FORESTRY IDEAS, 2012, vol. 18, No 2 (44): 125–131

VARIATION IN A SILVER BIRCH LOCALITY NEAR ARDINO (EASTERN RHODOPES)

Petar Zhelev[1] and Veselin Angelov[2]

[1]University of Forestry. 10 Kliment Ohridski Blvd., 1756 Sofia, Bulgaria.
E-mail: zhelev@ltu.bg
[2]State Forest Service – Ardino, 7 Republikanska str., Ardino 6750, Bulgaria.

Received: 22 October 2011 Accepted: 22 November 2012

Abstract

Phenotypic and allozyme variation of Silver Birch (*Betula pendula* Roth) was studied in three sub-populations in the locality near Ardino, which is one of the largest in Bulgaria. The 14 leaf traits studied were highly variable with coefficients of variation ranging from 14 to 33 %. Genetic variation, studied at 11 polymorphic allozyme loci, was well within the range reported for species with similar life-history characteristics; however, at the lower tail. Mean number of alleles per locus ranged from 1.8 to 2.2, and effective allele number – from 1.11 to 1.47. Expected and observed heterozygosities had similar values (0.137 and 0.135, respectively) and the inbreeding coefficient was not significantly different from zero. The overall differentiation was low (F_{ST} = 0.022). The results could be used for designing of management and gene conservation strategies.

Key words: *Betula pendula***,** genetic variation, leaf traits, allozymes.

Introduction

The Silver Birch (*Betula pendula* Roth) is a species abundant at northern latitudes, but is represented in Bulgaria by scattered localities in the mountainous regions. Because of its importance as timber and ornamental tree, it is cultivated quite frequently in the forest plantations and in parks, but its natural populations are restricted primarily to several larger localities, mainly in the western part of the country (Iliev 1988). The species has been extensively studied throughout its natural area of distribution (Eriksson and Jonsson 1986, Hattemer et al. 1990, Erilksson et al. 2003, Rusanen et al. 2004, Palme et al. 2003).

In Bulgaria the phenotypic variation of Birch was studied by Busov (1975) and in more detail by Iliev (1988). The latter author studied also the pollen and seed germination, phenology and caryotype. Different valuable ornamental forms and cultivars were successfully propagated *in vitro* (Iliev 1993; Iliev et al. 2001, 2010).

One of the regions where Silver Birch was not studied to date is situated in the Eastern Rhodopes near the city of Ardino (41º 32’ N, 25º 08’ E). It is probably the easternmost larger locality in the country. The species in this area reflects the characteristic physiognomy of the landscape and therefore, the area is called “Belite brezi” (The white birches). The Silver Birch stands in Ardino cover an area of about 2.2 km^2 forming an isolated population with regard to the species distribution in Bulgaria. This isolation could have

played a role in shaping the current population structure of Silver Birch.

The relatively small and isolated populations of tree species usually require special measures for conservation and/or management approach. The information about the level of diversity in such populations could be of use for designing appropriate management and conservation activities. Therefore, the objective of the present study was to assess the level of diversity in the population of Silver Birch near Ardino, Eastern Rhodopes, by using morphological and biochemical markers.

Material and Methods

Morphological variation

Three subpopulations were selected for this study. The morphological variation was studied on 5 individuals per subpopulation (15 for the whole population) and 5 leaves per individual. The following leaf traits were studied (abbreviations given in parentheses): 1. Petiole length (L_1, mm); 2. Lamina length (L_2, mm); 3. Lamina width (W_1, mm); 4. Number of lateral veins (N_1); 5. Distance from the base to the first tooth (L_3, mm); 6. Distance between the second and third vein (L_4, mm); 7. Number of teeth between the second and third vein (N_2); 8. Distance from the widest part of the leaf to the base (L_5, mm); 9. Distance from the widest part of the leaf to the tip (L_6, mm); 10. Angle at the leaf base (Ang_1, °); 11. Angle at the leaf tip (Ang_2, °); 12. Ratio L_1:L_2 (R_1); 13. Ratio L_2:W_1 (R_2); 14. Ratio L_2:L_3 (R_3); 15. Ratio L_6:L_5 (R_4).

After statistical processing (nested ANOVA and descriptive statistics), the variation within the subpopulations was approximately evaluated by the coefficient of variation for each trait. When the coefficient was higher than 20 %, the variation was evaluated as high (simplified from the scale of Mamaev 1972). The mean values for Ardino locality were compared to those obtained by Iliev (1988) for six other populations in Western Bulgaria.

