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INVESTIGATION OF THE SUGAR BEET (*Beta vulgaris* L. ssp. *vulgaris*) MICROSATELLITE LOCI STRUCTURE TO DEVELOP A TECHNOLOGY FOR GENETIC ANALYSIS OF SUGAR BEET LINES AND HYBRIDS

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Abstract

The quality control in the course of maintenance and reproduction of sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) hybrid parent lines upon seed production is highly important. The method of microsatellite analysis seems to be very perspective tool to provide genotyping during breeding and seed production. Different research groups reported about microsatellite loci in the sugar beet genome. However, the implementation of this technique into the breeding process requires the development of robust and high-throughput technology of analysis. To develop a technology for obtaining stable DNA profiles, a more detailed study of the sugar beet genome microsatellite loci is required using a large set of verified breeding material. The sequencing a number of sugar beet genome regions containing microsatellite loci to clarify the nature of polymorphism as well as ability for providing the stable DNA profiles has been made in this study. Together with breeders (Pervomayskaya Selection and Experimental Station, Krasnodar Krai), a collection of 146 sugar beet plant samples was selected, including 28 male-sterile (MS) lines, 28 O-type lines, 82 pollinator lines, 6 hybrids of Russian selection (Azimut, Corvette, Pervomaisky, Rubin, Fregate, Uspekh) as well as Dobrava and Dorothea hybrids. Five plants of each sample were analyzed for 12 microsatellite loci, FDSB 502, FBSB 1001, FDSB 1033, Unigene 27833, Unigene 26753, Unigene 16898, Unigene 17623B, Unigene 15915, Unigene 17923, SB 04, SB 09, and SB 15. Allelic variants of each locus were amplified, cloned into the pAL2-T plasmid vector and sequenced. The results of sequencing the microsatellite loci FDSB 1001, FDSB 1033, Unigene 16898, Unigene 17623B, Unigene 26753, Unigene 17923, Unigene 27833, and SB 04 revealed that their length polymorphism is solely due to the different number of tandem repeats in the amplified DNA fragment. The locus Unigene 15915 was excluded from further work because of insertions and deletions in the flanking regions of microsatellite repeats $(AC)_n$ in its allelic variants. The polymorphism of allelic variants of the microsatellite loci SB 09, SB 15, and FDSB 502 is due to the complex (composite) repeats. Nevertheless, the SB 09 and SB 15 loci were approved for further study, since they produced stable DNA profiles. The allelic variants of the locus FDSB 502 contained the $(TC)_n(GAT)_n(AAG)_n$ sequence, which in some cases may complicate the analysis. To use this locus for the genetic analysis of sugar beet lines and hybrids, we propose the primers flanking only variable microsatellite repeatsб the $(GAT)_n$ and $(AAG)_n$ separately. The results we report here are prospective to develop a technology for the genetic analysis of sugar beet lines and hybrids as a reliable tool for both breeding and seed production.

Keywords: *Beta vulgaris*, sugar beet, fingerprinting, microsatellite analysis, DNA-profile

Sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) is an important industrial crop, accounting for approximately 40% of global sugar production. It is also used as a

high-energy animal feed (beet molasses and beet pulp) and grown for biofuel production [1, 2].

In the recent past, the main indicators of the effectiveness of the breeding process were non-flowering and sugar yield per unit amount of raw materials and sowing area. Currently, economic priorities are increasing technological suitability of raw materials, seeds with high sowing and physical properties, tolerance to herbicides, resistance to diseases, pests, environmental factors, and, most importantly, the profitability of seed production and the cultivation of commercial crops [3-5].

In this regard, modern sugar beet hybrids are created on the basis of dioecious forms with cytoplasmic male sterility (CMS) and the so-called fixers of the CMS trait (O-type lines) and are multicomponent [6]. Therefore, commercial seed production of sugar beets is very complex and requires compliance with a number of conditions. Firstly, it is necessary to strictly monitor the high-quality maintenance and reproduction of all components, which, at a minimum, include components of the maternal form, the MS line (a line with cytoplasmic male sterility) and the O-type fixative line, as well as the component of the paternal form, the line pollinator.

