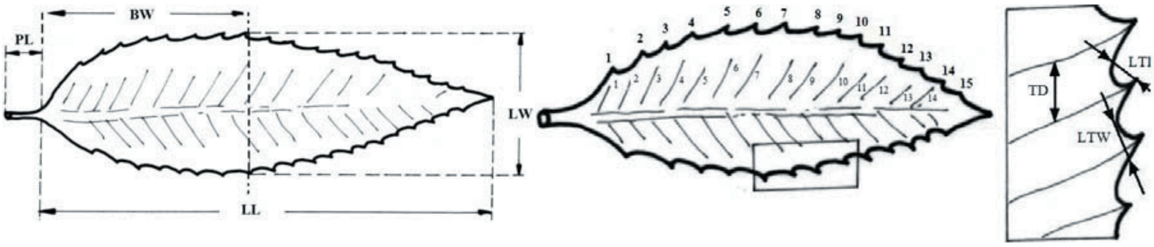

Fig. 2. Presentation of the assessed leaf morphological variables. Lamina length (LL). Lamina width (LW). Petiole length (PL). Distance from leaf base to the leaf maximum width (BW). Leaf tooth width (LTW). Leaf tooth length (LTL). Tooth distance (TD). Teeth (count variable) in bold numbers and vein (count variable) in non bold numbers

(15%). Sixty percent of the total variance could be observed in the first two principal components, indicating a high degree of correlation among the characteristics analyzed. Separate percentages of variation attributed to the first four components by decreasing order are 31%, 29%, 14% and 11%. PC1 was negatively related to LW, TI, LL/PL and BW/BL and positively correlate with PL. PC2 was positively related with LL, V, T, TD and LL/LW. PC3 was positively related with BW and negatively with LL/BW while PC4 was negatively related with LTW and LTI.

Variance analysis presented in Table 4 shows that significant variance among morphometric traits was attributable to differences between the populations. Varia-

Table 2. Means, standard deviation and CV for fourteen leaf parameters in chestnut trees in three natural populations of Hyrcanian forest (in the north of Iran). (Variable abbreviations are explained in the text and Fig. 2.)

	Siamazgi			Visrod			Ghalerodkhan		
	Mean	St. deviation	CV	Mean	St. deviation	CV	Mean	St. deviation	CV
LL [cm]	22.7	1.07	4.7	21.3	1.49	6.9	23.4	0.58	2.4
LW [cm]	7.6	0.54	7.1	8.2	1.09	13.2	7.2	0.24	3.3
BW[cm]	14.8	0.92	6.2	14.1	1.52	10.7	14.1	0.98	6.9
PL [cm]	1.3	0.11	8.4	1.3	0.12	9.2	1.5	0.20	13.3
V	20.8	1.29	6.2	19.4	1.84	9.4	22.9	0.77	3.3
T	19.7	1.25	6.3	19	2.06	10.8	22.4	0.99	4.4
LTW [cm]	0.7	0.03	4.2	0.7	0.05	7.1	0.7	0.02	2.8
LTI [cm]	0.3	0.01	3.3	0.3	0.01	3.3	0.3	0.02	6.6
TI [cm]	2.2	0.17	7.7	2.1	0.13	6.1	2.1	0.17	8.0
TD [cm]	1.07	0.05	4.6	1.03	0.06	5.8	0.9	0.04	4.4
LL/LW	3.01	0.27	8.9	2.6	0.27	10.3	3.2	0.14	4.3
LL/PL	17.6	1.54	8.7	16.7	2.31	13.8	16.02	1.64	10.2
LL/BW	1.5	0.03	2	1.5	0.04	2.6	1.6	0.09	5.6
BW/PL	11.4	0.95	8.3	11	1.58	14.3	9.6	1.26	13.1

Table 3. Correlation coefficients between leaf traits of the *C. sativa* trees and four principal components analysis and proportion of variability by the first four components, at 5% probability level. Only significative coefficients at $p < 0.05$ are shown.

