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ВОСПРОИЗВОДСТВО СОСНЫ КЕДРОВОЙ СИБИРСКОЙ НА ГЕНЕТИКО-СЕЛЕКЦИОННОЙ ОСНОВЕ*

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В статье приведены результаты изменчивости микросателлитных локусов ядерной ДНК клонового потомства плюсовых деревьев сосны кедровой сибирской 91/55, 94/58 и 100/64, произрастающее на прививочной плантации Караульного участкового лесничества Учебно-опытного лесхоза СибГУ им М. Ф. Решетнева (юг Средней Сибири).

По результатам исследований проведен отбор 8 стабильных и надежных праймеров методом полимеразной цепной реакции и электрофореза. Индетифицированы генотипы ДНК образцов сосны кедровой сибирской. Установлена $1{,}5\times10^{-6}$ вероятность случайного совпадения аллелей у неродственных генотипов. Выявлены 4 общих ДНК-профиля.

Ключевые слова: *сосна кедровая сибирская, плюсовые деревья, клоновое потомство, прививочные плантации, микросателлиты ядерной ДНК.*

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REPRODUCTION OF *PINUS SIBIRICA* DU TOUR ON A GENETIC AND BREEDING BASIS

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The article presents the results of variability of microsatellite loci of nuclear DNA of clone progeny of Siberian cedar pine trees 91/55, 94/58 and 100/64, growing on the grafting plantation of the Sentry district Forestry of the Educational and Experimental Forestry of the Reshetnev University (south of Central Siberia).

Based on the research results, 8 stable and reliable primers have been selected by polymerase chain reaction and electrophoresis. DNA genotypes of Siberian cedar pine samples have been identified. The probability of a random coincidence of alleles in unrelated genotypes was 1.5×10^{-6}. 4 common DNA profiles were identified.

Keywords: *siberian cedar pine, plus trees, clone progeny, grafting plantations, nuclear DNA microsatellites.*

INTRODUCTION

Siberian pine *Pinus Sibirica* Du Tour is one of the most important forest-forming species, as well as a valuable nut-bearing species in Russia [12]. Many studies have been performed to research the selection, variability and softening of Siberian pine *Pinus Sibirica* Du Tour in terms of productivity and reproductive development, and subsequently to the cultivation of selective planting material to create genetically valuable high-yielding nut-producing plantations [9; 6–13]. Grafting plantations cre-

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ated by ramets from plus trees certified for seed production are a valuable gene pool to conduct genetic research of selectable traits, which is an urgent task in the field of forest selection based on molecular genetics to create high-yielding industrial plantations [10–12].

Studying the genetic material of the clonal progeny of plus trees of Siberian cedar pine, growing at the educational and scientific places of the Karaulny district forestry of the Educational and Experimental Forestry Enterprise of the Reshetnev University is important for the conservation and rational use of the gene pools of this species.

The accumulated long-term data on the selection of Siberian cedar pine require genetic studies of the results obtained. In recent years, due to the development of specific primers, the SSR method, in which codominantly inherited nuclear microsatellite loci (nSSRs) are used as DNA markers, has become widely used in genetic studies of conifers. High polymorphism of microsatellites makes it possible to identify an organism with high accuracy and reveal biological relationships.

The purpose of the research is to select genetic markers of Siberian cedar pine and test them by carrying out genetic certification of the offspring of plus-trees.

OBJECTS AND RESEARCH METHODS

The research objects were the clonal offspring of three plus-trees 91/55, 94/58 and 100/64, growing on the grafting plantation of the Karaulny district forestry of the Educational and Experimental Forestry Enterprise of Reshetnev University. (southern Central Siberia).

Plus-trees were certified for yield in the Kolyvan forestry enterprise of the Novosibirsk region in 1977. Table 1 provides taxation characteristics of the plus-tree stand, where the studied plus trees grow. Table 2 characterizes the plus-trees at the certification time.

