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POLYMORPHISM OF SSR-LOCI IN SOYBEAN VARIETIES OF DIFFERENT COUNTRIES OF ORIGIN

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Topicality*. Microsatellite DNA sequences are widely used to identify the genotypes of living organisms. In 2014, the Chinese standard 'NY/T 2595-2014. Identification of soybean varieties. SSR marker method' on the identification of soybean varieties by polymorphism of 36 SSR loci was approved. In Ukraine, there are no approved regulatory documents on the identification of crops using DNA markers, including soybeans. Therefore, the study of the level of diversity and differentiation capacity of SSR markers proposed in the standard NY/T 2595-2014 in soybean varieties common in Ukraine is of interest.* ***Purpose.*** *Study of polymorphism of nine SSR loci in nine soybean varieties and divergence between soybean varieties bred in different countries.* ***Materials and Methods****. Nine soybean varieties were used in the work. The variability of nine microsatellite loci specified in the standard NY/T 2595-2014 as the most polymorphic in soybean varieties was evaluated. Polymorphism of SSR loci was studied using polymerase chain reaction with detection of results in agarose gel. The level of polymorphism in soybean varieties was assessed using the Nei genetic diversity index. The classification of soybean varieties was carried out by the nearest neighbour joining method in the PHYLIP program.* ***Results.*** *It was found that the total level of polymorphism of the studied SSR loci in nine soybean varieties, estimated by the Nei index, was 0.57±0.04. According to the studied SSR loci, no identical varieties were found; the values of genetic distances between all varieties were above 0. The Ukrainian varieties Raiduha and Hospodynia were the most genetically related, and the most distant were pairs of varieties Yunka (Canada) and Ultra (USA), Raiduha (Ukraine) and Yunka (Canada), Hospodynia (Ukraine) and Commandor (France). It was established that the Ukrainian varieties of Raiduha and Hospodynia are more genetically distant from all other soybean varieties studied. European and North American soybean varieties were divided into 2 groups. No grouping of varieties according to geographical origin was found.* ***Conclusions.*** *A significant resolution of SSR loci in the investigated soybean varieties was shown. The obtained results can be effectively used to identify soybean varieties, determine the efficiency of artificial hybridization, determine genetic purity, as well as in drawing up hybridization schemes taking into account remote ecological and geographical combinations.*

Key words: *DNA markers, microsatellite loci, molecular genetic diversity, genetic distances, divergence*

Introduction. Microsatellite DNA sequences (Simple Sequence Repeats, SSRs) are the most accessible, simple, convenient markers that are widely used to identify genotypes of living organisms.

A series of scientific papers have shown that SSR loci in soybeans exhibit a higher level of polymorphism compared to other markers such as RFLP, AFLP and RAPD [1–6], as well as SNP [6]. The linkage of some soybean SSR-loci, in particular, with soybean mosaic virus resistance genes [7], photoperiodic sensitivity genes (E-genes) [8] for the development of valuable economic traits is discussed.

Over the past decades, scientists have developed thousands of microsatellite markers for soybeans, and more than 2000 SSR-loci have been included in the overall integrated soybean genetic map [9].

In 2014, China approved a standard for genetic purity of soybean varieties based on polymorphism of 36 SSR loci [10]. At the same time, the typicality of most crop seeds in Ukraine is regulated by morphological traits according to DSTU 2240-93 [11]. No approved regulatory documents on the identification of crops with DNA markers, including soybeans, are available in Ukraine.

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Thus, the study of the diversity level and differential capacity of SSR markers proposed in the standard NY/T 2595-2014 in soybean varieties is of scholarly interest [10].

This study was aimed to investigate the polymorphism of nine SSR-loci in nine soybean varieties, which were bred in different countries, and the divergence between these varieties.

Materials and Methods. Nine soybean varieties originating from different countries (Table 1) and not previously analysed for the selected SSR loci were used in this study.