	Factor 1	Factor 2	Factor 3	Factor 4
LL [cm]		0.87*		
LW [cm]	–0.86*			
BW [cm]			0.88*	
PL [cm]	0.95*			
V(counted variable)		0.90*		
T(counted variable)		0.89*		
LTW [cm]				–0.80*
LTI [cm]				–0.82*
TI [cm]	–0.61*			
TD [cm]		0.79*		
LL/LW		0.79*		
LL/PL	–0.88*			
LL/BW			–0.77*	
BW/PL	–0.82*			
Eigenvalue	4.47	4.15	2.09	1.55
Explained variance	0.31	0.29	0.14	0.11
Cumulative variance [%]	31	60	74	85

tion due to populations was significant in the first three principal components extracted ($P < 0.05$, Table 4). A post-hoc analysis compared factor scores, and revealed nuances in the differences between the populations (Table 5). In the first factor, the Ghalerodkhan Population was significantly different from Siamazgi and Visrod populations whereas these two populations did not differ from each of other. In regards to the second factor, all three populations differed significantly from each other. In regards to the third factor, the Ghalerodkhan Population showed no significant difference from the Visrod Population (Table 5).

We performed a discriminant analysis on the data for all populations. Two ones of all roots (discriminant functions) had significantly associated eigenvalues (Table 6). The population cases were plotted on root1/ root2 (Fig. 3). Function 1 accounting for 31% of total variance clearly separates the Siamazgi Population from the other two. Function 2 represents another 29% of the total variance, and separates the Ghalerodkhan Population from the other two.

Results of classification discriminant analysis showed a high percentage of correctly classified cases for all actual populations (in total 93%, Table 7).

The low plasticity was found in most of the characters. Particularly, tooth distance (TD) and leaf length/ distance from leaf base to the widest point (LL/BW) showed very low values (Fig. 4).

Discussion

The main finding of this study is the high morphological differentiation among *Castanea sativa* stands in Hyrcanian forest located in north of Iran. This is a statistically robust result because we have transformed all the measured variables in order to account for ontogenetic factors related to the variable leaf size. As demonstrated by several studies (Blue and Jensen, 1988; Bruschi et al., 2003) most trees produce a wide range of leaf sizes as a result of positional effects and epigenetic factors (i.e., micro-environmental differences within the population). If the variation observed in linear measures (Fig. 2) was only a function of the difference existing among leaf sizes, then the analysis of residuals carried out from linear regression against the total size should highly limit the number of significant effects. Thus, the observed pattern of morphological variation should have strictly reflected the real genetic and adaptive differences among the studied populations. These findings coincide with the results of Aravanopoulos et al. (2005) who examined leaf variability in *Castanea sativa* populations in Greece.

Table 4. One-way Analysis of Variance carried out on each factor extracted

		SS	G	MS	F	P
Factor 1	Population	7.892	2	3.946	4.401	0.016
	Error	51.107	57	0.896		
Factor 2	Population	25.951	2	12.975	22.379	0.000
	Error	33.048	57	0.579		
Factor 3	Population	15.347	2	7.673	10.020	0.000
	Error	43.652	57	0.675		
Factor 4	Population	2.915	2	1.457	1.481	0.235
	Error	56.084	57	0.983		

Table 5. Post- hoc comparison LSD (Least Significant Differences) test

		Visrod Population	Ghalerodkhan Population
Factor 1	Siamazgi Population	0.631	0.025
	Visrod Population		0.007
Factor 2	Siamazgi Population	0.002	0.0009
	Visrod Population		0.0000
Factor 3	Siamazgi Population	0.003	0.00005
	Visrod Population		0.206