Population structure

The number of individuals sampled in the three subpopulations was 57, 57, and 55, respectively. The enzymes were extracted from dormant winter buds using Tris-HCl extraction buffer (pH 7.3), containing 1 % β-mercaptoethanol and 2 % Polyvinil-pyrrholidone 10K, water-soluble. Also, 5 mg Na_2EDTA, 15 mg dithiothreitol and 300 mg saccharose were added to 10 ml of extraction buffer prior to homogenization. Standard 12 % starch gel-electrophoresis was applied in two buffer systems: (1) Lithium borate – Tris-citrate discontinuous buffer system pH 8.1 (Ashton and Braden 1961) and (2) Tris-citrate continuous buffer system pH 7.0 (Shaw and Prasad 1970). The enzyme systems were the following ones (buffer system in parentheses): Alcohol dehydrogenase, ADH, 1 locus (2); Isocitric dehydrogenase, IDH, 2 loci (2); Leucine aminopeptidase, LAP, 1 locus (1); Malate dehydrogenase, MDH, 1 locus (2); Menadione reductase, MNR, 1 locus (1); 6-phosphogluconate dehydrogenase, 6-PGD, 1 locus (2); Phospho-glucomutase, PGM, 1 locus (2); Phospho-glucose isomerase, PGI, 2 loci (1); and Shikimate dehydrogenase, SkDH, 1 locus (2). Only polymorphic loci are listed above. The staining procedures followed Cheliak and Pitel (1984) and Wendel and Weeden (1989) with slight modifications.

Table 1. Descriptive statistics of the traits of Silver Birch (pooled results of all experimental plots).

Statistics	Traits														
	L_1	L_2	W_1	N_1	L_3	L_4	N_2	L_5	L_6	Ang_1	Ang_2	R_1	R_2	R_3	R_4
Mean	17.2	47.8	39.8	5.1	15.9	8.5	5.0	15.0	32.8	143.8	75.6	0.3	1.2	3.1	2.3
SE	0.4	0.8	0.7	0.1	0.4	0.2	0.1	0.4	0.7	3.0	0.9	0.0	0.0	0.1	0.1
SD	3.6	7.3	5.8	0.7	3.1	1.6	0.8	3.5	6.2	26.2	8.4	0.1	0.1	0.5	0.7
CV	20.8	15.3	14.7	13.7	19.7	18.8	16.9	23.1	18.8	18.2	11.1	15.5	9.6	16.0	32.0
p	2.4	1.8	1.7	1.6	2.3	2.2	1.9	2.7	2.2	2.1	1.3	1.8	1.1	1.8	3.7

* *SE* – standard error; *SD* – standard deviation; *CV* – coefficient of variation; $p = SE \cdot Mean^{-1} \cdot 100$.

Diploid genotypes were scored from electrophoregrams and the genetic parameters expressing intra- and interpopulation diversity were calculated using the software BIOSYS (Swofford and Selander 1989).

Results and Discussion

Leaf morphology

The morphological variation in the subpopulations and in the entire population was relatively high with coefficient of variation ranging from 14.7 to 23.1 for the metric traits and from 13.7 to 18.2 for the categorical variables and angles (Table 1). ANOVA test revealed that there were only few statistically significant effects of the subpopulation variation (results not shown) for traits L_3 (p=0.02), L_5 (p=0.02) and Ang_1 (p=0.02). All the effects caused by differences among individuals within a subpopulation, and among leaves within the individuals were statistically non-significant.

Iliev (1988) showed for six populations from Western Bulgaria that there were significant differences in the traits W_1, N_1, L_3, L_4, N_2, L_5 and L_6. This could be due to the fact that the populations included in the present study represents only small part of the species' variation in Bulgaria, while populations studied by Iliev (1988) covered much larger area with substantial variation among populations. However, our results are well within the range of the results for Western Bulgaria (Table 2).

Leaf morphology and morphometrics have been successfully applied in taxonomic, ecological and genetic studies of many plant species (e.g., Aravanopoulos 2005, Dickinson et al. 1987, Neophytou et al. 2007), including birches. Such studies are especially useful for resolving taxonomic problems and hybridization within a species complex, like *Betula pendula*-*Betula pubescens* in central and Western Europe (Jentys-Szaferowa 1950; Gardiner 1972, 1981; Howland et al. 1995). The leaf traits are also important for studying the species response to different environmental factors (Franiel and Więski 2005, Kovačić and Nikolić 2005, Migalina et al. 2010).

Table 2. Comparison of the means obtained in the present study with the overall mean reported by Iliev (1988) for six populations from Western Bulgaria.