The creation of a commercial sugar beet hybrid is multi-step and involvs crossing specific parental lines to produce simple intermediate hybrids. The selection of each component for hybridization is carried out based on the specific combinative ability which is determined experimentally in test crosses. Thus, to consistently produce a commercial hybrid, it is necessary to control all components used to generate the final hybrid, as well as intermediate hybrids.

Genetic analysis can be used to evaluate the quality of breeding material at various stages of creating a sugar beet hybrid. Such an analysis is necessary for the genetic identification of lines that are components of the hybrid, as well as for assessing their homogeneity.

A number of molecular genetic methods can be used to analyze plant genomes: RFLP (restriction fragment length polymorphism) [7], AFLP (amplified fragment length polymorphism) [8], RAPD (random amplified polymorphic DNA) [9], SCAR (sequence characterized by an amplified region) [10], SNP (single nucleotide polymorphism) [11], DArT (diversity array technology) [12], SSR (simple sequence repeat). or microsatellite analysis [13]. Among them, the most commonly used methods for identifying plant genotypes are SNP and SSR. Note, the method of studying single nucleotide polymorphisms (SNP) was used to analyze the genome of both sugar beet [14-16] and a number of other crops, e.g., cocoa [17], cucumber [18], cauliflower [19], honeysuckle [20]. However, the use of this method for reliable identification of genotypes requires the development and subsequent recording of a large number of markers (from hundreds to several thousand) and expensive equipment for obtaining and processing the results.

For the purposes we mentioned, the most promising is the analysis of microsatellite loci polymorphisms in the sugar beet genome. The microsatellite markers give a stably reproducible DNA profile (primers are complementary to conservative regions of the genome). In addition, these markers are codominant, allowing their use to track the inheritance of the genomes of parental lines in intermediate and final hybrids.

Despite a number of works have been published on the microsatellite analysis method in sugar beet breeding programs both abroad [21-26] and in Russia [27-29], this technology is not convenient. To create a technology that allows unique and stable DNA profiles to be generated, an in-deep study of genomic microsatellite profiles on a large sample of verified sugar beet breeding material is

required.

In the presented study, we for the first time carried out a detailed analysis of the primary structure of a number of microsatellite loci in the sugar beet genome to determine the nature of the polymorphism of these regions and their suitability for obtaining stable DNA profiles.

The goal of our work was to study the structure of microsatellite loci of the sugar beet genome for subsequent use in creating a technology for genetic analysis of lines and hybrids.

Materials and methods. The study was performed on 146 samples of sugar beet (*Beta vulgaris* L.) plants, including 28 MS lines, 28 O-type lines, 82 pollinator lines, 6 hybrids of domestic selection (Azimut, Korvet, Pervomaisky, Rubin, Fregat, Uspekh), hybrids Dobrava and Dorotea (provided by the Pervomaisk Selection and Experimental Station of Sugar Beet, Gulkevichi, Krasnodar Territory). For reliable results, five different plants of each sample were used.

Genomic DNA was isolated from green leaves by CTAB extraction with additional purification with chloroform [30]. DNA in the resulting preparations was detected by electrophoresis in a 1% agarose gel, followed by staining with ethidium bromide. The quality and quantity of isolated DNA were determined on a SPECTROstar Nano plate spectrophotometer (BMG LABTECH GmbH, Germany).

Amplification of target DNA fragments was carried out with locus-specific primers FDSB 502 [21], FDSB 1001, FDSB 1033, 521.6 [24], SB 04, SB 09, SB 15 [25], Unigene 15915, Unigene 16898, Unigene 17623B, Unigene 17923, Unigene 26753, Unigene 27833 [23], labeled with fluorescent dyes FAM, R6G, TAMRA and ROX. PCR was run in a 25 μl reaction mixture containing 67 mM Tris-HCl, pH 8.8; 16.6 mM $(NH_4)_2SO_4$ (AppliChem, USA); 2.5 mM $MgCl_2$ (AppliChem, USA); 5 units/μl of Taq-DNA polymerase (DNA-Technology LLC, Russia), 25 mM dNTP (Medigen LLC, Russia), 10 pmol of each primer (NPK Syntol, Russia) and 2 μl of DNA solution. The PCR protocol was 95 °C for 5 min; 30 cycles: 94 °C for 30 s, 53 °C for 30 s, 72 °C for 30 s; 72 °C for 5 min (a CFX-96 thermal cycler, Bio-Rad, USA).