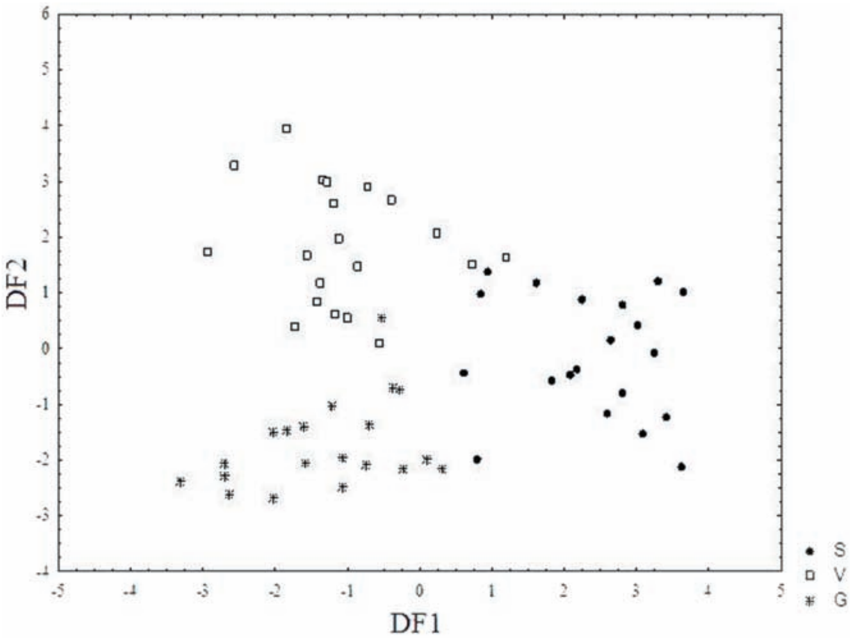

Fig. 3. Scatter plot of the discriminant analysis. Discriminant scores of the considered cases from different populations in the plot of the first two discriminant functions (DF1, DF2) are shown on the axes. (S) Siamazgi Population; (V) Visrod Population; (G) Ghalerodkhan Population

Table 6. Standardized coefficients and characteristics of discriminant analysis for first two discriminant functions (DF1, DF2)

	DF1	DF2
TI	0.98	0.06
TD	0.04	1.42
LL/BW	–1.47	0.05
N	0.32	–0.71
T	–0.40	0.11
BW	3.84	2.15
LTI	0.06	–0.21
LL/PL	11.34	3.15
LW	–2.81	1.72
LL	–3.79	–2.26
BW/PL	–9.18	–3.12
Autoval	2.95	2.26
Prop. Cum	0.56	1.00
Eigenvalue	2.95	2.26
Wilks Lambda	0.07	0.30
CHi-Sguare	133.01	61.50
p-level	0.000	0.000

Table 7. Number and percentage of cases correctly classified by discriminant analysis

	Siamazgi	Visrod	Ghalerodkhan	% of correct cases
Siamazgi	18	1	1	90
Visrod	1	19	0	95
Ghalerodkhan	0	1	19	90
Total	19	21	20	93

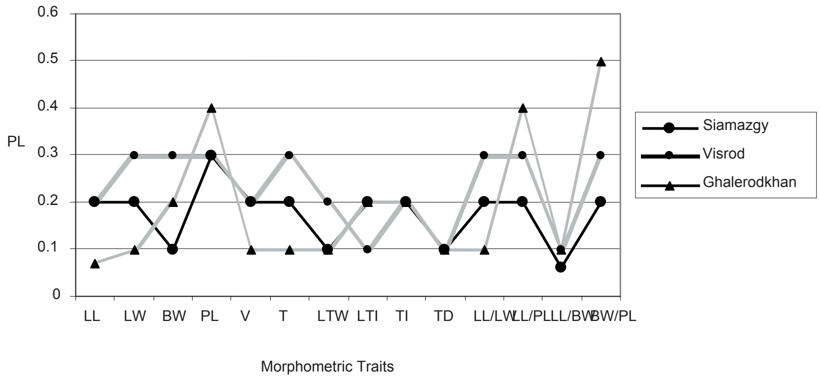

Fig. 4. Plot of plasticity (Pl) for all characters and characters ratios studied measured

Also Ertan (2007), Bolvansky and Uzik (2005) and Aravanopoulos et al. (2001) reported significant differences between accessions and populations of *C. sativa* based on leaf and fruit morphology. In general, there are several studies using different morphological and molecular techniques for estimating diversity of chestnut populations (Villani et al., 1999; Pereira-Lorenzo et al., 1996; Orgauzie et al., 1998; Mancuso et al., 1999; Goulao et al., 2001; Yuqing and Dane, 2003). However, none of the reported morphological studies used a statistical approach in order to correct for allometric and epigenetic effects.

