Short note: Genetic diversity and differentiation in Ural populations of the aspen, *Populus tremula* L., as revealed by inter-simply sequence repeat (ISSR) markers

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Abstract

Genetic diversity and differentiation of six *Populus tremula* L. populations in the Ural mountains (Russia) were investigated using ISSR-markers. Analyses revealed relatively high genetic diversity and significant ($p = 0.985$) genetic isolation by distance of foothill and plain cis-Ural populations in the zone of forests with mixed coniferous and broad-leaved tree species. The genetic drift may be a factor that lead to comparatively low genetic variability and genetic singularities of isolated small populations of the aspen in dark coniferous taiga of the Ural mountains.

Key words: inter-simply sequence repeat (ISSR) markers, genetic diversity, genetic differentiation, *Populus tremula* L., Ural mountains.

Populus tremula L. (Salicaceae), a typical pioneer forest tree species, has got comparatively high genetic diversity and relatively low variation among European populations (SALVINI et al., 2001; INGVARSSON, 2005; HALL et al., 2007). The trembling aspen is one of the most widely distributed in forests of Russia and area of its stands exceeds 20 millions hectares. Unfortunately the gene pool of the species have not been studied in the country with the use of DNA based genetic markers. The aim of this study was partially to fill this gap and to carry out analysis of Ural populations of *Populus tremula* by using inter-simply sequence repeat (ISSR) markers.

Genetic differentiation of populations was quantified in 174 adult trees from six aspen stands. They are located on the western macroslope of the Ural mountains

(the cis-Ural: stands Pt1-Pt4; the dark coniferous Ural taiga: Pt5 and Pt6) on the area with geographic coordinates 57°017'–58°813 E and 54°956'–57°569 N. The average distance between populations is 125±11 km with the maximal value 235 km. Fresh leaves of adult trees were dried with silica gel, and genomic DNA was isolated according to a procedure (ROGERS, 1985) with our nonessential modifications. Quantitative analysis and spectral characterization of the DNA were carried out on a Spectrofotometr™ NanoDrop 2000 ("Thermo scientific", USA). PCR amplification was carried out (Boronnikova, 2009) by using GeneAmp® PCR System 9700 ("Applied Biosystems", USA). The separation of the DNA was carried out by electrophoresis in 1.7 % agarose gel (RESolute Wide Range, BIOZym). Amplification products were stained using with ethidium bromide and visualized under UV light (Gel Doc XR, BioRad, USA). PCR reactions were conducted thrice to guarantee the reproducibility of results. In a pre-screen with 20 synthesized ISSR primers, 5 primers with clearly identifiable amplification products were selected for further analysis: M1 (AC)₈CG, M27 (GA)₈C, X9 (ACC)₆G, X10 (AGC)₆C, X11 (AGC)₆G. Gene parameters were calculated using POPGENE 1.31 (YEH et al., 1999) and GenAlEx 6.3 (PEAKALL and SMOUSE, 2006).

Five primers produced a total of 119 clearly identifiable bands of which 87 were polymorphic. The estimations of genetic diversity for the separate primers generally were comparable: in average Nei's gene diversity $H = 0.264 \pm 0.012$ (the parameter varies in the range 0.241–0.295); observed number of alleles $N_A = 1.900 \pm 0.029$ (1.828–1.961); effective number of alleles $N_E = 1.437 \pm 0.021$ (1.394–1.494), Shannon's information index $I = 0.4060.018$ (0.388–0.448).

The cis-Ural's stands Pt1, Pt2, Pt3 and Pt4 have a tendency to a higher genetic diversity (*table*) in comparison to the populations Pt5 and Pt6 from dark coniferous Ural taiga. We revealed one more feature in the study of the allelic profile of the stand Pt6 when considering fre-

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Table. – Genetic diversity within populations of *P. tremula*.

