A study of the genetic diversity in the world soybean collection using microsatellite markers associated with fungal disease resistance

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Изучение генетического разнообразия мировой коллекции сои с использованием микросателлитных маркеров, связанных с устойчивостью к грибным болезням

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Background. Soybean (*Glycine max* (L.) Merr.) gradually becomes one of the leading legume crops in Kazakhstan. The area under soybeans in the country has been increasing annually and requires the development of adapted cultivars with a higher yield, improved quality characters, and resistance to emerging fungal diseases. The enlargement of the crop's gene pool also suggests the need to study and document local soybean accessions to meet the standards of the available world soybean collection by using reliable and informative types of DNA markers. **Materials and methods.** In this study, the soybean collection consisting of 288 accessions from different countries, including 36 cultivars and promising lines from Kazakhstan, was studied. The molecular genetic analysis was performed using nine polymorphic SSR (simple sequence repeats) markers, seven of which (Satt244, Satt565, Satt038, Satt309, Satt371, Satt570 and Sat_308) were associated with resistance to three main fungal diseases of soybean – frogeye leaf spot, fusarium root rot, and purple seed stain. **Results.** The average PIC (polymorphism information content) value of the analyzed SSR markers constituted 0.66 ± 0.07, confirming their high-level polymorphism. The principal coordinate analysis suggested that the local accessions were genetically most close to the accessions from East Asia. As the collection showed a robust resistance to three studied fungal diseases in Almaty Region during 2018–2019, the distribution of the studied SSR markers in the population was not significantly associated with resistance to the analyzed diseases under field conditions. **Conclusion.** SSR genotyping of the soybean collection helped to identify accessions that potentially possess resistance-associated alleles of fungal disease resistance genes. The data obtained can be further used for the development of DNA documentation and the breeding the promising cultivars and lines of soybean.

Key words: *Glycine max*, SSR markers, frogeye leaf spot, fusarium root rot, purple seed stain.

Актуальность. Соя (*Glycine max* (L.) Merr.) становится одной из ведущих зернобобовых культур в Казахстане, что в свою очередь требует создания адаптированных сортов, характеризующихся более высокой урожайностью, улучшенными признаками качества и устойчивостью к новым грибным болезням. В связи с расширением генофонда культуры местные образцы, вовлекаемые в селекционные программы, необходимо изучать и документировать с использованием надежных и информативных типов ДНК-маркеров. **Материалы и методы.** В настоящем исследовании изучена коллекция сои, включающая 288 образцов, в том числе 36 сортов и перспективных линий из Казахстана. Молекулярно-генетический анализ выполнен с использованием девяти полиморфных SSR-маркеров, семь из которых (Satt244, Satt565, Satt038, Satt309, Satt371, Satt570 и Sat_308) сцеплены с генами устойчивости к трем основным грибным болезням сои: церкоспорозу, корневой гнили и пурпурному церкоспорозу. **Результаты.** Среднее значение индекса PIC (polymorphism information content) анализируемых SSR-маркеров составило 0,66 ± 0,07, что подтверждает высокий уровень их полиморфизма. Анализ методом главных координат показал, что местные образцы генетически наиболее близки к сортам из Восточной Азии. В 2018 и 2019 годах в условиях Алматинской области наблюдали высокую устойчивость образцов коллекции к трем изученным грибным болезням. Распределение полиморфных вариантов изученных SSR-маркеров в популяции не было статистически значимо связано с наличием устойчивости в полевых условиях. **Заключение.** SSR-генотипирование коллекции сои позволило выявить образцы, обладающие аллелями SSR-локусов, ассоциированных с генами устойчивости к грибным болезням. Результаты исследований могут быть в дальнейшем использованы для ДНК-паспортизации и селекции перспективных сортов и линий сои.

Ключевые слова: *Glycine max*, SSR-маркеры, церкоспороз, корневая гниль, пурпурный церкоспороз.

Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most economically important legume crops, which provides plant protein for more than a quarter of all food and feed in the world (Garcia et al., 1997). Over the past ten years, the worldwide soybean production increased 1.2 times due to the ever-rising demand for this crop. In 2019, it was at the level of 358,650 million metric tons, and the major part of this yield belonged to Brazil, USA, and Argentina (U.S. Department of Agriculture…, 2020). Soybean is considered a highly profitable, export-oriented crop in Kazakhstan. Nowadays, over 90% of soybean production has been concentrated in Almaty Region (Makulbekova et al., 2017). In 2019, Kazakhstan harvested 229,000 metric tons of soybean. For further development of the soybean industry, the Government of Kazakhstan has declared a new program "Northern Soybean" for diversification of soybean to the north part of the country. Implementation of this program would make it possible over five years to enlarge sown areas to 1.5 million ha, raise productivity, and increase the production over 3 million tons (North Kazakhstan…, 2019).