Table 1. Soybean varieties under study

Variety name	Originator	Country of origin
Abelina	SAATBAU (Saatbau Probstdorfer)	Austria
Candy	Cangro-Genetics Inc.	Canada
Prudence	HURON COMMODITIES INC.	Canada
Yunka	Sevita Genetics	Canada
Ultra	ASGROW &Monsanto	USA
Hospodynia	Yuriev Plant Production Institute of NAAS	Ukraine
Raiduha	Yuriev Plant Production Institute of NAAS	Ukraine
Commander	EURALIS SEMENCES	France
Mentor	EURALIS SEMENCES	France

The variability of nine microsatellite loci Satt197, Satt429, Satt112, Satt380, Satt239, Satt588, Satt300, Satt373, Satt514, which are the most polymorphic in soybean varieties according to the NY/T 2595-2014 standard, was assessed [10]. DNA was extracted from a mixture of embryonic root segments of 30 soybean seeds by the sorbent method using spin columns. DNA amplification was carried out in tubes with lyophilised sets with PCR reagents (Bioneer, South Korea) in TP4-PTsR-01 'Tertsik' amplifier. The final volume of the reaction mixture was 20 µl and contained 20 ng of DNA and 1 µM of each primer.

For the amplification of SSR loci, we used the program proposed in the standard [10]. The amplification was performed with an initial denaturation for 5 min at 94 °C, and then 35 cycles in the following mode: denaturation for 45 s at 94 °C, hybridisation of primers at Tm of 45 s, elongation for 45 s at 72 °C, and final elongation for 7 min at 72 °C.

The amplification products were separated by electrophoresis in a 2% agarose gel with ethidium bromide in a borate buffer with low ionic strength [12].

Electrophoresis of amplification products was performed with a horizontal HoeferSuper-Sub100 device (USA). M combi (Isogen) molecular weight markers were used. The prepared gels were photographed.

The number and size of amplification products were determined using the demo version of Totallab 120 (https://www.totallab.com).

The allele frequency was calculated using the formula:

$$p_i = P_{ii} + \sum P_{ij}\ /2, \text{ where}$$

p_i – frequency *of* allele *i;*

P_{ii} P_{ij} – frequencies of genotypes *ii* and *ij*.

According to the values of allele frequencies, the number of rare, general and frequent allelic variants was determined. Rare allelic variants were found in the studied sample of soybean varieties with a frequency of ≤ 1%, and general allelic variants – with a frequency of 1–20%. Amplification products that were found in more than 20 % of the soybean varieties studied were considered to be frequent alleles.

The polymorphism of SSR markers in soybean varieties was estimated using the Nei's genetic diversity index (H*e*) or theoretical heterozygosity, which was calculated by the formula:

$$He = 1 - \sum p_{Li}^2, \text{ where}$$

p_{Li} - frequency allele *i* in locus *L*.

The divergence between soybean varieties was estimated by calculating the Nei-Li's genetic distances with PHYLIP programme using the formula:

$D_{ij} = 1 - S_{ij} = \frac{2a}{2a+b+c}$, where D_{ij} – Nei and Li genetic distance between samp-les *i* and *j;*

S_{ij} – Nei and Li similarity coefficient between samples *i* and *j;*

a – number of common gradations of a trait in two compared objects;

b – number of one gradation that is typical only for the first object;

c – number of one gradation that is typical only for the second object.

Based on the distance matrices between populations of soybean varieties and individual samples, a dendrogram was created using the neighbour-joining method in the PHYLIP software.

Results and Discussion

Diversity of soybean varieties by polymorphism of SSR loci. During the amplification of nine microsatellite loci, 26 allelic variants were identified in soybean varieties. All loci showed polymorphism. The maximum number of allelic variants (5) was identified in soybean varieties using the Satt373 marker, and the minimum number of allelic variants (2) was found at the Satt380, Satt239, Satt300, and Satt514 markers (Fig. 1, Table 2).

The average number of allelic variants per locus was 2.89±1.05. The sizes of amplification products were determined relative to the molecular weight marker M combi and ranged from 134 to 326 bp for different SSR markers (Table 2). Similar sizes of amplification products were found by other authors [10] when studying the same SSR markers in other soybean samples.

Most of the allelic variants of the studied microsatellite loci were found in the sample of soybean varieties with a frequency of more than 20 % (frequent alleles) and 1–20 % (general alleles).

In addition, 1 rare allelic variant at the Satt373 locus (297 bp) was found only in the sample of Yunka variety (Fig. 1).