PCR products were detected by high-resolution capillary electrophoresis under denaturing conditions (an ABI PRISM 3130XL genetic analyzer, Applied Biosystems, USA). To determine the size of PCR fragments using the DNA Fragment Analysis software (IAP RAS, Russia), 1 μl of the PCR product was mixed with 0.5 μl of the molecular weight marker GeneScan™ 600 LIZ (Applied Biosystems, USA) and 8 μl of Super DI formamide (MCLab, USA) and denatured for 5 min at 95 °C.

Preparation of samples for sequencing included amplification of each allelic variant with unlabeled primers and subsequent purification of the resulting amplified DNA fragment using the Cleanup Mini kit (JSC Evrogen, Russia). Purified PCR products were cloned into the plasmid vector pAL2-T (JSC Evrogen, Russia), which was delivered into *Escherichia coli* XL1-Blue strain by electroporation. Clones after blue-white selection were tested for the presence of the insert using PCR. Plasmid DNA was isolated by a standard method [31]. Plasmid inserts were sequenced by the Sanger method with a standard pair of primers, the M13F 5′-GTTGTAAAACGACGGCCAGTG-3′, M13R 5′-AGCGGATAACAATTTCACACAGGA-3′ (Synthol Research and Production Company, Russia). To ensure the reliability of sequencing results for each allelic variant, two DNA samples were taken from unrelated samples and two clones were selected from a Petri dish.

Nucleotide sequence analysis was performed using Chromas 2.6.6 (Tech-

nelysium Pty, Ltd., Australia) and Clustal Omega (EMBL's European Bioinformatics Institute, EMBL-EBI, UK) software.

The design of new primers for the FDSB502 microsatellite locus, flanking only tandem repeat regions, was carried out with Primer3Plus software, EMBL (https://www.primer3plus.com), the absence of secondary structures in the sequence was checked with Oligo Calc software: Oligonucleotide Properties Calculator (http://biotools.nubic.northwestern.edu/OligoCalc.html).

Results. The polymorphism of microsatellite loci in the sugar beet genome was studied using plant material from the collection of the Pervomaisk Sugar Beet Breeding and Experimental Station (Krasnodar Territory, Gulkevichi), used in breeding in 2018-2022.

For reliable discrimination and identification of plants, the selection of the most informative microsatellite loci is of decisive importance. For this purpose, based on an analysis of literature data, 40 microsatellite loci were initially selected [27]. Selection was carried out by the following criteria: the number of alleles in the locus is at least three; the location of loci on different chromosomes, which should ensure independent inheritance of DNA markers; DNA fragment size from 100 bp up to 400 bp for reliable determination of PCR fragment lengths.

The polymorphism of the selected loci was studied experimentally on a set of 129 sugar beet samples. Loci that were monomorphic, difficult to amplify, or that gave ambiguous and unstable results were excluded from the study. As a result, 13 microsatellite loci remained, the 521.6, FDSB 502, FBSB 1001, FDSB 1033, Unigene 27833, Unigene 26753, Unigene 16898, Unigene 17623B, Unigene 15915, Unigene 17923, SB 04, SB 09, SB 15 which were highly polymorphic (from 3 to 11 detected alleles for each locus). Using them, unique DNA profiles were generated for each sample of sugar beet [27].

A study of a larger set of 146 sugar beet samples confirmed the suitability of these loci for genetic analysis. The exception was the 521.6 locus [24] the amplification of which in some cases gave nonspecific DNA fragments in addition to the target product (Fig. 1). Therefore, locus 521.6 was excluded from tests.