At the time of certification, the plus tree 91/55 was distinguished by a spherical crown with powerful branches with horns. The cones are large and late in maturity. The condition is good. Tree 94/58 had a powerful oval-oblong shape with powerful branches. The needle-foliage is thick. Female type of sexualization. The condition is good. Tree 100/64 had a spherical crown, dense foliage and an early ripening shape. The condition is very good. The survival rate of cuttings 91/55 is 88 %, 94/58 – 90 %, 100/64 – 97 %[**].

In 1988, plus-trees were propagated by grafting using the core method for cambium according to E. P. Prokazin. Siberian pine seedlings of local (Biryusa) origin were taken as a rootstock [1].

In May 2021, an inventory of ramets of plus trees was carried out at the grafting plantation and cuttings were cut for genetic research. Experimental material was collected from 47 clonal trees.

The methodological basis for the work was the microsatellite analysis method based on the polymerase chain reaction (PCR). Microsatellite loci are regions of the genome with repeating short (2–6 nucleotides) sequences called motifs [5]. The number of repeats at each locus varies from organism to organism, so each individual is characterized by a virtually unique multilocus genotype. Using the electrophoresis method gives a possibility to detect the variability of this parameter by determining the molecular weight of the variable fragment in this case the allele. Heavier molecules (with a larger number of repeats) migrate more slowly and polymorphism can be observed in the resulting electropherogram: the presence of different discrete variants in individual individuals, reflecting a different number of repeating motifs.

To study the variability of microsatellite loci of nuclear DNA, we studied pine needles, previously dried in a dry-heat oven at a temperature of +40 ºC for 2–3 days. DNA extraction from needles was carried out according to the standard protocol for plant tissues (CTAB method) using cetyltrimethylammonium bromide [15].

To select nuclear DNA microsatellite loci, developed specifically for studying the variability of Siberian cedar pine, 12 nuclear microsatellite loci were selected for testing [1], the characteristics of which are presented in Table. 3.

When performing PCR, a commercial GenPak®PCR Core kit (scientific manufacturing laboratory “Genlab” LLC, Russia) was used for all primers, according to the manufacturer’s instructions.

PCR-amplification of selected microsatellite loci was carried out using the following regime: preliminary DNA denaturation was at 94 °C – 15 min; then 10 cycles, including 1 min of melting at 94 °C, annealing of primers for 1 min at 60–50 °C (–1 °C for each cycle) and 1 min. elongation at 72 °C, the next 25 cycles consisted of 1 min of melting at 94 °C, 1 min of primer annealing at 53 °C, and 1 min of elongation at 72 °C. The final elongation cycle took place at 72 °C for 20 min.

Electrophoretic separation of amplification products was carried out in 6 % polyacrylamide gel (PAGE), using 1xTAE buffer in chambers for vertical electrophoresis (VE-20, LLC “HELICON COMPANY”) at a voltage of 200V for 2.5 hours. E. coli plasmid PBR322 DNA treated with HpaII restriction enzyme was used as a standard length marker. The gels were stained with ethidium bromide solution with further visualization of the amplicons in UV-light using the Gel-Imager gel documentation system. The analysis results were read using the Photo-Capt V.12.4 program (Vilber Lourmat). Analysis of the identified genotypes was carried out using the program (macro) GenAlEx, a freely distributed add-in for MS Excel [16].

[1] ** Data provided upon request in 2021 in the Kolyvan forestry of the Novosibirsk region (condition assessment cards).

Table 1
Taxation characteristics of a positive forest stand

Structure	Age class/years	Average height, m	Average diameter, cm	Bonitet	Stand type	Density	Selection category
9K	4/160	18	52	III	thv	0,6	Plus for seed production
1C	6/110	20	44				

Table 2
Biometric indicators of *Siberian cedar pine*, selected for seed productivity

Number of a plus-tree	Age, years	Height		Stem diameter		Crown diameter, m
		m	% to Xav.	cm	% to Xav.	
91/55	140	19	105	72	138	8,5
94/58	150	23	128	72	138	10,0
100/64	110	17	94	44	84	7,0

Table 3
Characteristics selected for testing the nuclear microsatellite loci of the Siberian cedar pine