Despite the fact that soybeans are obligate

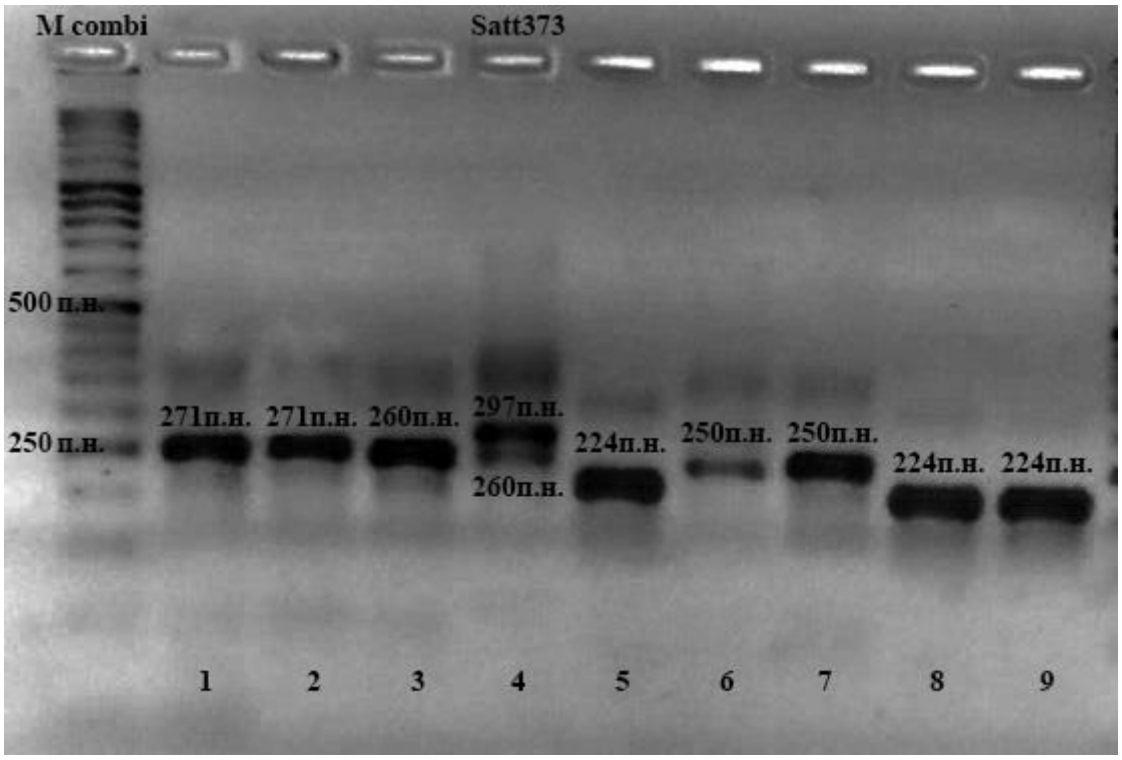

Fig. 1. Electrophoregram of amplification products of the most polymorphic SSR locus Satt373 in soybean varieties (M combi – marker, 1 – Candy, 2 – Abelina, 3 – Commander, 4 – Yunka, 5 – Ultra, 6 – Prudence, 7 – Mentor, 8 – Hospodynia, 9 – Raiduha).

self-pollinators [13], one heterogeneous variety Yunka was found among the studied samples, in which two DNA products were amplified at the Satt373 locus during the analysis of seed mixture DNA (Fig. 1). To find out the nature of the heterogeneity in this sample, a seed analysis is required. But this was not part of the objectives of this study. The level of heterozygosity in the total sample of soybean samples at all microsatellite loci was 1.2 %.

According to the Nei index, the most polymorphic loci in soybean varieties were Satt373 and Satt429 (D=0.80 and 0.72, respectively). High values of the Nei index of genetic diversity were also characterised by such markers as Satt112 and Satt588 (D=0.63 and 0.60, respectively).

The SSR locus Satt380 (D=0.37) showed the lowest variability in terms of diversity index.

The total level of polymorphism in the stu-

Table 2. Allelic variants and Nei genetic diversity index calculated by SSR-marker polymorphism in soybean varieties

Locus	Amplification products, bp	Allele frequency, %	Nei genetic diversity index
Satt197	169	11	0.52
	175	22	
	183	67	
Satt429	206	11	0.71
	220	11	
	231	44	
	245	33	
Satt112	317	11	0.63
	321	44	
	326	44	
Satt380	134	22	0.37
	142	78	
Satt239	178	56	0.52
	190	44	
Satt588	230	11	0.60
	234	33	
	245	56	
Satt300	145	56	0.52
	175	44	
Satt373	224	33	0.80
	250	22	
	260	17	
	271	22	
	297	6	
Satt514	198	44	0.52
	240	56	

died soybean varieties was 0.57±0.04.