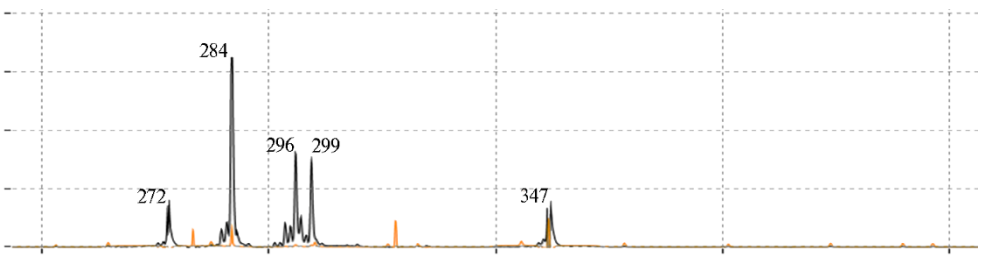

Fig. 1. Electropherogram of PCR products of the microsatellite locus 521.6, labeled with the fluorescent dye TAMRA, in the sugar beet line Op 66279 7/10 from the working collection of the Pervomaisk Research and Development Station of Sugar Beet (Gulkevichi, Krasnodar Territory, 2018-2022). During amplification, nonspecific DNA fragments (272 bp, 296 bp, 299 bp, 347 bp) appeared in addition to the target 284 bp PCR product.

As a result of genetic analysis of 146 sugar beet lines, 35 lines with a high homogeneity were selected. Homogeneous lines (all plants had an identical DNA profile for microsatellite loci) were involved in the breeding as components for the creation of new hybrids. Lines with incomplete homogeneity (less than 80%) were subjected to further self-pollination, followed by annual control of uniformity using microsatellite analysis.

All allelic variants of the studied microsatellite loci that we identified are submitted in Table 1.

1. **Polymorphism of microsatellite loci of sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) identified in a set of 146 samples from the working collection of the Pervomaisk Breeding and Experimental Station of Sugar Beet** (Gulkevichi, Krasnodar Territory, 2018-2022)

Locus	Alleles, bp	References
FDSB 502	265, 271, 273, 276, 279, 283, 286, 293, 314	[21]
FDSB 1001	315, 323, 325, 333, 347, 351	[24]
FDSB 1033	167,177, 193, 195, 197, 199, 221, 229	[24]
SB 04	180, 186, 189, 192, 195, 198, 201	[25]
SB 09	130, 133, 136	[25]
SB 15	146, 150, 154, 160, 166, 170, 174	[25]
Unigene 15915	299, 305, 314, 321, 339, 342, 345, 349, 383	[23]
Unigene 16898	276, 279, 285, 291	[23]
Unigene 17623B	147, 153, 156, 159, 162, 165, 168, 171, 174, 177, 180	[23]
Unigene 17923	193, 195, 197, 199, 201, 203, 205, 209, 215, 219, 225	[23]
Unigene 26753	282, 285, 288, 291, 294, 297, 303	[23]
Unigene 27833	190, 199, 205, 208, 211, 214, 217	[23]

N o t e. The size of PCR products was determined by high-resolution capillary electrophoresis under denaturing conditions on an ABI PRISM 3130XL genetic analyzer (Applied Biosystems, USA). The GeneScan™ 600 LIZ molecular weight marker (Applied Biosystems, USA) was used as a size standard.

To obtain reliable results of genetic analysis, the length polymorphism of microsatellite loci must be caused only by the microsatellite repeats without additional insertions or deletions outside the repeat region in the amplified fragment. Therefore, at the next stage, we assessed the primary structure of the 12 microsatellite loci used in the analysis.