Our results clearly show that the highest mean value in the majority of characters studied, belonged to Ghalerodkhan individuals, especially lamina length, petiole length, teeth (count variable) and vein (count variable), although climate (rainfall and temperate) and soil type are similar in the other two studied stands. In contrast to the European chestnut, as studied in other countries (Aravanopoulos et al., 2005; Ertan, 2007), the leaf size in the Iranian chestnut is larger but length of the petiole is shorter.

In our PCA, the major proportion of variation (60%) was accounted for the first two principal components. Based on the analysis of the eigenvectors from the PCA it can be concluded that the first three components separate populations by inferring differences in leaf size (LL, LW, and BW), while the fourth component did not separate populations by inferring differences in leaf size. In PCA 4, populations were separated by inferring differences in teeth variables.

In this study, lamina size variables (LL, LW, BW and PL) and lamina shape parameters appear to be more important compared to the other parameters, since their high loading characterizes the low space eigenvectors. Similarly, leaf size and shape variables have been used repeatedly in the literature for similar studies – with a notable success (Rajora et al., 1991; Ahmed and McNeil, 1996; Khasa and Bousquet, 2000; Aravanopoulos et al., 2005).

Low values in plasticity were observed among all of the characteristics studied. In particular, tooth distance (TD) and leaf length/distance from leaf base to the widest point (LL/BW) showed values lower than the others. Because the populations studied are situated in the Hyrcanian region within the same biogeographical ranges, isolation and environmental homogeneity could explain the low plasticity – because plant phenotypic plasticity involves changes in physiology, morphology or development of the same genotype growing in different environments (Gianoli and Gonzalez-Teuber, 2005). However, the mere observation of plasticity in a phenotypic trait does not necessarily imply that the response is adaptive (Schwaegerle and Bazzaz, 1987; Sultan, 1995). In controlled field trial conditions, there have been no reports of a lack of plasticity for any morphometric characters such as was evidenced in this study. Further investigation is needed to compare our findings with those in controlled field conditions.

In general, our results indicate considerable leaf variation in the populations studied. Moreover, we can conclude that leaf parameters are suitable variables to detect levels of phenotypic variability among Chestnut natural populations. Future studies should be focused on nut morphology, enzyme electrophoresis and molecular markers in natural Chestnut populations in Iran.

The high diversity observed in the populations studied is very important for the conservation of the species genetic resources, and it should be also considered by botanists and taxonomists.

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References

AHMAD, M., MC NEIL, D.L. 1996. Comparison of crossability, RAPD, SDS-PAGE and morphological markers for revealing genetic relationships within and among Lens species. *Theor. appl. Genet*., 93: 788–793.

ARAVANOPOULOS, F.A. 2005. Phenotypic variation and population relationships of chestnut (Castanea sativa) in Greece, revealed by multivariate analysis of leaf morphometrics. *Act hort.* 693: 230–240.

ARAVANOPOULOS, F.A., DROUZAS, A.D., ALIZOTI, P.G. 2001. Electrophoretic and quantitative variation in chestnut (Castanea sativa Mill.) in Hellenic populations in old-growth natural and coppice stands. *For. Snow Landsc. Res.*, 76 (3): 429–434.

ASHTON, P.M.S., OLAMDER, L.P., BERLYN, G.P., THADANI, R., CAMERON, I.R. 1998. Change in leaf structure in relation to crown position and tree size of Betula papyrifera within fire region stands of interior cedar-hemlock. *Can. J. Bot.*, 76: 1180–1187.

BLUE, M.P., JENSEN, R.J. 1988. Positional and seasonal variation in Oak (Quercus: Fagaceae) leaf morphology. *Am. J. Bot*., 75: 939–947.

BOLVANSKY, M., UZIK, M. 2005. Morphometric variation and differentiation of European Chestnut (Castanea sativa Mill.) in Slovakia. *Biologia (Bratislava)*, 60 (4): 423–429.