Divergence of soybean varieties by polymorphism of SSR loci. The minimum genetic distance (0.004274) was found between the Ukrainian varieties Hospodynia and Raiduha (Table 3). These results are explained by the fact that these varieties have a common genetic basis, as they are registered by the same originator, the Yuriev Plant Production Institute (Kharkiv).

The maximum genetic distance (0.041894) for SSR loci polymorphism was found between pairs of varieties Ultra and Yunka, Raiduha and Yunka, and Hospodynia and Commandor. Ultra variety is the only one originating from the USA, Yunka variety is the only Canadian variety registered by the originator Sevita Genetics. The Raiduha and Hospodynia varieties are both of Ukrainian origin. Therefore, it is reasonable to assume that these pairs of varieties were developed using source material of different origins.

As a result of clustering soybean samples by SSR loci polymorphism, three groups of samples can be conditionally distinguished (Fig. 2). Cluster 1 includes the Ukrainian varieties Raiduha and Hospodynia, which are spatially furthest from the Commandor (France) and Yunka (Canada) varieties-representatives of cluster 2. Cluster 3 was formed due to the close location of soybean varieties Candy (Canada), Mentor (France), Prudence (Canada), and Abelina (Austria). At the same time, cluster 3 gravitated towards cluster 2. The Ultra variety is closer to cluster 1 in terms of its location on the dendrogram.

When studying the divergence of soybean varieties by SSR loci polymorphism, it was found that the genetic distances between all varieties are greater than 0. This makes it possible to differentiate varieties in determining genetic purity, to assess their genetic distance in crosses planning and breeding, and to register varieties.

Table 3. Nei and Li genetic distances between soybean varieties based on SSR loci polymorphism

Genetic distance	Candy	Abelina	Commandor	Yunka	Ultra	Prudence	Mentor	Hospodynia	Raiduha
Candy	0.00000	0.010246	0.019794	0.010246	0.019794	0.010246	0.019794	0.019794	0.019794
Abelina	0.010246	0.000000	0.027728	0.019794	0.014349	0.006976	0.014349	0.019794	0.019794
Commandor	0.019794	0.027728	0.00000	0.01217	0.014349	0.019794	0.014349	0.041894	0.027728
Yunka	0.010246	0.019794	0.01217	0.00000	0.041894	0.014349	0.014349	0.019794	0.041894
Ultra	0.019794	0.014349	0.014349	0.041894	0.00000	0.019794	0.019794	0.014349	0.006976
Prudence	0.010246	0.006976	0.019794	0.014349	0.019794	0.00000	0.006976	0.027728	0.027728
Mentor	0.019794	0.014349	0.014349	0.014349	0.019794	0.006976	0.00000	0.019794	0.019794
Hospodynia	0.019794	0.019794	0.041894	0.019794	0.014349	0.027728	0.019794	0.00000	0.004274
Raiduha	0.019794	0.019794	0.027728	0.041894	0.006976	0.027728	0.019794	0.004274	0.00000

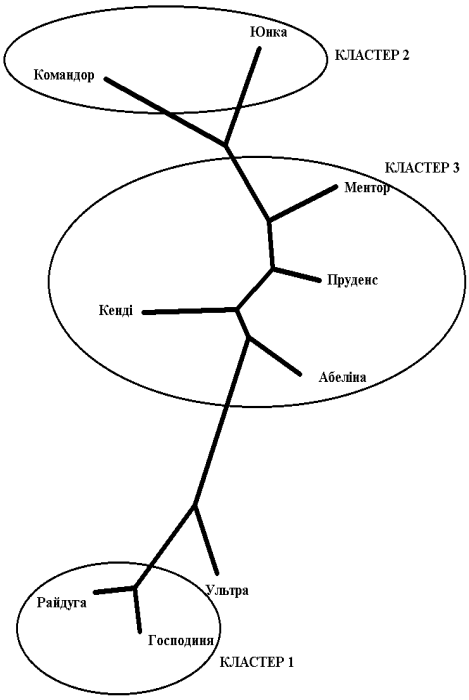

Fig. 2. Dendrogram with divergence of soybean varieties by SSR loci polymorphism.