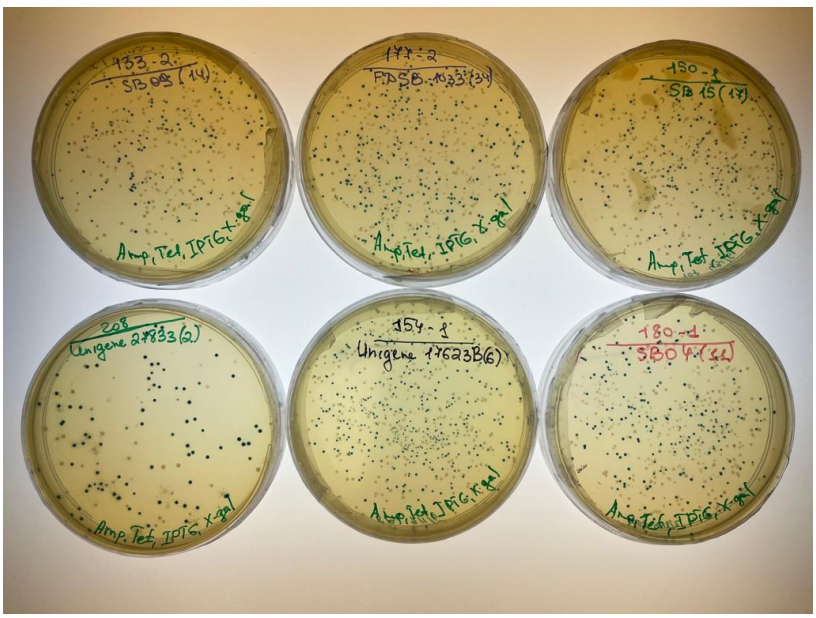

Fig. 2. Petri dishes with *Escherichia coli* XL1-Blue transformants carrying the pAL2-T plasmid vector with inserts of microsatellite loci SB 09, FDSB 1033, SB 15, Unigene 27833, Unigene 17623B and SB 04 target fragments (white clones). DNA fragments of micro-satellite loci were obtained from the analysis of the working collection of sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) of the Pervomaisk Sugar Beet Breeding and Experimental Station (Gulkevichi, Krasnodar Territory, 2018-2022).

Allelic variants of each of the 12 loci were individually amplified and cloned into the pAL2-T plasmid vector (JSC Evrogen, Russia). The resulting *E. coli* transformants with inserted target DNA fragments (Fig. 2, white colonies) were selected and their plasmid DNA was sequenced for each allelic variant of the corresponding locus.

The results of the analysis of nucleotide sequences of all microsatellite loci

studied in our work are schematically shown in Figure 3.

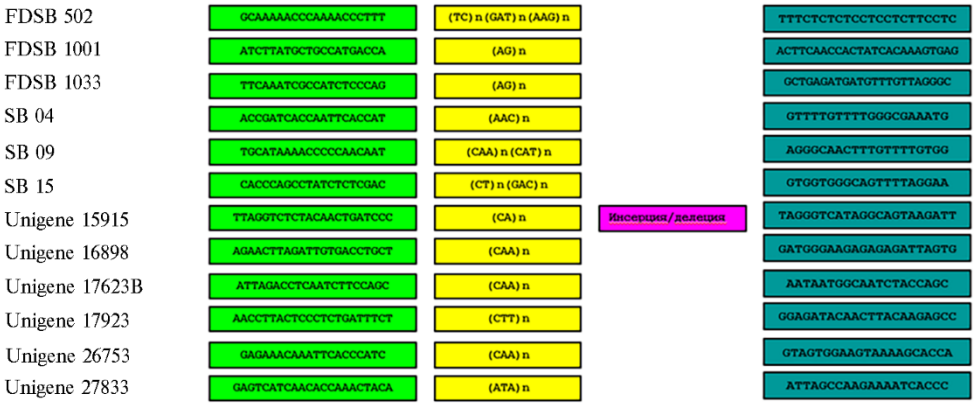

Fig. 3. The structure of microsatellite loci in the genome of sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) samples from the working collection of the Pervomaisk Sugar Beet Breeding and Experimental Station (Gulkevichi, Krasnodar Territory, 2018-2022). Sequences marked in light green and dark green are primer regions, in yellow are tandem repeat regions, and in pink are insertions/deletions.

