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GENETIC DIVERSITY BETWEEN THREE SPECIES OF *SANGUISORBA* L. FROM WEST SIBERIA BASED ON RANDOMLY AMPLIFIED DNA FINGERPRINTS

ГЕНЕТИЧЕСКИЕ РАЗЛИЧИЯ МЕЖДУ ТРЕМЯ ВИДАМИ *SANGUISORBA* L. ИЗ ЗАПАДНОЙ СИБИРИ НА ОСНОВЕ МЕТОДА СЛУЧАЙНО АМПЛИФИЦИРОВАННЫХ ФРАГМЕНТОВ ДНК (RAF)

Summary. Genetic differences between *Sanguisorba officinalis* L., *S. alpina* Bunge and *S. azovtsevii* Krasnob. et Pschen. were studied. Randomly amplified DNA fingerprints (RAF) technique demonstrated a high degree of genetic identity between *S. alpina* and *S. azovtsevii*. Placement of *S. officinalis* and *S. azovtsevii* into the same species is shown to be unjustified. Allopolyploid origin of *S. azovtsevii* on the basis of *S. alpina* genome with a small contribution of *S. officinalis* is confirmed.

Key words: *Sanguisorba*, genetic diversity, randomly amplified DNA fingerprints, species differentiation.

Аннотация. Изучены генетические различия между *Sanguisorba officinalis* L., *S. alpina* Bunge и *S. azovtsevii* Krasnob. et Pschen. Метод случайно амплифицированных фрагментов (RAF) показал высокую степень генетической идентичности *S. alpina* и *S. azovtsevii*. Объединение видов *S. officinalis* и *S. azovtsevii* под одним названием также неоправданно. Подтверждено аллополиплоидное происхождение *S. azovtsevii* на основе генома *S. alpina* с небольшим вкладом генома *S. officinalis*.

Ключевые слова: *Sanguisorba*, генетические различия, метод случайно амплифицированных фрагментов ДНК, видовая дифференциация.

Introduction. The genus *Sanguisorba* L. (Rosaceae) includes about 20 species. For our investigation, we chose three species: *S. officinalis* L., *S. alpina* Bunge and *S. azovtsevii* Krasnob. et Pschen.

Within the territory of Russia, the most important species is *S. officinalis*. It is a medicinal plant, which is widely used. *Sanguisorba officinalis* is broadly distributed from Europe to Far East and North America. In traditional Russian medicine, *S. officinalis* roots were used to treat intestinal problems (bloody dysentery, stomatitis and others). The methanol extract of *S. officinalis* demonstrated anticancer and antithrombin activity (Goun et al., 2002). Inflorescences erect, spicate, 1–2 cm long, 0.5–1 cm wide. Chromosome numbers of *S. officinalis* in Siberia: 2n=28 (Seminskiy Pass), 56 (Azovtsev et Zaitseva, 1971).

Sanguisorba alpina is common in the Siberian mountains, and differs clearly from *S. officinalis* by its long inflorescence. *Sanguisorba alpina* is not used as a medicinal plant although it is a potentially valuable source of biologically active substances, triterpenoids (Jia et al., 1993). Inflorescence nodding, spicate, cylindric, rarely ellipsoid, 1.5–7 cm long, 1–1.5 cm wide, pendent. Chromosome number of *S. alpina* in Siberia: 2n=28 (Mishima et al., 2002).

Sanguisorba azovtsevii (Pschenichnaja et Krasnoborov, 1986) was described as an interspeci-

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Fig. 1. Inflorescence shape of three studied species of *Sanguisorba*.

fic hybrid between *S. officinalis* and *S. alpina*. This species is allied to *S. officinalis*, but differs from it by having longest stamens and inflorescences. Inflorescence cylindrical, 2–5 cm long, 1–1.5 cm wide, erect, sometimes pendent. However, *S. azovtsevii* differs from *S. alpina* by different biochemical content of the stamens. Chromosome number of *S. azovtsevii*: 2n=42 (Azovtsev et Zaitseva, 1971).

The species status of *S. azovtsevii* has been doubted; it was suggested that this species is one of the polyploid forms of *S. officinalis* since morphological differences between these two species are insignificant (Cherepanov, 1995) (Fig. 1). In order to establish taxonomic status of *S. azovtsevii*, we conducted population analysis of all three species with the goal to investigate their molecular-genetic differences.

In our work, we used RAF (Randomly amplified DNA fingerprints) technique, which is a modified DAF protocol (Waldron et al., 2002). Recently RAF was used effectively for evaluation of genetic diversity and interpopulational relationships both in animals and plants (Chan et al., 2008; Cunningham et al., 2002; Nand et al., 2005; Ramage et al., 2004).

