Analysis of intraspecific polymorphism of *Nitraria sibirica* Pall. using the ISSR technique

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Abstract. The analysis of DNA polymorphism of *Nitraria sibirica Pall.* was carried out at 13 natural populations of the Republic of Altai and Altai Territory using the ISSR technique. Seven effective ISSR primers have been identified to analyze DNA polymorphism in *N. sibirica.* 99 DNA fragments were yielded at DNA amplification with these primers, 66 of them were polymorphic. The genetic distance Nei (D) between the studied populations of *N. sibirica* averaged 0.32, at mean 0.09 - within populations. An identification ISSR marker has been revealed, which can be used to study the genetic variability of the genus *Nitraria L. (Nitrariaceae)* species.

1 Introduction

The genus *Nitraria L. (Nitrariaceae)* is a representative of the ancient desert flora and includes about 10 species [1,2]. The genus is widespread in the steppe, semi-desert and desert regions of Minor, Central, North and Central Asia, Southeast Europe, North Africa, Australia. This plant populations are confined to intrazonal soils highly salted [3].

Nitraria sibirica Pall. is the most widespread and polymorphic species growing in Central Asia, Kazakhstan, China, Mongolia, Siberia. Despite its significant range, these species populations are usually small numbered and vulnerable state in Siberia. To preserve local populations, *N. sibirica* is included in the regional Red Data Books of the Republic of Altai, Republic of Khakassia, Irkutsk Region, Transbaikal Territory, where this species has the 2nd or 3rd nature conservation status [4,5,6,7].

Studying the species genetic polymorphism is important to establish regularities of evolutionary transformations under certain environmental conditions, and elaborate techniques for specific genotypes conservation [8]. To research polymorphism in populations, DNA marker systems are the most convenient, characterized by significant polymorphism and non-dependent on environment conditions where the plant grows. The most practical and easily reproducible tools include techniques for amplifying genomic DNA with a high intraspecific variability [9]. The method of the intermicrosatellite sequences (ISSR) analysis is used to study the genetic relationship of objects, analysis of taxa genetic diversity and phylogeny, and is most effective to investigate variability within a single species [10]. When assessing the genetic diversity in natural populations of the genus *Peganum*, close to *Nitraria*, it was noted that ISSR markers reveal higher-level

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polymorphism compared to ITS and RFLP systems [12]. At the same time, applying ISSR marker system does not always allow tracing the correlation between the genetic divergence of samples and their geographic origin [13]. Choosing a molecular marker, adequate to a given task and a taxonomic level of objects, similar to the choice of a trait or a trait group in comparative morphological analysis, has a great influence on the topology of phylogenetic trees [14].

The study objective is to select ISSR primers to analyze *Nitraria sibirica* genetic variability.

2 Materials and Methods

DNA samples for genetic analysis were obtained of 22 samples of *Nitraria (N. sibirica, N. schoberi L.)* collected during field studies in 2011-2020 at 13 natural populations of the Republic of Altai and Altai Territory (Table 1).

Table 1. Location of *Nitraria* populations

№	Symbol	Species	Title of populations	Collecting place
1	A1	*N. sibirica*	Dzhira	Altai Territory, Kulundina District, Dzhira Lake eastern shore
2	A2			
3	A3			
4	A4			
5	B1		Kuchuk	Altai Territory, Blagoveshchensk District, Nizhny Kuchuk village environs
6	B2			
7	C		Uglovskoe	Altai Krai, Uglovsky District, Uglovskoe village environs
8	D		Balansor	Altai Territory, Uglovsky District, Kruglyansky village, the Balansor Lake shore
9	E		Tassor	Altai Territory, Uglovsky District, the Tassor Lake shore
10	F		Chinkussor	Altai Territory, Uglovsky District, the Chinkussor Lake shore
11	G		Veseloyarsk	Altai Territory, Rubtsovsk District, Veseloyarsk village environs
12	H		Novenkoe	Altai Territory, Loktevsky District, Novenkoe village environs
13	I		Gornyak	Altai Territory, Loktevsky District, Gornyak village environs
14	J1		Rubtsovsk	Altai Territory, Rubtsovsk city environs
15	J2			
16	J3			
17	K		Pospelikha	Altai Territory, Pospelikhinsky District, Pospelikha village environs, turn to Babylonsky village
18	L		Tobeler	Altai Republic, Kosh-Agach District, Tobeler village environs
19	N1		Kosh-Agach	Altai Republic, Kosh-Agach District, 13 km of Kosh-Agach village, a lake shore in the Chuya river valley
20	N2			
21	N3			
22	X	*N. schoberi*	Kuchuk	Altai Territory, Blagoveshchensk District, New Kuchuk village environs

DNA was isolated of 5 mg leaf tissue dried by Liston et al (1990) methodology with CTAB protocol (Doyle and Doyle, 1987). PCR was carried out in a BIS-N M111-02 amplifier (BIS, Russia). The reaction mixture contained 10×Taq-buffer for PCR (BIORON), 10 mM $MgCl_2$ (BIORON), 10 mM ISSR primers, mixed (5 mM each) dNTPs (Medigen), 2 µl of template DNA, 5U / µl DFS-Taq DNA Polimerase (BIORON), water. The mixture volume for a sample was 25 µL. Samples were stained with SYBR Green.