Trait	Range		Difference (WB–A)	Statistical significance
	Western Bulgaria	Ardino		
L_1, mm	7–32	12–29	–0.3	ns
L_2, mm	21–80	28–63	–2.2	ns
W_1, mm	22–69	26–56	–2.7	*
N_1, no	3–10	3–6	+1.1	***
L_3, mm	6–26	10–25	–1.6	*
L_4, mm	3–15	5–14	–0.9	*
N_2, no	0–6	3–7	–2.3	***
L_5, mm	5–29	7–23	+2.0	*
L_6, mm	12–60	19–50	–4.0	*
Ang_1, °	71–203	98–203	–6.3	ns
Ang_2, °	50–116	58–102	–1.4	ns

Legend: * – $p \leq 0.05$; *** – $p \leq 0.001$; ns – non significant as tested by Student's t-test.

Table 3. Allele frequencies in the sub-populations studied.

Locus	Allele	Subpopulation		
		1	2	3
Adh	1	0.000	0.009	0.010
	2	0.921	0.946	0.784
	3	0.079	0.045	0.206
Idh1	1	0.974	0.974	0.991
	2	0.026	0.026	0.009
Idh2	1	0.026	0.000	0.036
	2	0.974	1.000	0.964
Lap-1	1	0.044	0.000	0.045
	2	0.956	1.000	0.955
Mdh-2	1	0.143	0.077	0.255
	2	0.857	0.885	0.745
	3	0.000	0.038	0.000
Mnr	1	0.009	0.000	0.027
	2	0.991	0.982	0.973
	3	0.000	0.018	0.000
Pgi-1	1	0.000	0.000	0.027
	2	1.000	1.000	0.973
Pgi-2	1	0.339	0.327	0.463
	2	0.661	0.673	0.500
	3	0.000	0.000	0.037
Pgm	1	0.856	0.911	0.856
	2	0.144	0.089	0.144
6pgdh	1	0.018	0.000	0.036
	2	0.982	1.000	0.964
Skdh	1	0.956	0.956	0.929
	2	0.044	0.044	0.071

Population structure

The differences in allele frequencies among the subpopulations were of small magnitude. The most polymorphic locus was *Pgi-2*, and some more expressed differences were detected in locus *Adh* but these loci still showed minor polymorphism, i.e., the most represented allele being the same (Table 3). Mean allele number ranged from 1.8 to 2.2 and effective allele number – from 1.11 to 1.47 (Table 4). Percent of polymorphic loci was low in all three subpopulations studied when 0.05 criterion was applied (mean 39.4), but it could be seen that without applying restriction criterion there were only 4 of 33 cases of "real" monomorphic loci (Table 3). There was some variation in the heterozygosity, which ranged from 0.096 to 0.178, and for the whole population it was 0.135. The differences between the observed and expected heterozygosities were not significant (mean = 0.013) indicating random mating and population status close to Hardy-Weinberg equilibrium. The values for the polymorphism and genetic diversity parameters are well within the range of these summarized by Hamrick et al. (1992) for the woody plant species with similar life-history characteristics.

F-statistics (Table 5) showed that in most loci the inbreeding coefficient within populations (F_{IS}) was negative, as proven by the overall values (Table 4). More noticeable positive deviations from zero were found at loci *Pgm* and *Adh*. The overall differentiation measure (F_{ST}) indi-

cates that only 2.2 % of the total variation was due to among-subpopulation differences.

The results obtained in our study concord well with the results reported for *Betula pendula* and other birch species. Wang (1996) reported heterozygosity 0.17, and Rusanen et al. (2003) – 0.141 for natural populations of *B. pendula*. The latter authors (Rusanen et al. 2003) found level of differentiation similar to our study (F_{ST} = 0.032). Alsos et al. (2002) found similar level of diversity in *Betula nana* (*He* = 0.141 at species level) but substantially higher level of differentiation (F_{ST} = 0.19), which could be attributed to the vegetative mode of reproduction of the species. Zeng et al. (2003) reported heterozygosity level 0.206 and differentiation (F_{ST}) 0.04 for *Betula alnoides*. These results indicate that the population from Ardino expresses levels of diversity and differentiation well within the range reported for *Betula pendula* and other birch species.

The results of weak genetic differentiation agree with these concerning morphological variation, where only one-fifth of the values were significantly different among the tree sub-populations. This could be an indicator of existence of adequately large and variable meta-population in the area, which is situated very close to the rear edge of species' distribution in Europe. The results concerning the patterns of distribution of genetic variation could be of use for development of conservation strategy for the species (Ganopoulos et al. 2011).