Conclusions. The results of determining the genetic diversity of nine SSR loci proposed in the NY/T 2595-2014 standard for nine soybean varieties originating from different countries are presented in this study. The general level of polymorphism estimated by the Nei's genetic diversity index was found to be 0.57±0.04. The significant separation capacity of SSR loci in soybean varieties was revealed. No identical varieties were found at the studied SSR loci, and the genetic distances between all varieties were above 0. The most genetically related were the Ukrainian varieties Raiduha and Hospodynia; the most distant were the varieties Yunka (Canada) and Ultra (USA), Raiduha (Ukraine) and Yunka (Canada), Hospodynia (Ukraine) and Commander (France).

The divergence of soybean varieties based on SSR loci polymorphism was estimated by the neighbour-joining method. It was found that the Ukrainian varieties Raiduha and Hospodynia are more genetically distant from all other soybean varieties studied. European and North American soybean varieties were divided into 2 groups according to their geographical origin. The results obtained are useful for the identification of soybean varieties, copyright protection, determination of artificial hybridisation efficiency and genetic purity, as well as for the development of hybridisation schemes for distant ecological and geographical combinations.

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Чернишенко П. В. [1]***, Чернишенко Г. Є.***[2]***, Скобля Є. В.***[3] ***Поліморфізм SSR-локусів у сортах сої, які походять з різних країн.*** *Зернові культури. 2024. 8 (1). 37–43.*

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Актуальність. Мікросателітні послідовності ДНК широко використовуються для ідентифікації генотипів живих організмів. В 2014 році в Китаї затверджено стандарт NY/T 2595-2014:'Identification of soybean varieties. SSR marker method' щодо ідентифікації сортів сої за поліморфізмом 36 SSR-локусів. Затверджені нормативні документи щодо ідентифікації сільськогосподарських культур за допомогою ДНК-маркерів, у тому числі сої, в Україні відсутні. Отже, представляє інтерес вивчення рівня різноманіття та диференційної здатності SSR-маркерів, запропонованих в стандарті NY/T 2595-2014, в сортах сої, поширених в Україні. **Мета.** Вивчення поліморфізму дев'яти SSR-локусів в дев'яти сортах сої та дивергенції між сортами сої, селекція яких велась у різних країнах. **Матеріали і методи.** В роботі використано дев'ять сортів сої. Оцінювали мінливість дев'яти мікросателітних локусів, що показані в стандарті NY/T 2595-2014 як найбільш поліморфні в сортах сої. Поліморфізм SSR-локусів вивчали за допомогою полімеразної ланцюгової реакції з детекцією результатів в агарозному гелі. Оцінювання рівня поліморфізму в сортах сої здійснювали за допомогою індексу генетичного різноманіття Nei. Класифікацію сортів сої здійснювали методом приєднання найближчих сусідів у програмі PHYLIP. **Результати.** Виявлено, що загальний рівень поліморфізму досліджених SSR-локусів в дев'яти сортах сої, оцінений за індексом Nei, становить 0,57±0,04. За вивченими SSR-локусами не виявлено ідентичних сортів, значення генетичних відстаней між усіма сортами були вище 0. Найбільш генетично спорідненими між собою виявилися українські сорти Райдуга

та Господиня, найбільш віддаленими – сорти Юнка (Канада) і Ультра (США), Райдуга (Україна) і Юнка (Канада), Господиня (Україна) і Командор (Франція). Встановлено, що українські сорти Райдуга та Господиня є більш генетично віддаленими від усіх інших вивчених сортів сої. Європейські та північно-американські сорти сої об'єднувалися в 2 групи. Групування сортів згідно з географічним походженням не виявлено. **Висновки.** Показано значну роздільну здатність SSR локусів в досліджених сортах сої. Отримані результати можна ефективно використовувати для ідентифікації сортів сої, визначення ефективності штучної гібридизація, визначення генетичної чистоти, а також в при складанні схем гібридизації з урахуванням віддалених еколого-географічних комбінацій.

Ключові слова: *ДНК-маркери, мікросателітні локуси, молекулярно-генетичне різноманіття, генетичні відстані, дивергенція*