№	Locus	Motif	Primer sequence (5'–3')	Number of alleles, pcs.
1	Ps_80612	$(AAG)_{10}$	F:CTTCTAAGTGGGTCATCTTGGC R:CTGTCTAGGCTTTTGGCCTTTA	4
2	Ps_364418	$(TGA)_{10}$	F:TCGGACCTAAAGAAAAGAGGTG R:AAGATTCGTCTGAGTGGACGTT	7
3	Ps_1502048	$(AAT)_{11}$	F:AGATCCATCCCAATCACAGTTC R:AGGGACCTAGCACTTTCATCCT	3
4	Ps_1915155	$(TAT)_{11}$	F:TTGTTGGATTGGCTCATGG R:TCTCCAGTACACACCTCGATTG	4
5	Ps_2040062	$(GAT)_{12}$	F:TGGTATGAAAACCTTCAGCCTC R:TAATGTCGTCTTCGTCGTCGT	2
6	Ps_264432	$(TTC)_{13}$	F: GCATTGTTGATTTGTGTCCCTA R: GAGGCTGAAAAGGAGAAGATGA	4
7	Ps_1375177	$(CAT)_{10}$	F: ATGGGCTAGATGGTAGCAGTTC R: GGTGGTTTGGCTCTCTTAAATG	3
8	Ps_31489	$(AGA)_6$	F: CACCCAAACAAGACAAACCTCT R: TTCTTCCTCCTTCCCCTTATTC	2
9	Ps_25981	$(TATT)_5$	F: TTGAGTGGGATGGACATAGAG R: TTGCCCCAAGTCTACAAGAT	3
10	Ps_39709	$(ATGT)_5$	F: GTTCTCTTAACCTCGAACTTGTGAT R: CTGAAAACCCTGTCAAACAACA	4
11	Ps_718958	$(TAT)_{10}$	F: CTATGTATGGGTCAATGGTGTCC R: GATGCAACAAATGCACATGACT	2
12	Ps_650842	$(TTA)_{11}$	F: ATGCACTCTAACTCCAAGCACA R: ATAATGACCCAAGCATGAAACC	4

RESULTS AND THEIR DISCUSSION

The main criterion to select primers at the stages of PCR and electrophoresis was the stable and reliable manifestation of loci alleles and a sufficiently high level of polymorphism on the tested samples. On DNA preparations isolated from the needles of 47 trees, 12 nuclear microsatellite loci were tested (Table 3). Primary analysis of these loci demonstrated that Ps_650842 and Ps_264432 had 3 amplification zones, which made it difficult to read variants of the variable zone and could lead to future errors when interpreting the data obtained. The Ps_2040062 locus was polymorphic, but it clearly contained “null alleles” (the lack of amplification of some samples may be due to mutations in the “landing” zone of the primer). Another primer (Ps_718958) turned out to be monomorphic (Fig. 1). After excluding the loci described above, 8 loci that demonstrated the most stable interpretable spectra were taken into further work (Fig. 2 and 3, Table 4).

Based on the results of a DNA study of 47 Siberian cedar pine samples for 8 polymorphic nuclear microsatellite loci, data was analyzed in the GenAlEx program to identify genotypes and assess the probability of a random coincidence of multilocus genotypes. The probability of a random coincidence of alleles in unrelated genotypes, calculated for each locus, should not exceed 5 %, and when using the entire set of markers, the probability of erroneously establishing genetic identity should not exceed one millionth of a percent [2]. The probability of a random coincidence of alleles in unrelated genotypes, calculated for each locus, should not exceed 5 %, and when using the entire set of markers, the probability of erroneously establishing genetic identity should not exceed one millionth of a percent [2]. The method is also called population matching probability. It is widely used in DNA forensics as an indicator of the statistical power of a particular set of marker loci [6].

Calculations based on a set of 8 loci showed that the probability of a random coincidence of unrelated genotypes was 1.5×10^{-6}. This value confirms the effectiveness of using this set of microsatellite loci for genetic certification of the offspring of plus Siberian cedar pine trees.