Our study on the level of genetic variation within the locality near Ardino indicate that even a relatively small and isolated habitat of Silver Birch still possesses relatively high level of diversity, which could be a precondition for successful adaptation to uncertain future environmental changes.

Table 4. Polymorphism and diversity in the subpopulations of Silver Birch.

Subpopulations	*N*	n_A	n_e	*P*	*Ho*	*He*	*F*
S_1	55.7 (0.8)	1.9 (0.1)	1.47	36.4	0.132 (0.045)	0.129 (0.041)	–0.023
S_2	56.2 (0.5)	1.8 (0.2)	1.11	36.4	0.096 (0.038)	0.100 (0.041)	0.040
S_3	53.0 (0.9)	2.2 (0.1)	1.22	45.5	0.178 (0.050)	0.182 (0.052)	0.022
Mean	55.0 (0.7)	2.0 (0.13)	1.27	39.4	0.135 (0.044)	0.137 (0.045)	0.013

Legend: *N* – average sample size; n_A – mean allele number; n_e – effective allele number; *P* – percent of polymorphic loci (0.05 criterion); *Ho* – observed heterozygosity; *He* – expected heterozygosity; *F* – inbreeding coefficient ($F = 1–Ho \cdot He^{-1}$). In parentheses – standard errors.

Table 5. *F*-statistics for the studied sub-populations.

Loci	F_{IS}	F_{IT}	F_{ST}
Lap-1	–0.047	–0.031	0.015
Pgi-1	–0.028	–0.009	0.018
Pgi-2	0.029	0.05	0.021
Mnr-1	–0.022	–0.014	0.007
Idh-1	–0.024	–0.021	0.003
Idh-2	–0.033	–0.021	0.011
Pgm-1	0.138	0.143	0.006
Skdh	–0.06	–0.056	0.003
Adh	0.112	0.154	0.048
6Pgdh	–0.031	–0.018	0.012
Mdh-2	–0.179	–0.14	0.033
Mean	**0.002**	**0.024**	**0.022**

Acknowledgments

This study was a part of M.Sc. thesis submitted by V. Angelov to the University of Forestry, Sofia. We thank Mira Georgieva for the lab assistance and two anonymous reviewers for their useful comments on an earlier draft of the manuscript.

References

Alsos I.G., Engelskjøn T., Brochmann C. 2002. Conservation genetics and population history of *Betula nana*, *Vaccinium uliginosum*, and *Campanula rotundifolia* in the Arctic Archipelago of Svalbard. Arctic, Antarctic, and Alpine Research 34(4): 408–418.

Aravanopoulos F.A. 2005. Phenotypic variation and population relationships of Chestnut (*Castanea sativa*) in Greece, revealed by multivariate analysis of leaf morphometrics. Acta Horticulturae 693: 233–240.

Ashton G.C., Braden A.W.H. 1961. Serum β-globulin polymorphism in mice. Australian Journal of Biological Sciences 14: 248–253.

Busov B. 1975. Studies on the intraspecific ecotype variation of the Birch (*Betula verrucosa* Ehrh.) in the basin of river Rilska. Gorskostopanska nauka 12(5): 43–48 (in Bulgarian).

Cheliak W.M., Pitel J.A. 1984. Techniques d'électrophorèse sur gel d'amidon des enzymes d'essences d'arbres forestiers. Rapport d'information PI-X-42. IFNP, Petawawa, Canada, 49 p.

Dickinson T.A., Parker W.H., Strauss R.E. 1987. Another approach to leaf shape comparisons. Taxon 36: 1–20.

Eriksson G., Jonsson A. 1986. A review of the genetics of *Betula*. Scandinavian Journal of Forest Research 1: 421–434.

Eriksson G., Black-Samuelsson S., Jensen M., Myking T., Rusanen M., Skrøppa T., Vakkari P., Westergaard L. 2003. Genetic variability in two tree species, *Acer platanoides* L. and *Betula pendula* Roth, with contrasting life-history traits. Scandinavian Journal of Forest Research 18: 320–331.

Franiel I., Więski K. 2005. Leaf features of silver birch (*Betula pendula* Roth). Variability within and between two populations (uncontaminated vs Pb-contaminated and Zn-contaminated site). Trees – Structure and Function 19: 81–88.