BRUSCHI, P., GROSSONI, P., BUSSOTII, F. 2003. Within- and among-tree variation in leaf morphology of Quercus petraea (Matt.) Liebl. natural populations. *Trees*, 17: 164–172.

COUSENS, J. E., 1963. Variation in some diagnostic characters of the sessile and pedunculate oaks and their hybrids in Scotland. *Watsonia*, 5: 273–286.

DIAMANDIS, S., PERLEROU, C. 1996. Biological control of Chestnut blight and resin top disease of Scots pine in Greece. In *Proceedings of the Greek-Bulgarian Conference*. Drama: GEOTTE Publ., p. 93–99.

DICKINSON, T.A., PARKER, W.H., STRAUSS, R.E. 1987. Another approach to leaf shape comparisons. *Taxon,* 36: 1–20.

ERTAN, E. 2007. Variability in leaf and fruit and in fruit composition of Chestnut (Castanea sativa) in the Nazilli region of Turkey. *Genet. Resour. Crop. Evol.*, 54: 691–699.

GIANOLI, E., GONZÁLEZ-TEUBER, M. 2005. Environmental heterogeneity and population differentiation in plasticity to drought in Convolvulus chilensis (Convolvulaceae). *Evol. Ecol*., 19: 603–613.

GOULAO, L., VALDIVIESSO, T., SANTANA, C., OLIVEIRA, C.M. 2001. Comparison between phonetic characterizations using RAPD and ISSR markers and phenotypic data of cultivated Chestnut (Castanea sativa Mill.). *Genet. Resour. Crop Evol*., 48 (4): 329–338.

ISHIDA, T.A., HATTORI, K., SATO, H., KIMURA, M.T. 2003. Differentiation and hybridization between Quercus crispula and Q. dentata (Fagaceae): Insights from morphological traits, amplified fragment length polymorphism markers, and leaf-miner composition. *Am. J. Bot*., 90 (5): 769–776.

JAZIREIE, M.H. 1961. *Chestnut (a tree for Iran)*. Iranian forestry press. 14 p.

KHASA, P.D., BOUSQUET, J. 2001. Multivariate analysis of allozyme and morphometric variability in Racosperma auriculiformae and R. manguim. *Silvae Genet*., 50: 191–199.

KREMER, A., DUPOUEY, J.L., DEANS J.D., COTTERELL, J., CSAIKL, U., FINKELDEY, R., ESPINEL, S., JENSEN, J., KLEINSCHMIT, J., VAN DAM, B., DUCOUSSO, A., FORREST, I., DE HEREDIA, U.L., LOWE, A.J., TUTKOVA, M., MUNRO, R.C., STENHOFT, S., BADEAU, V. 2002. Leaf morphological differentiation between Quercus robur and Quercus petraea is stable across western European mixed oak stands. *Ann. Forest Sci*., 59: 777–787.

LANG, P., HUANG, H. 1999. Genetic variation and population structure of three endemic Castanea species in China. *Act. Hort*., 494: 269–276.

MANCUSO, S., FERRINI, F., NICESE, F.P. 1999. Chestnut (Castanea sativa Mill.) genotype identification: an artificial neural network approach. *J. Hort. Sci. Biotechnol.*, 74 (6): 777–784.

NEOPHYTOU, C.H., PALLI, G., DOUNAVI, A., ARAVANOPOULOS, F.A. 2007. Morphological differentiation and hybridization between Quercus alnifolia Poech and Quercus coccifera L. (Fagaceae) in Cyprus. *Silvae Genet*., 56 (6): 271– 277.

OLSSON, U. 1975. A morphological analysis of phenotypes in populations of Quercus (Fagaceae) in Sweden. *Bot. Not*., 128: 55–68.

ORAGUZIE, N.C., MCNEIL, D.L., PETERSON, A.M., CHAPMAN, H. 1998. Comparison of RAPD and morphonut markers for revealing genetic relationships between Chestnut species and New Zealand Chestnut selection. *N.Z. J. Crop Hort. Sci*., 26: 109–115.