Analysis of multilocus allele combinations by 8 microsatellite loci showed that out of 47 studied samples, 26 belonged to different genotypes (Table 5). Four common DNA profiles were identified: genotype A – samples No. 100/64_21-16 (2) and 100/64_21-16 (4); genotype B – samples No. 100/64_2-19, 100/64_3-19, 100/64_4-17, 100/64_4-18, 91/55_6-17, 100/64_7-18; genotype C – 91/55_3-15, 91/55_3-16, 91/55_5-15a, 91/55_8a-16, 91/55_8-16, 91/55_9a-16, 91/55_10a-16, 91/55_11-16 , 91/55_12-16; genotype D – samples No. 91/55_9-16 (1), 91/55_10-16 (2).

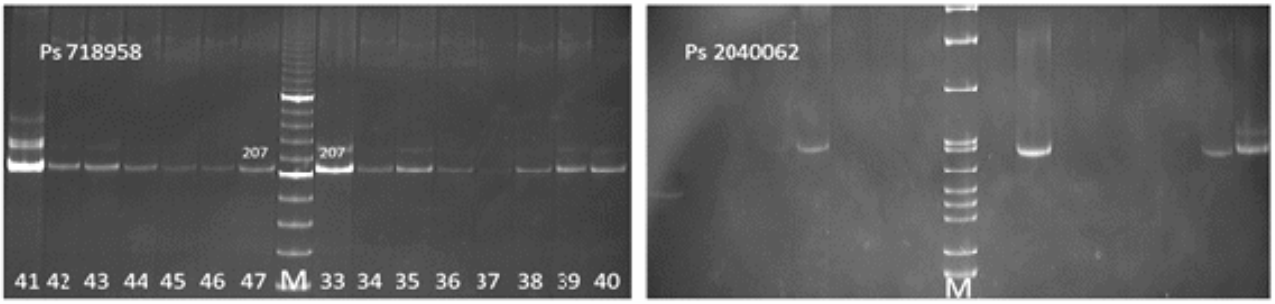

Fig. 1. Electropherograms of loci amplification products of Ps_718958 and Ps_2040062.
33–47 – serial numbers of tree samples. M – standard fragment length marker

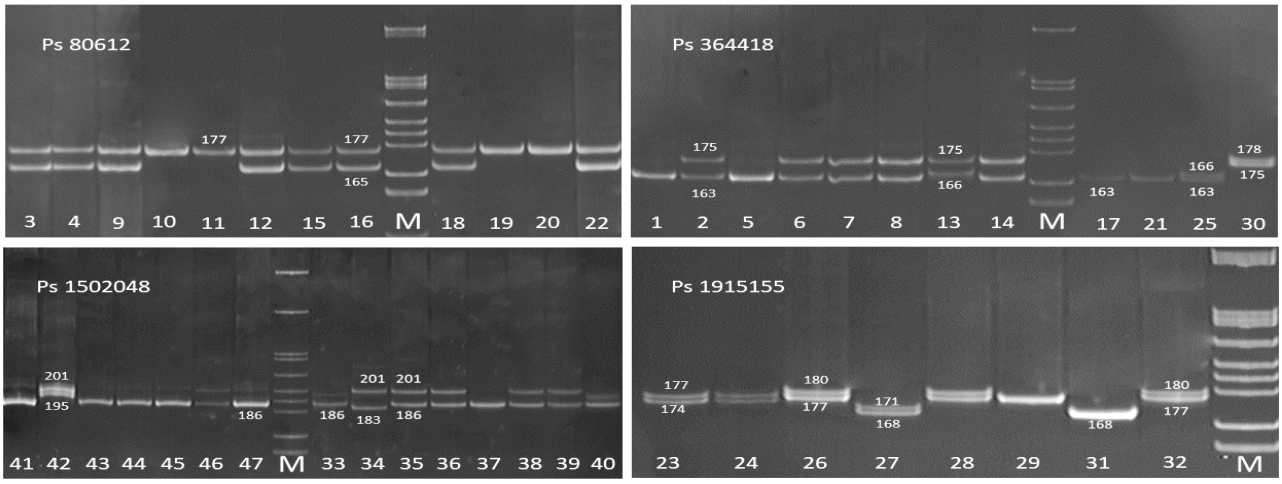

Fig. 2. Electropherograms of loci amplification products of Ps_80612, Ps_364418, Ps_1502048, Ps_1915155.
1–47 – serial numbers of tree samples. M – standard fragment length marker

