Genetic analysis of the homogeneity of soybean varieties by microsatellite loci

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Abstract. Soybean is the main protein and oil crop in the world. The production of soybeans is increasing every year. The special attention is given to the breeding and seed production of this crop due to its high economic importance. Currently, the morphological characteristics are used to characterize new varieties, applied for a patent. The analysis of polymorphism of the lengths of microsatellite loci (SSR) allows both to identify genotypes exactly and to evaluate genetic homogeneity within a variety. Soybean is a self-pollinating plant species; cross-pollination is extremely rare. The study of the genetic homogeneity of modern varieties of cultivated soybean showed the presence of genetic variability in five of the twenty studied varieties. We observed the heterogeneity of the varieties from one to four DNA SSR loci. We found the maximum level of intravarietal polymorphism in the variety Vilana beta – 46 % by the Soypr1 locus.

1. Introduction
In modern economic conditions, soybean is becoming one of the most important agricultural crops. The demand for soybean and the products of its processing is increasing every year. The soybean acreage is also increasing [1]. As a result, both the demand for new varieties and the requirements for them are increasing, these requirements include high productivity, resistance to biotic and abiotic stresses. Considering the complex breeding problems, there is a need for a complete and objective information on the source material used to develop new varieties.

A variety is a group of plants that, regardless of its protection, is determined by the traits characterizing a given genotype or combination of genotypes and differs from other plant groups of the same botanical taxon by one or more traits. According to the UPOV Convention (the International Union for the Protection of New Varieties of Plants), each new variety for which a patent application is filed must differ from existing well-known varieties, be sufficiently uniform and stable, that is, to meet the requirements of DUS criteria (distinctness, uniformity, stability). For example, a variety is considered uniform if, taking into account the variations that may arise due to the peculiarities of its cultivation, it is sufficiently uniform in its respective characteristics [2].

Traditionally, plant varieties are evaluated for uniformity based on morphological characteristics using field trials. The use of modern genotyping technologies based on the analysis of polymorphism of the lengths of microsatellite loci (SSR - simple sequence repeat) allows to identify the genotypes exactly, to evaluate the genetic homogeneity of a variety, and to control the transfer of genetic information from parental forms to hybrids. These technologies are effective both with phenotypic evaluation and without it [3, 4]. The identification of hybrids is also possible using the microsatellite analysis, since, in most cases, SSR markers are inherited codominantly. The amplified fragments of
both parental forms are present in F1 hybrids, provided that their parents have the different lengths of it.

The genotyping technology based on the analysis of polymorphism of microsatellite DNA loci is already used to identify almost all main agricultural plants [5-12]. However, there is little information in the specialized literature on studies of the intravarietal variability of cultivated plant varieties by SSR loci. T. Satina et al. evaluated the genetic homogeneity of the collection of rapeseed varieties of domestic and foreign breeding based on the analysis of polymorphism of 15 SSR loci. The results of their research showed that only 18 of 79 varieties were genetically homogeneous [13]. Apparently, this is due to the method of pollination, since rapeseed is an optional self-pollinated plant, and cross-pollination reaches 30 %, depending on the growing conditions. Information on the genetic homogeneity of varieties is necessary both for the certification of varieties and in breeding work for developing new varieties and hybrids.

It is well known that soybean is a self-fertile and obligate autogamous (self-pollinating) plant species with cleistogamous (closed flowering) flower [14-16]. However, many researchers have found cases of cross-pollination in soybean varieties. Under unfavorable growing conditions, such as low relative humidity, high air temperature during budding, late sowing etc., the frequency of cross-pollination can reach 5.2 % [16]. To confirm the assumption about the genetic homogeneity of soybean varieties, we studied their intravarietal polymorphism.

2. Materials and methods
We selected 20 soybean varieties for the research. Most of them were of the breeding of V.S. Pustovoit All-Russian Research Institute of Oil Crops (VNIIMK) and its branches: the Don experimental station (DOS VNIIMK), the Armavir experimental station (AOS VNIIMK) and one variety of Delta Agrar, Serbia (Table 1). We isolated DNA from the fragments of green leaves or from the corcule of seeds of each plant variety separately and from a mixture of 10-30 plants. We used a modified method with the CTAB (Cetyltrimethylammonium-bromide) lysis buffer for DNA isolation [17].