Materials and methods. Plant material was collected in August 2010 within Russian Federation: 9 individuals of *S. officinalis* from Tuva Republic, at the bank of Uyuk River (55º 04’ 10’’ N, 94º 11’ 01’’ E); 10 individuals of the same species from Altai Republic, at Chibit Village (50º 21’ 29’’ N, E 87º 22’ 47’’ E); and 9 individuals of each *S. azovtsevii* and *S. alpina* from Altai Republic, at Seminskiy Pass (51º 05’ 50’’ N, E 85º 36’ 51’’ E). Fresh leaf material was dried using silica gel.

DNA was isolated using Diamond DNA kit (ABT Llc., Russia) according to the manufacturer’s instructions. Experimental amplifications were done using primers of series A – 01-10 and B – 01-11 (Carl Roth, Germany). For further work, we selected primers A04 (5’ – AATCGGGCTG – 3’) and B06 (5’ – TGCTCTGCCC – 3’).

PCRs were carried out in 25 μL reaction mix included 5 ng DNA, 2.5 μL 10x Buffer and 25 mM $MgCl_2$ (Sibenzyme Llc., Russia), 1 μL 5mM of mix dNTPs (Medigen Llc., Russia), 1 μL of each 10mM primer and 1 unit Taq DNA polymerase (Sibenzyme Llc., Russia) in the MyCycler thermal cycler (Bio-Rad, USA) using RAF protocol: 94.0ºC for 5 min. [94.0ºC for 30 sec., 57.0ºC for 1 min., 56.0ºC for 1 min., 55.0ºC for 1 min., 54.0ºC for 1 min., 53.0ºC for 1 min.]x35, 72.0ºC for 10 min., 4.0ºC until the end of the process.

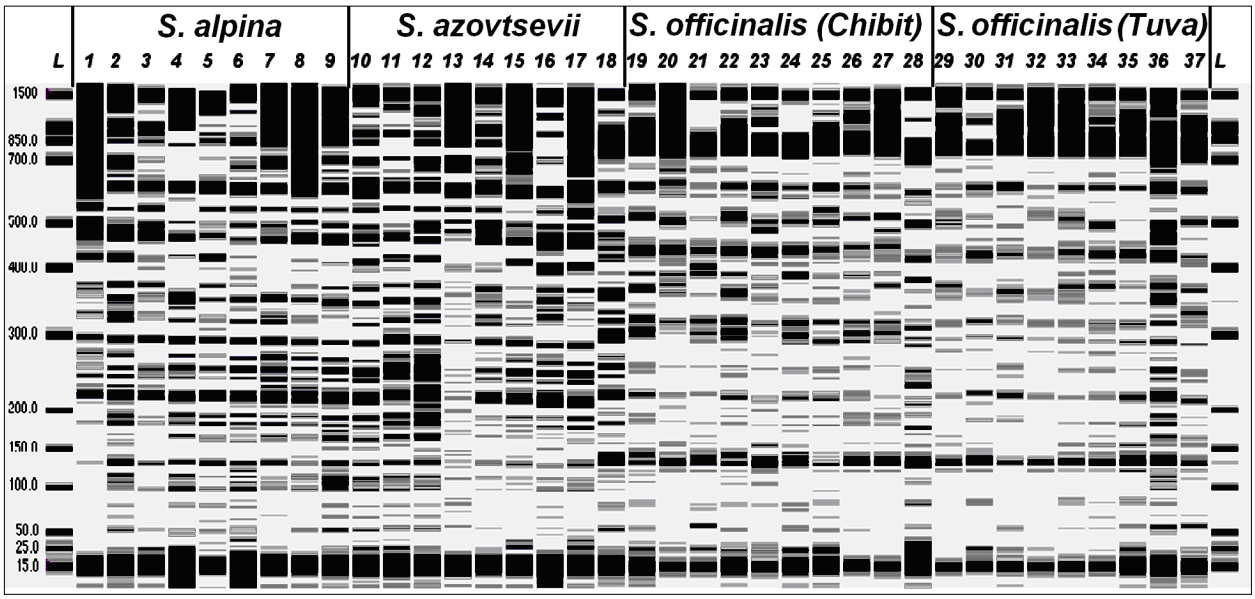

Fig. 2. The virtual gel photography of *Sanguisorba* individuals’ RAF-polymorphisms.

Table 1

Nei's unbiased measures of genetic identity and genetic distance between different population of *Sanguisorba*

Population	*S. alpina*	*S. azovtsevii*	*S. officinalis* (Chibit)	*S. officinalis* (Tuva)
S. alpina	***	0.9682	0.5887	0.5823
S. azovtsevii	0.0323	***	0.6726	0.6499
S. officinalis (Chibit)	0.5298	0.3966	***	0.9340
S. officinalis (Tuva)	0.5407	0.4310	0.0682	***

Note: Nei's (1978) genetic identity (above diagonal) and genetic distance (below diagonal).

DNA fragments were separated by microfluidic electrophoresis in Automated Electroforesis Station Experion (Bio-Rad, USA) using Experion DNA 1K Analysis Kit (Bio-Rad, USA). Resulting image (virtual gel) of fragments separation is presented on Fig. 2.