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Unigene 26753_288-1     GAGAAACAAATTCACCCATCAACATCTTCAACAACAACAACAACAACAACGACAACAACC   60
Unigene 26753_288-2     GAGAAACAAATTCACCCATCAACATCTTCAACAACAACAACAACAACAACAACAACAACC   60
Unigene 26753_294-3     GAGAAACAAATTCACCCATCAACATCTTCAACAACAACAACCACAACAACAACAACAACA   60
Unigene 26753_294-4     GAGAAACAAATTCACCCATCAACATCTTCAACAACAACAACCACAACAACAACAACAACA   60
Unigene 26753_303-5     GAGAAACAAATTCACCCATCAACATCTTCAACAACAACAACAACAACAACCCACAACG     60
Unigene 26753_303-6     GAGAAACAAATTCACCCATCAACATCTTCAACAACAACAACAACAACGACAACCACAACA   60

Unigene 26753_288-1     ACAACAA---------------AGGAGCAAGTTCTTACCAATGCTATGCTCCTCATCAAG  105
Unigene 26753_288-2     ACAACAA---------------AGGAGCAAGTTCTTACCAATGCTATGCTCCTCATCAAG  105
Unigene 26753_294-3     ACAACAACAACAA---------AAGAGCAAGTCCTTACCAAAGCTATGCTCCTCATCAMC  111
Unigene 26753_294-4     ACAACAACAACAA---------AAGAGCAAGTTCTTACCAATGCTATGCTCCTCATCAAC  111
Unigene 26753_303-5     ACAACAACAACAACAACAACAAAAGAGCAAGTTCTTACCAATGCTATGCTCCTCATCAAC  120
Unigene 26753_303-6     ACAACAACAACAACAACAACAAAAGAGCAAGTTCTTACCAATGCTATGCTCCTCATCAAC  120

Unigene 26753_288-1     CAAAGAAGTGACCAAGCTTTCGCTTTCGCATCGGCGTAGCTCCTCCGCCTGCTCCACCAC  165
Unigene 26753_288-2     CAAAGAAGTGATCAAGCTTTCGCTTTCGCATCGGCGTAGCTCCTCCGCCTGCTCCACCAC  165
Unigene 26753_294-3     CAAGGAAGTGATCAAGCTTTCGCTTTCGCATCAGCGTAGCTCCTCCACACATGTTCCACCAC  171
Unigene 26753_294-4     CAAAGAAGTGATCAAGCTTTCGCTTTCGCATCGGCGTAGCTCCTCCACATGTTCCACCAC  171
Unigene 26753_303-5     CAAAGAAGTGATCAAGCTTTCGCTTTCGCATCGGCGTAGCTCCTCCACCTGTTCCACCAC  180
Unigene 26753_303-6     CAAAGAAGTGATCAAGCTTTCGCTTTCGCATCGGCGTAGCTCCTCCACCTGTTCCACCAG  180

Unigene 26753_288-1     CTCCTCCACCGTCTCCGAGGACCCTTTATCACCAAAAGTTGGGTGTATGGGCCAAGTTAA  225
Unigene 26753_288-2     CTCCTCCACCGTCTCCGAGGACCCTTTATCACCAAAAGTTGGGTGTATGGGCCAAGTTAA  225
Unigene 26753_294-3     CTCCTCCACCGTCTCCGAGGACCCTTTATCACCAAAAGTTGGGTGTATGGGCCAAGTTAA  231
Unigene 26753_294-4     CTCCTCCACCGTCTCCGAGGACCCTTTATCACCAAAAGTTGCGTGTATGGGCCAAGTTAA  231
Unigene 26753_303-5     CTCCTCCACCGTCTCCGAGGACCCTTTACCACCAAAAGTTGGGTGTATGGGCCAAGTTAA  240
Unigene 26753_303-6     CTCCTCCACCGTCCCCGAGGACCCTTTATCACCAAAAGTTGGGTGTATGGGCCAAGTTAA  240