We carried out the DNA amplification with 13 pairs of SSR primers flanking microsatellite DNA loci, developed and published by P.B. Gregan et al. [18]. Their characteristics and nucleotide sequences are presented in the Table 2. The selection and testing of these loci for identification of soybean genotypes and certification of varieties have been described earlier [19].

We carried out the PCR (polymerase chain reaction) in a volume of 25 ml of a reaction mixture containing: 67 mM of tris-HCl, pH8.8; 16.6 mM of ammonium sulfate; 1.5-3 mM of MgCl₂; 0.01 % of Tween 20; 0.2 mM of deoxyribonucleoside phosphates; 10 pM of primers; 10 ng of template DNA and 1 unit of recombinant thermostable DNA polymerase (NPO “SibEnzyme”, Russia). We carried out the polymerase chain reaction in a S1000™ thermal cycler (BioRad, USA) under the following temperature conditions: initial denaturation at 96 °C for 2 min, then 32 cycles at temperature-time regime: denaturation at 94 °C for 30 sec, primer annealing at 45-60 °C, depending on the primer – 40 sec, elongation at 70 °C for 1 min, final elongation at 70 °C for 2 min. We determined the concentration of the obtained DNA by the intensity of its staining with ethidium bromide in 1 % agarose gel.

We carried out the electrophoretic separation of amplified DNA fragments in the agarose gel (2 % agarose, 1x SB buffer) using a horizontal electrophoresis chamber (SE.1, Helikon, Russia) for 1.5-2 hours at a current strength of 50 mA and a voltage of 90-100 V and in the polyacrylamide gel (8 %) for 3 hours at a current of 38-45 mA and 230 V in a chamber for vertical electrophoresis (VE-20, Helikon, Russia). We documented the results of electrophoresis using the BIO-PRINT digital video documentation system (Vilber Lourmat, France).

Table 1. The soybean varieties used for studying the genetic homogeneity.

| Variety | Originator |
|---------|------------|
| Greya   | VNIIMK     |
| Lira    | VNIIMK     |
3. Results and discussion

To study the intravarietal genetic homogeneity of soybean, we mainly selected the new varieties of the breeding of V.S. Pustovoit All-Russian Research Institute of Oil Crops. We individually isolated DNA from 100 plants for varieties Vilana and Vidra, and from 5 plants for the other varieties. At the same time, for each variety, we isolated DNA from a mixture of 10-30 plants and amplified it with 13 primers flanking the microsatellite regions.

The soybean variety Vilana has a high productivity and is widely used by soybean producers. The genetic analysis of the homogeneity of this variety was carried out twice: in 2008 and in 2019. The results showed that in 2008 the genetic polymorphism within the variety was detected by two SSR loci Satt9 and Soypr1. Moreover, by the Satt9 locus, out of hundreds of analyzed plants, six were found in which the DNA fragments differed in length from the rest. And by the Soypo1 locus, only one plant

Table 2. The characteristics of the studied microsatellite loci.