Amplification took place according to the following protocol: Primary denaturation at 95° C – 2 min; 40 cycles including DNA denaturation at 94° C – 0.40 min, primer annealing – 1 min, chain elongation at 72° C – 2 min; final extension of chains at 72° C – 5 min. The amplified fragments were separated in an electrophoresis chamber in 1.5% agarose gel, the chamber was filled with 1×TBE buffer. Electrophoresis results were visualized using a UV gel documentation system (Bio-Rad GelDoc XR+). Sixteen primers were tested for amplification efficiency of PCR products at 22 selected *Nitraria* samples. The level of polymorphism for each primer in percent was calculated by the formula P = Np/N*100, where Np is the number of polymorphic fragments, N is the total number of fragments [15].

The dendrogram was created using software TREECON (version 1.3 b) by the UPGMA method with bootstrap support – 100 pseudoreplicas [16]. Genetic distances were calculated according to Ney [17].

3 Results

Seven ISSR primers were identified, which reproduced the clearest profiles on electrophoregrams (Table 2).

Table 2. Characteristics of ISSR primers used to study the variability of *N. sibirica*.

Name of a primer	Nucleotide sequence 5`-3`	$T_{annealing,}$ (ºC)	Number of amplified fragments	Number of polymorphic fragments	P, %
UBC 807	$(AG)_8T$	52	10	8	80
M 11	$(CA)_6R$	49	13	7	53,8
17899 A	$(CA)_6$A-<G>	47	12	10	83,3
17898 B	$(CA)_6GT$	42	9	5	55,5
HB 10	$(CAC)_3GC$	51	20	14	86,6
HB 12	$(GA)_6CC$	42	18	8	55,5
17898A	$(CA)_6AC$	45	17	14	82,4

4 Discussion

All profiles revealed 99 fragments, 66 of them were polymorphic. A ISSR primer initiated the synthesis of averaged 14.4 DNA fragments in *N. sibirica*. The number of amplified DNA fragments varied depending on the primer from 9 to 20, their sizes are 400–1530 bp. The primer HB 10 detected the largest number of loci, the primer 17898 B - the smallest one. The number of polymorphic loci detected by primers varied from 5 (primer 17898 B) to 14 (the primer HB 10 and 17898 A).

Interspecies and interpopulation differences were revealed only by the primer UBC 807 (Fig. 1). Unique to *N. schoberi* fragments range from 600 to 700 bp, as well as in the spectrum region from 1000 bp. At the same time, *N. schoberi* lacks fragments in 900 bp region, which are inherent in *N. sibirica* samples.

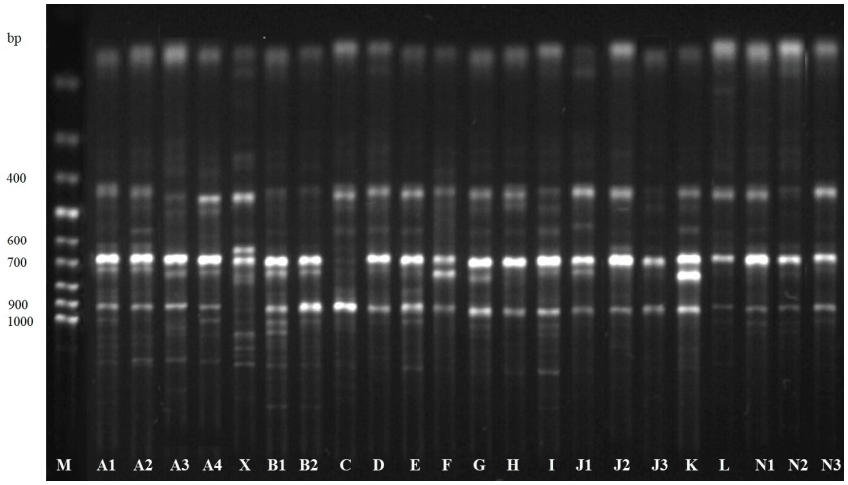

Fig. 1. ISSR profiles of fragments amplified with UBC 807 primer.

The genetic distance according to Ney (D) between *N. sibirica* and *N. schoberi* averaged D=0.63, between populations of *N. sibirica* – D=0.32, within populations of *N. sibirica* – D=0.09. In general, the intergroup differences are consistent with the geographic differentiation of *N. sibirica* populations (Fig. 2). The largest distances have been revealed between the populations of the high-mountainous Chuya steppe and the Kulunda flatland.

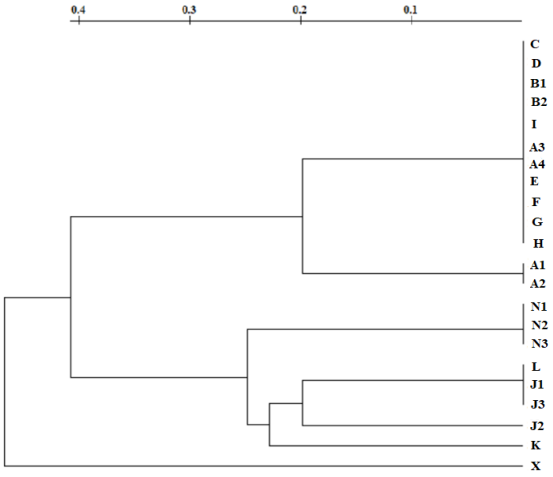

Fig. 2. Dendrogram of similarity of *Nitraria* samples, constructed by the UPGMA method based on the polymorphism of the ISSR marker UBC-807.

This study suggests the possibility of using the ISSR primer UBC 807 to analyze the phylogeography of *N. sibirica*. The revealed interpopulation differences in *N. sibirica*

samples of geographically distant natural populations evidence their genetic differentiation during isolation in intrazonal communities.

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