Populations	H	N_A	N_E	I
Pt1	0.139±0.018	1.345±0.478	1.248±0.376	0.202
Pt2	0.160±0.018	1.465±0.501	1.275±0.367	0.239
Pt3	0.142±0.017	1.417±0.495	1.239±0.340	0.215
Pt4	0.138±0.016	1.449±0.500	1.225±0.319	0.211
Pt5	0.114±0.015	1.354±0.480	1.186±0.299	0.174
Pt6	0.088±0.014	1.315±0.466	1.141±0.269	0.137
Mean	0.129±0.009	1.381±0.023	1.217±0.016	0.193±0.013

Figure. – Distribution of the ISSR bands with different frequencies in a population of *Populus tremula* L. of the dark coniferous taiga.

quencies of the ISSR bands. It was found U-shaped form of the distribution (fig.), viz. bands with frequencies close to zero or 1.0 are dominated in number. Such type of distribution and the reduced genetic diversity in this population are largely in agreement with expectations under genetic drift (WRIGHT, 1984).

It was revealed that genetic differentiation among six populations was relatively high with the mean measure G_{ST} of 0.513. The estimations for the separate primers generally were comparable: 0.354 (M1), 0.491 (X10), 0.535 (X11), 0.566 (M27), and 0.619 (X9). Here we exclude the effect of clone composition on the calculated level of the genetic differentiation as each individual in our study has got its own unique set of ISSR bands. Thereto trees within a stand were at least of 100 m apart to decrease a probability of sampling two or more individuals of the same clone.

Measuring distances between pairs of the stands did not demonstrate a relation between population genetic structure and the geographic position of the populations. But analyses revealed significant and strong isolation by distance when the stands Pt5 and Pt6 were excluded from the analysis (coefficient of correlation $r = 0.90$, $p = 0.985$).

The level of the genetic differentiation among six populations seems quite high considering the geographic closeness of them and life history traits of *P. tremula*. It was determined that species with outbreeding, wind pollination, and long-distance wind dispersal of seeds and pollen has much less variation among populations (HAMRICK et al., 1996). Our estimations also are in contrast to results of recent investigations of the trembling aspen, which appear to have substantially lower variation among populations. For instance, cpDNA polymorphism has been described in ten Italian stands (Salvini et al., 2001). Molecular markers revealed relatively low genetic differentiation among populations (PCR-RFLP: $G_{ST} = 0.22$; SSR: $G_{ST} = 0.07$). It was demonstrated (HALL et al., 2007) that 18 neutral SSRs, 50 SNPs of 5 phenology candidate genes, 7 SSRs allowed to reveal among 12

European populations less values of genetic differentiation (F_{ST} was around 0.015). More genetic differentiation among populations of *Populus tremula* was found at five genes (an average estimate of $F_{ST} = 0.117$), coding three enzyme systems (INGVARSSON, 2005). One of reasons of relatively high differences of our calculated parameter G_{ST} and the mentioned estimations may be the specificity of the ISSR-sequences. Such differences of estimates of intraspecific genetic diversity derived by various DNA markers of plants were demonstrated earlier (NYBOM, 2004).

It appears that the patterns of genetic variation within and among Ural populations of *P. tremula* are also determined by such reason as location of the stands in different areas of vegetation of the region. The samples Pt1-Pt4 with similar levels of genetic diversity and the positive correlation between pairwise values of G_{ST} and geographic distances are located on the southern part of the studied region in foothill plains. Forests are composed here of both conifers (*Picea obovata* Ledeb., *Abies sibirica* Ledeb. and *Pinus sylvestris* L.) and broad-leaved forest tree species such as *Tilia cordata* Mill., *Quercus robur* L., *Acer platanoides* L., *Ulmus glabra* Huds. *Populus tremula* L. The populations Pt5 and Pt6 are situated in the mountainous dark coniferous taiga with a almost complete predominance of *P. obovata* and *A. sibirica*. In such boreal forests, aspen colonize only areas disturbed by fire or logging. Therefore its possibilities for a territorial expansion and genetic realization of movement of pollen and seeds strongly limited by availability of suitable ecological conditions. That is why the species is presented here only in small scattered island stands or single trees. Perhaps populations of the aspen in dark coniferous taiga have less genetic variation and above mentioned distribution of ISSR bands in comparison to cis-Ural stands owing to genetic drift.

Populus tremula L. has a great interest in Russian forestry due to large potential in biomass cultivation. Therefore it is important to include into breeding and conservation programs genetic resources of populations with acceptable gene fund such as the cis-Ural stands. Our results demonstrated that the use of populations from dark coniferous taiga for this purpose can be not appropriate in comparison to populations of the cis-Ural. In this regard, it is necessary to confirm the revealed trends by examining in the Urals a larger number of populations of the species.

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