PEREIRA-LORENZ, S., FERNANDEZ-LOPEZ, J., MORENO-GONZALES, J. 1996. Variability and grouping of Northwestern Spanish Chestnut cultivars. II. Isoenzymatic traits. *J. Am. Soc. Hort. Sci*. 121: 190–197.

POORBABAEI, H. 2008. *Stand structure and spatial pattern of sweet Chestnut (Castanea sativa) trees in the Gilan forests, North of Iran*. Paper presented at the Global Conference on Global Warming, July 6–10, Istanbul, Turkey.

ROJARA, O.P., ZSUFFA, L., DANCIK, B.P. 1991. Allozyme and leaf morphological variation of eastern Cottonwood at the Northern limits of its range in Ontario. *J. For. Sci*., 37: 668–702.

RUTTER, P.A., MILLER, G., PAYNE, J.A., 1990. Chestnut. In MOORE J.N, BALLINGTON J.R. (eds). *Genetic resources of temperate fruit and nut crops*. Act. Hort., 290. Wageningen: The International Society for Horticulture Science, p. 761–788.

Schwaegerle, K.E., Bazzaz, F.A. 1987. Differentiation among nine populations of Phlox: response to environmental gradients. *Ecology*, 68: 54–64.
Sultan, S. E. 1995. Phenotypic plasticity and plant adaptation. *Acta bot. neerl*., 44: 363–383.
Villani, F., Sansotta, A., Cherubini, M., Cesaroni, D., Sbordoni, V. 1999. Genetic structure of natural populations of Castanea sativa in Turkey: evidence of hybrid zone. *J. Evol. Biol.*, 12: 233–244.
Yuqing, F., Dane, F. 2003. Allozyme variation in endangered Castanea pumila var. pumila. *Ann. Bot*., 92: 223–230.

Fenotypická variabilita prirodzených populácií gaštana (*Castanea sativa* Mill.) v pohorí Hyrcanian (severný Irán) na základe morfometriky listov

Súhrn

Gaštan jedlý je rozšírený v pohorí Hyrcanian na severe Iránu ako okrajová drevina. Je to hospodársky významná drevina z hľadiska produkcie dreva a plodov a preto jej zachovanie je veľmi dôležité. Neexistujú však informácie o variabilite populácií gaštana v Iráne. Cieľom práce bolo preskúmať variabilitu morfológie listov pri troch prirodzených populáciách gaštana. Pri 20 stromoch v každej populácii bolo zobraných po 40 listov. Pri každom liste bolo zaznamenaných 9 parametrov (dĺžka čepele, šírka čepele, dĺžka stopky, vzdialenosť od bázy listu k maximálnej šírke listu, šírka zúbku, dĺžka zúbku, vzdialenosť medzi zúbkami, počet žiliek, počet zúbkov) a 4 parametre boli vypočítané (dĺžka listu / šírka listu, dĺžka listu / dĺžka stopky, dĺžka listu / vzdialenosť od bázy listu k maximálnej šírke listu, vzdialenosť od bázy listu k maximálnej šírke listu / dĺžka stopky). Na nahradenie pôvodného súboru premenných súborom nových, vzájomne nekorelovaných, umelých premenných bola použitá metóda hlavných komponentov (PCA). Väčšina variability (85 %) sa dala vysvetliť prvými štyrmi komponentmi a veľkosť listov bola premenná s najväčšou váhou pri vlastných vektoroch. Hodnoty hlavných komponentov z PCA boli použité pri jednoduchej analýze variancie. Pri väčšine faktorov sa ukázali štatisticky významné rozdiely medzi populáciami. Výsledky diskriminačnej analýzy ukázali na vysoké percento správne klasifikovaných prípadov vo všetkých aktuálnych populáciách (v priemere 93 %). Parametre plasticity listov vykazovali nízke hodnoty pri všetkých sledovaných znakoch. Môže sa zhrnúť, že znaky listov sú vhodné premenné na detekciu úrovne fenotypickej variability medzi prirodzenými populáciami gaštana. Vysoká variabilita pozorovaná v sledovaných populáciách je dôležitá pre uchovanie genetických zdrojov druhu.

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