| Locus   | Localization | Chromosome | Repeat | Nucleotide sequences of flanking primers | Size of fragments (bps) |
|---------|--------------|------------|--------|----------------------------------------|------------------------|
| Sat1    | D2           | 17         | (AT)38 | GCGGATACGACAAAAATTGTT GCGAACTGGAAGATACTACCC | 188-235                |
| Satt1   | K            | 9          | (ATT)24| AGTACATAGATATAAAAGTGCTC AATGATGAAACGTGAATTATT | 141-150               |
| Satt2   | D2           | 17         | (ATT)25| AATATGGGAAAAACTAATGG TAATGTGGCTATCTGGTT | 140-152               |
| Satt5   | D1b          | 2          | (TTA)21| TATCTAGAAGAGACTAAAAA GTGCGATTAGCTGGAAATA | 157-177               |
| Satt9   | N            | 3          | (AAAT)13| CTAATGGATGFAATTAACCCTT CGGAAGCAGCTAGGCAATT | 142-221               |
| Soypr1  | K            | 9          | (ATT)20| GTTAGAAGAACCTCGGCCACAC | 163-188               |
| Sat36   | D1a          | 1          | (AT)19 | AAAGTCTAAACTGGCACTCAllATGTGAACATATAATATCAGCTC | 115-185               |
| Soyhspr | F            | 13         | (AT)15 | TGTTGGCCACAAAAGCTATAG CTAACGTTCAGTATGTC | 118-135               |
| Soysc   | 514          | M          | (AT)14 | AGTCSGTAGTCTAGCTACATGAC CAGTTGAATATGGAAAGCAATG | 179                   |
| Satt681 | C2           | 6          | (ATT)20 | GCCGTGCACTTGGTCACTGTGTG TCAGTGGAACCTGCCCTT | 241                   |
| Sat263  | C2           | 6          | (AT)17 | GCCGTGCAGTCCTTAATTAGTATG CCCGTGCAGCCCTTATTAC | 134                   |
| Satt141 | D1b          | 2          | (ATA)25 | CGCGTTGTGGTGCTGATATCC CGCGTTGCGAGCTCTTA | 205                   |
| Satt181 | H            | 12         | (ATT)19 | GGGCTAGCAGATATGGAC GGGGTACGCTGAGGT | 214                   |
| Satt161 | C1           | 4          | (ATT)11 | GGGTATATCACATATCTCCACCTTT GGGCTGTTGTTAATGTGTT | 225                   |
with a different fragment size was identified. The genetic purity of the variety is 92.93 % [20]. Studies carried out in 2019 showed that there is no polymorphism in the Satt9 locus, and in the Soypr1 locus the number of samples with the fragment sizes different from the main ones was about 46 %. At the same time, these plants do not differ from each other by morphological characteristics since microsatellite loci are located in non-coding regions of the genome.

The same level of polymorphism in the same locus was found in the new variety Vilana beta. This variety originated from a recombinant plant with an increased level of chlorophyll \(\beta\), which was found in the sowings of the variety Vilana. The genetic profiles of these two varieties were identical in the studied SSR loci, they have not been distinguished yet. As an example, Figure 1 shows the results of DNA amplification of the soybean variety Vilana beta with the Soypr1 primer.

![Figure 1](image1.png)

**Figure 1.** The electropherogram of separation of PCR products obtained with the primer Soypr1, tracks 1-9 for the soybean variety Vilana beta, M – molecular weight marker.

We also revealed the genetic heterogeneity in the soybean variety Vidra (originator is Delta Agrar, Serbia). We analyzed 100 plants individually and several samples of a mixture of DNA of 10-30 plants. The results showed the genetic heterogeneity by two loci – Satt36 and Satt5. Moreover, by the Satt36 locus only one of 100 plants was atypical; by the Satt5 locus, the percentage of heterogeneity was about 30 (Fig. 2).

![Figure 2](image2.png)

**Figure 2.** The electropherogram of separation of PCR products obtained with the primer Satt5, tracks 1-15 for the soybean variety Vidra, 16 – Olimpiya, 17 – Vilana, M – molecular weight marker.

In case of the soybean variety Lada, the analysis results showed the genetic heterogeneity in four loci: Satt2, Satt9, Soyhsp176 and Satt5. By the Satt5 locus, three of 10 analyzed plants had DNA fragments that differed in length from the others (tracks 5, 6, 9, Fig. 3, C). For the remaining loci Satt2, Satt9 and Soyhsp178, we identified one plant with a different profile for each of them (Fig. 3, A, B, D).
Figure 3. The electropherogram of separation of PCR products obtained with the primers Satt2 (A), Satt9 (B), Satt5 (C), Soyhsp176 (D).

The soybean variety Duar was obtained from the hybrid population F4 (Khersonskaya 8 × Rannyaya 5) × line 303 by intraspecific hybridization. The results of polymorphism analysis of 13 microsatellite loci showed that the presence of the fragments of various lengths in the Satt5 and SoySC514 loci. The level of polymorphism was about 30%.

The analysis of allelic state of microsatellite loci of the remaining 15 varieties showed that they are homogenous by all 13 studied loci. All DNA fragments identified as a result of amplification are of the same length. In the samples containing the DNA of the mixture of plants within a variety, there were no double fractions, which indicates the genetic heterogeneity of a variety.

4. Conclusion

The study of intravarietal polymorphism of soybean varieties by 13 microsatellite DNA loci revealed diversity in allelic composition in the varieties Vilana, Vilana beta, Lada, Vidra, and Duar. This is probably due to the genetic heterogeneity of varieties. As described above, this heterogeneity does not affect the morphological characteristics of the variety; however, this must be considered during the development of a molecular genetic passport.

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