Ganopoulos I., Aravanopoulos F.A., Argiriou A., Kalivas A., Tsaftaris A. 2011. Is the genetic diversity of small scattered forest tree populations at the southern limits of their range more prone to stochastic events? A wild cherry case study by microsatellite-based markers. Tree genetics and genomes 7: 1299–1313.

Gardiner A.S. 1972. A biometric study of leaf variation in some British Birch populations. Forestry 45: 37–47.

Gardiner A.S. 1981 *Betula pubescens* – the affinities of types in the Scottish highlands with those of continental Europe: a study of leaf morphology or the application of morphometrics. In: Last F.T.; Gardiner A.S., (eds.) Forest and woodland ecology: an account of research being done in ITE. Cambridge, NERC/Institute of Terrestrial Ecology: 89–93.

Hamrick, J.L. Godt M.J.W., Sherman-Broyles S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests 6: 95–124.

Hattemer H.H., Steiner W., Kownatzki D. 1990. Genetic markers in birch. Silvae genetica 39: 45–50.

Howland D.E., Oliver R.P., Davy A.J. 1995. Morphological and molecular variation in natural populations of *Betula*. New Phytologist 130: 117–124.

Iliev I. 1988. Study on the natural populations of Silver Birch (*Betula pendula* Roth) in Western Bulgaria and selection of valuable ornamental forms. PhD thesis, University of Forestry, Sofia, 167 p. (in Bulgarian).

Iliev I. 1993. Effect of different factors on the cloning of Silver Birch (*Betula pendula* Roth) *in vitro*. Nauka za gorata 31(4): 73–84 (in Bulgarian).

Iliev I., Kitin P., Funada R. 2001. Morphological and anatomical study of *in vitro* root formation of Silver birch (*Betula pendula* Roth). Propagation of Ornamental Plants 1: 10–19.

Iliev I., Scaltsoyiannes A., Tsaktsira M., Gajdosova A. 2010. Micropropagation of *Betula pendula* Roth cultivars by adventitious shoot induction from leaf callus. Acta Horticulturae 885: 161–173.

Jentys-Szaferowa J. 1950. Analysis of the collective species *B. alba* L. on the basis of leaf measurements. Part II: *B. pubescens* Ehrh., *B. tortuosa* Ledeb., *B. carpatica* Wadst. et Kit. Bulletin International de l'Academie Polonaise de Sciences et des Lettres B1 (1–3): 1–48.

Kovačić S., Nikolić T. 2005. Relations between *Betula pendula* Roth (Betulaceae) leaf morphology and environmental factors in five regions of Croatia. Acta Biologica Cracoviensia, Series Botanica 47/2: 7–13.

Mamaev S.A. 1972. Forms of intraspecific variation of woody plants. Moscow, 284 p. (in Russian).

Migalina S.V., Ivanova L.A., Makhnev A.K. 2010. Changes of leaf morphology in *Betula pendula* Roth and *B. pubescens* Ehrh. along a zonal-climatic transect in the Urals and Western Siberia. Russian Journal of Ecology 41: 293–301.

Neophytou C., Palli G., Dounavi A., Aravanopoulos F.A. 2007. Morphological differentiation and hybridization between *Quercus alnifolia* Poech and *Quercus coccifera* L. (Fagaceae) in Cyprus. Silvae genetica 56(6): 271–277.

Palme A.E., Su Q., Rautenberg A., Manni F., Lascoux M. 2003. Postglacial recolonization and cpDNA variation of silver birch, *Betula pendula*. Molecular ecology 12: 201–212.

Rusanen M., Vakkari P., Blom A. 2003. Genetic structure of *Acer platanoides* and *Betula pendula* in northern Europe. Canadian Journal of Forest Research 33: 1110–1115.

Shaw C.R., Prasad R. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochemical Genetics 4: 297–330.

Swofford D., Selander R. 1989, Biosys-1 (Release 1.7): A computer program for the comprehensive analysis of Allelic Variation in Population Genetics and Biochemical Systematics, INHS. Illinois, USA, 43 p.

Wang T.L. 1996. Allozyme variation in populations, full-sib families and selfed lines of *Betula pendula* Roth. Theoretical and Applied Genetics 92: 1052–1058.

Wendel J., Weeden N. 1989. Visualization and interpretation of plant isozymes. In: Soltis D.E., Soltis P.S. (eds.). Isozymes in Plant Biology. Dioscorides Press, Portland, OR: 5–45.

Zeng J., Wang Z., Zhou S., Bai J., Zheng H. 2003. Allozyme variation and population genetic structure of *Betula alnoides* from Guangxi, China. Biochemical Genetics 41: 61–75.