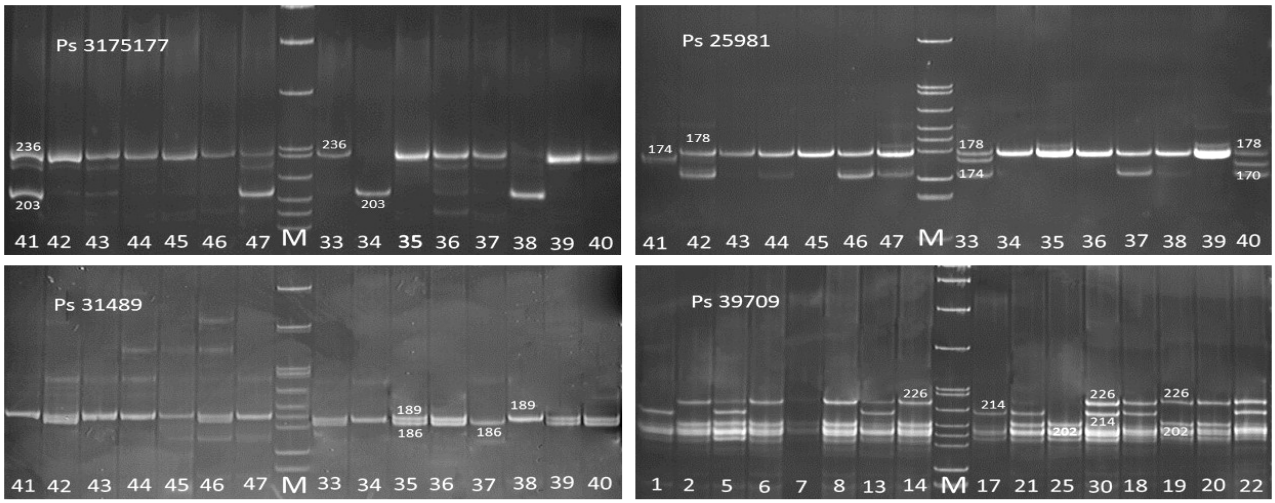

Fig. 3. Electropherograms of loci amplification products of Ps_3175177, Ps_25981, Ps_31489, Ps_39709.
1–40 –serial numbers of tree samples. M – standard fragment length marker

Table 4
Characteristics of nuclear microsatellite loci selected for further researching *Siberian cedar pine*

№	Locus	Motif	Fragment size	Number of alleles, pcs.
1	Ps_80612	$(AAG)_{10}$	162–180	5
2	Ps_364418	$(TGA)_{10}$	163–178	5
3	Ps_1502048	$(AAT)_{11}$	183–201	5
4	Ps_1915155	$(TAT)_{11}$	162–183	7
5	Ps_1375177	$(CAT)_{10}$	203–236	4
6	Ps_31489	$(AGA)_6$	186–189	2
7	Ps_25981	$(TATT)_5$	170–178	3
8	Ps_39709	$(ATGT)_5$	202–226	3

Table 5
Number of genotype matches in samples of progeny of plus *Siberian cedar pine* trees