In the analysis we formed a matrix on the basis of presence (1) or absence (0) fragments of the equal length. There were 52 fragments for 37 individuals (4 populations) for further analysis: in populations of *S. alpina*, *S. azovtsevii* and *S. officinalis* from Tuva we used only 9 individuals. This dataset was used to calculate genetic diversity estimates, G_{ST} (Nei, 1973) and genetic distances (Nei, 1978) among populations and individual samples using the Popgene (v. 1.31), a software package of Yeh and Boyle (1997).

Multidimensional scaling (MDS) (Kruskal, 1964) was carried out using program for phenetic analysis NTSYS-pc, Numerical Taxonomy System, version 2.1 (Rohlf, 1992). Pairwise genetic distances were calculated using the coefficient of Lynch (1990).

Results and discussion. As a result of this study, we have revealed genetic diversity within populations of all three species of *Sanguisorba*. The highest genetic diversity was in *S. azovtsevii* (GST= 0.2830), which indicates a high intrapopulational polymorphism of this species. *Sanguisorba alpina* had GST=0.2137; and *S. officinalis*, GST=0.2569 (Chibit) and GST= 0.2289 (Tuva). Total genetic diversity for all populations GST=0.4053, therefore one cannot speak about free gene exchange among *S. alpina*, *S. azovtsevii* and *S. officinalis*. We interpret high genetic diversity in *S. azovtsevii* as a result of its hybrid origin from *S. alpina* and *S. officinalis*. At the same time, combined genetic diversity for *S. alpina* and *S. azovtsevii* was much lower (0.2678) than combined genetic diversity for *S. azovtsevii* and *S. officinalis* (GST=0.3913). This attests to high similarity between genomes of *S. alpina* and *S. azovtsevii*. The same is demonstrated by the analysis of genetic identity and genetic distance between different populations of *Sanguisorba* (Table 1).

The lowest genetic distance is observed between *S. alpina* and *S. azovtsevii*, and is very close to the genetic distance between populations of *S. officinalis*. It must be noted that in angiosperm species Nei's unbiased measure of genetic distance between populations based on RAF analysis do not exceed 0.3 even when populations are geographically distant (Gao et al., 2000; Huang et al., 2000; Hu et al., 2010; Yamskikh et al., 2011). However, distance-matrix methods (measures of gene diversity, genetic identity and genetic distance) do not always provide a correct estimate of genetic similarity since different characters in RAF analysis have different weight, which is not taken into account here. One of the methods that allows to minimize errors of statistical analysis in case of unequal weights is Multidimensional Scaling (MDS) (Weising, Nybom, 2005). A result of MDS analysis of RAF data is a three-dimensional graph that reflects the degree of similarity between the specimens (Fig. 3).

Studied specimens of *Sanguisorba* are differentiated into two highly separate groups. One group is formed by *S. officinalis* while the second includes *S. alpina* and *S. azovtsevii*; this is comparable to the data of genetic identity and genetic diversity analysis. Therefore, we established

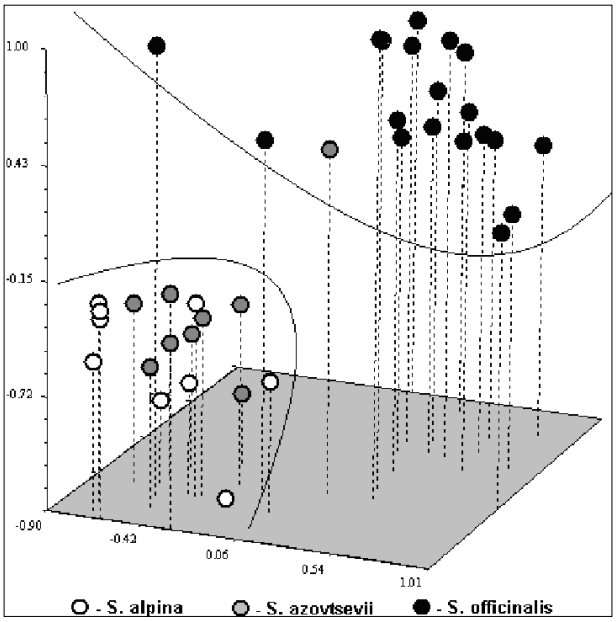

Fig. 3. Differentiation of *Sanguisorba* species based on multidimensional scaling analysis.

a very high degree of relatedness of *S. alpina* and *S. azovtsevii*. Based on the results of RAF DNA analysis, we confirm cytogenetic data on allopolyploid origin of *S. azovtsevii* on the basis of *S. alpina* genome, but not that of *S. officinalis*. Placement of *S. officinalis* and *S. azovtsevii* into the same species, suggested by Cherepanov (1995), is therefore unjustified. However, in our opinion, placement of *S. alpina* and *S. azovtsevii* into the same species is also not justified due to the existence of numerous cytogenetic, molecular-genetic and morphological differences that have been observed over more than 30 years.

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