Unigene 26753_288-1     GAGAAACAACAAAGTTGTTGGTTTTCCAAGTAAGAGTTTTGTTGGTGCTTTTACTTCCAC  285
Unigene 26753_288-2     GAGAAACAACAAAGTTGTTGGTTTTCCAAGTAAGAGTTTTGTTGGTGCTTTTACTTCCAC  285
Unigene 26753_294-3     GAGAAACAACAAAGTTGTTGGTTTTCCCAGTAAGAGTTTCGTTGGTGCTTTTACTTCCAC  291
Unigene 26753_294-4     GAGAAACAACAAAGTTGTTGGTTTTCCAAGTAAGAGTTTTGTTGGTGCTTTTACTTCCAC  291
Unigene 26753_303-5     GAGAAACAACAAAGTTGTTGGTTTTCCAAGTAAGAGTTTTGTTGGTGCTTTTACTTCCAC  300
Unigene 26753_303-6     GAGAAACAACAAAGTTGATGGTTTTCCAAGTAAGAGTTTTGTTGGTGCTTTTACTTCCAC  300

Unigene 26753_288-1     TAC         288
Unigene 26753_288-2     TAC         288
Unigene 26753_294-3     TAC         294
Unigene 26753_294-4     TAC         294
Unigene 26753_303-5     TAC         303
Unigene 26753_303-6     TAC         303
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Fog. 4. Comparison of nucleotide sequences of the Unigene 26753 microsatellite locus allelic variants identified in the genome of sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) samples from the working collection of the Pervomaisk Sugar Beet Breeding and Experimental Station (Gulkevichi, Krasnodar Territory, 2018-2022). The primer regions are highlighted in light green and dark green; the tandem repeat region is highlighted in yellow. Sample 1 (allele 288 bp) is line Ot 7994, sample 2 (allele 288 bp) is line Op 19962, sample 3 (allele 294 bp) is line Ot 12122, sample 4 (allele 294 bp) is line MS 11348, sample 5 (allele 303 bp) is line Op 10632, sample 6 (allele 303 bp) is line Op SP-1P2.

Figure 4, as an example of the analysis of a typical microsatellite locus the fragment length polymorphism of which is due only to a different number of repeats (in this case, CAA), shows the alignment of nucleotide sequences of the

most common alleles of the Unigene 26753 microsatellite locus.

The results of sequencing allelic variants of the microsatellite loci FDSB 1001, FDSB 1033, Unigene 16898, Unigene 17623B, Uni-gene 17923, Unigene 27833, SB 04 also confirmed that their polymorphism is caused solely by the number of microsatellite repeats in the amplified DNA fragment.

Analysis of the nucleotide sequences of allelic variants of the microsatellite locus Unigene 15915 showed that the length polymorphism of the amplified fragments is caused not only by a different number of tandem repeats $(CA)_n$, but also by additional insertions and deletions in the DNA regions flanking the repeats (see Fig. 3). This complicates the interpretation of the results of microsatellite analysis; thereof, the indicated Unigene 15915 locus was excluded from further work.

The results of sequencing allelic variants of microsatellite loci SB 09, SB 15 and FDSB 502 showed that these loci contain complex (compound) repeats (see Fig. 3). However, amplification of microsatellite loci SB 09 and SB 15 resulted in stable and reproducible DNA profiles, so these two loci were involved in further tests.

The polymorphism of the FDSB 502 locus is due to quantitative changes in the complex (composite) tandem repeat $(TC)_n(GAT)_n(AAG)_n$ (see Fig. 3). Note, in some cases, the analysis of polymorphism of a locus containing hree types of tandem repeats in the amplified DNA fragment is diffecult. We have previously shown that to obtain stable DNA profiles, it is advisable to simultaneously amplify no more than two polymorphic regions in one locus [32-34].

This locus can be used for genetic analysis of sugar beet lines and hybrids by amplifying each tandem repeat region separately. In this case, the likelihood of obtaining a reliably interpretable DNA profile is much higher. Therefore, we selected primers flanking different groups of tandem repeats in the FDSB 502 locus.