№	Identification number		Multilocus genotypes	Genotype designation	Number of matches
	clone (plus-tree)	ramet			
1	100/64	21-16 (2)	16316317717718918917717723623618618617817821422 6g	A	2
2	100/64	21-16 (4)			
3	100/64	2-19	16317518018318918916517723623618319217417820222 6g	B	6
4	100/64	3-19			
5	100/64	4-17			
6	100/64	4-18			
7	100/64	6-17			
8	100/64	7-18			
9	91/55	3-15	16317817718018618916517723623618618617017821422 6g	C	9
10	91/55	3-16			
11	91/55	5-15a			
12	91/55	8a-16			
13	91/55	8-16			
14	91/55	9a-16			
15	91/55	10a-16			
16	91/55	11-16			
17	91/55	12-16			
18	91/55	10-16 (1)	16617217417718618916517723623618618617017820221 4g	D	2
19	91/55	10-16 (2)			
20	91/55	9-16 (1)	17517817418318618617717720623618619517817820222 6g	E	2
21	91/55	9-16 (2)			
22	100/64	2-18	16316317118018618917717720623618319217817821421 4g	1	0
23	100/64	3-18	16316317718018618916517720623618620117417820221 4g	2	0
24	91/55	5-16	16316317417418618617717723623620120117017820220 2g	3	0
25	91/55	6-16	16317816217418618916517720623618618617817820221 4g	4	0
26	100/64	6-19	16617517417718618916817720623618620117817821422 6g	5	0
27	100/64	8-18	16316317717718918916817720923618618617817821421 4g	6	0
28	100/64	9-18	16316317718018918916516520623618618617817820221 4g	7	0
29	100/64	10-18	16316617717718618917717720623618618617817820220 2g	8	0
30	91/55	11a-16	16317516817118618616517720620918618617817820221 4g	9	0
31	91/55	13-15	16316317717718918917717720623618618617817821421 4g	10	0
32	100/64	13-17	17517817717718618917718020320318618617017821422 6g	11	0
33	91/55	14-15	16316616816818618916518023623618618617417821421 4g	12	0
34	91/55	14-16	16317517718018918917718023623618619517017820221 4g	13	0
35	94/58	15-14	16316317717718618916517423623618618617417821422 6g	14	0
36	94/58	16-15 (1)	16317817417718918917717720620618320117817821421 4g	15	0
37	94/58	16-15 (2)	17817817717718618916517723623618620117817820221 4g	16	0
38	94/58	17-13	16617517718018618916817723623618620117817820222 6g	17	0
39	94/58	17-15	16317517417418618616217723623618618617817821422 6g	18	0
40	94/58	18-13	17517517718318918917718020620618620117817820221 4g	19	0
41	94/58	18-14	16317817718018618916216523623618620117817820221 4g	20	0
42	94/58	19-13	16317517718018618917718023623618619517017821422 6g	21	0
43	100/64	19-18	16617217718018918916517720623618618617417421421 4g	22	0

End of table 5

№	Identification number		Multilocus genotypes	Genotype designation	Number of matches
	clone (plus-tree)	ramet			
44	100/64	21-16 (1)	1631751771801861891771802362361952011781782266226g	23	0
45	100/64	21-16 (3)	1751751741801891891771802362361861861781782142266g	24	0
46	100/64	21-16 (5)	1631631711801861891651652362361862011781782266226g	25	0
47	100/64	22-17	1631751771771891891771772062061861861781782142146g	26	0

To confirm the correspondence of the obtained clone genotypes with the supposed plus trees No. 91/55, 94/58 and 100/64 growing in the Novosibirsk region, it is necessary to select material (needles or wood) from these trees. The authors plan to select control samples from plus-trees to determine their genetic characteristics and more detailed comparison of their genotypes with the genotypes of the clonal offspring.

CONCLUSION

During the research, 8 nuclear microsatellite loci of Siberian pine were selected, demonstrating the most stable interpretable spectra. The probability of a random coincidence of unrelated genotypes at these loci was 1.5×10^{-6}.

Four common DNA profiles were identified: genotype A – samples No. 100/64_21-16 (2) and 100/64_21-16 (4); genotype B – samples No. 100/64_2-19, 100/64_3-19, 100/64_4-17, 100/64_4-18, 91/55_6-17, 100/64_7-18; genotype C – No. 91/55_3-15, 91/55_3-16, 91/55_5-15a, 91/55_8a-16, 91/55_8-16, 91/55_9a-16, 91/55_10a-16, 91/55_11- 16, 91/55_12-16; genotype D – samples No. 91/55_9-16 (1), 91/55_10-16 (2).

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