2. Novel primers for amplification of variable regions of the FDSB 502 locus in the sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) genome

Locus	Microsatellite repeats	Primer pair
FDSB 502-2	$(GAT)_n$	502-2F: 5′-ACAATGGCGAATCGCTTTTGGGG-3′
		502-2R: 5′-CGTACTCATCTTCATCGTCTTCTTC-3′
FDSB 502-3	$(AAG)_n$	502-3F: 5′-GAAGAAGACGATGAAGATGAGTACG-3′
		502-3R: 5′-GAATCAACCTTGCCGACATATCC-3′

3. Microsatellite loci that are promising for creating genotyping technology for sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) lines and hybrids

Locus	Microsatellite repeats	Detected alleles	
		size rank, bp	number
Unigene 16898	$(CAA)_n$	276-291	4
Unigene 17623B	$(CAA)_n$	147-179	11
Unigene 17923	$(CTT)_n$	193-225	11
Unigene 26753	$(CAA)_n$	282-303	7
Unigene 27833	$(ATA)_n$	190-217	7
FDSB 1033	$(AG)_n$	165-229	8
FDSB 1001	$(AG)_n$	315-351	6
SB 04	$(AAC)_n$	180-201	7
SB 09	$(CAA)_n(CAT)_n$	130-136	3
SB 15	$(CT)_n(GAC)_n$	146-174	7
FDSB 502-2	$(GAT)_n$	112-154	5
FDSB 502-3	$(AAG)_n$	223-241	4

N o t e. Obtaining stable and unambiguously interpreted DNA profiles when using microsatellite loci in this study is shown on 146 phenotypically characterized samples from the working collection of the Pervomaisk Breeding and Experimental Station of Sugar Beet (Gulkevichi, Krasnodar Territory, 2018-2022).

In a set of 146 sugar beet samples, for polymorphisms caused by different numbers of tandem repeats of each type, it was shown that the DNA region with the microsatellite repeat $(TC)_n$ is conservative (the number of TC repeats in all samples is the same and equal to 10) while the regions $(GAT)_n$ and $(AAG)_n$ are variable. Therefore, for genetic analysis of sugar beet lines and hybrids, only the

primers that flank the microsatellite repeats $(GAT)_n$ and $(AAG)_n$ in the FDSB 502 locus (Table 2) seem to be promising.

Therefore, 12 microsatellite loci provide stable and unambiguously interpreted DNA profiles and are promising for genotyping sugar beet lines and hybrids (Table 3).

So, 146 samples from the working collection of the Pervomaisk Selection and Experimental Station of Sugar Beet were analyzed for 12 microsatellite loci (FDSB 502-2, FDSB 502-3, FBSB 1001, FDSB 1033, Unigene 27833, Unigene 26753, Unigene 16898, Unigene 17623B, Unigene 17923, SB 04, SB 09 and SB 15). The tested samples were 28 MS lines, 28 O-type lines, 82 pollinator lines, hybrids of domestic selection Azimut, Korvet, Pervomaisky, Rubin, Fregat, Uspekh, hybrids Dobrava and Doroteya. The revealed allele length polymorphisms are 265-314 bp for FDSB 502, 315-351 bp for FDSB 1001, 167-229 bp for FDSB 1033, 180-201 bp for SB 04, 130-136 bp for SB 09, 146-174 bp for SB 15, 299-383 bp for Unigene 15915, 276-291 bp for Unigene 16898, 147-180 bp for Unigene 17623B, 193-225 bp for Unigene 17923, 282-303 bp for Unigene 26753, and 190-217 bp for Unigene 27833. Sequencing of allelic variants of microsatellite loci FDSB 1001, FDSB 1033, Unigene 16898, Unigene 17623B, Unigene 26753, Unigene 17923, Unigene 27833, and SB 04 confirmed that their polymorphisms are due to the number of tandem repeats in the amplified DNA fragment. In addition to $(CA)_n$ repeats, the Unigene 15915 locus contains insertions and deletions, so we do not recommend this locus for genotyping. The polymorphisms of the SB 09, SB 15 and FDSB 502 loci are caused by complex repeats. However, the SB 09 and SB 15 loci provide stable DNA profiles. The $(TC)_n(GAT)_n(AAG)_n$ polymorphism was detected in the FDSB 502 locus, which may distort the genotyping results. To use this locus in genotyping sugar beet lines and hybrids, we propose primers flanking only the variable microsatellite repeats $(GAT)_n$ and $(AAG)_n$. The results obtained will help create a reliable laboratory tool for sugar beet breeding and commercial seed production.

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