The important factor that severely limits the soybean productivity worldwide is susceptibility to harmful diseases (Wrather et al., 1996). In Kazakhstan, more than ten fungal diseases of soybean have been identified (Maui et al., 2016; Zatybekov et al., 2018). Due to the expanding area under this crop, it is an obvious necessity to study the genetic potential of soybean associated with the tolerance to harmful pathogens. In general, the total yield loss due to susceptibility to fungal diseases can reach 40% (Wrather et al., 1996). Frogeye leaf spot (FLS, caused by *Cercospora sojina* Hara), fusarium root rot (FRR, caused by *Fusarium solani* (Mart.) Sacc. f. sp. *glycines* Roy), and purple seed stain (PSS, caused by *Cercospora kikuchii* (Tak. Matsumoto & Tomoy.) M.W. Gardner) are the most dangerous and widespread fungal diseases of soybean worldwide (Zatybekov et al., 2017).

In local breeding programs targeted for soybean resistance to fungal diseases, phenotypic analysis is mainly carried out on the basis of field observations; there are practically no studies at the molecular level. Therefore, along with field assessment of the various gene pools for resistance to fungal diseases, the analysis of genetic diversity is also required.

The genetic marker is a DNA sequence with a known chromosome localization which is linked with a particular gene or is a part of a gene of interest (Idrees et al., 2014). They are useful tools to differentiate individuals in a population or to classify individuals representing different varieties or cultivars within a species. To date, there are a number of various classes of DNA markers applied for different purposes in plant molecular genetics, breeding, biotechnology, etc. (Idrees et al., 2014; Singh et al., 2010). Simple sequence repeats (SSRs), or microsatellites, have become the most widely used markers for DNA fingerprinting or genotyping plant accessions. SSRs were applied in many studies due to their high informativeness, codominance, multiallelism, reproducibility in different laboratories, and transferability among related species (Cregan et al., 1999). In particular, SSRs are considered as a reliable tool to identify the genetic diversity and relationships among soybean genotypes in different populations (Wang et al., 2006).

Assessment of genetic divergence and relatedness among genetic resources (as potential breeding material) has significant implications for the improvement of crop plants. Knowledge of genetic diversity could help soybean geneticists and breeders to understand the structure of germplasm, predict which combinations would produce the best offspring, and facilitate widening of the genetic base of breeding material for selection (Bisen et al., 2015). The survey of recent reports demonstrated the successful use of SSR markers for screening soybean in relation with resistance to fungal diseases (Zhong et al., 2018; Ghorbanipour et al., 2019).

Rouf Mian et al. (1999) used the NILs (near-isogenic lines) population created by backcrossing the *Rcs3* gene from cv. Davis to the FLS-susceptible cultivar Wright. In the cultivar Davis (showing complete resistance to all isolates of FLS causative agent), the donor parent of the *Rcs3* allele for FLS resistance, a 154 bp fragment of Satt244 was amplified (Rouf Mian et al., 1999). Iqbal with co-authors identified six QTLs responsible for SDS resistance and reported five of them to be related to the SSR markers Satt214, Satt309, Satt570 (on the linkage group G), Satt371 (LG C2), and Satt354 (LG I) (Iqbal et al., 2001). Closely located to the *Rfs* gene is the SSR marker Satt309, which produced a 165 bp band in cv. Forrest resistant to SDS. Jointly, these QTLs explained about 90% of the total variation in SDS disease incidence of RILs (recombinant inbred lines) from the Forrest × Essex cross, and they showed only the presence of additive genic action (Iqbal et al., 2001). Jackson et al. (2008) carried out genetic mapping of resistance to PSS in the F_2 population from crossing the susceptible cultivar Agripro 350 and resistant PI 80837. The candidate gene has been mapped between Sat_308 and Satt594 loci on molecular linkage group G. The Sat_308 primers produced a band of approximately 310 bp both in PI 80837 and the resistant bulk (Jackson et al., 2008). Hence, the involvement of polymorphic informative SSR markers into breeding is important for crop improvement programs, including targeting resistance to diseases. The purpose of this work was to study the genetic diversity in the soybean collection, consisting of 288 accessions, using microsatellite markers associated with resistance to common fungal diseases – FLS, FRR, and PSS.

Materials and methods

Plant material and field observations

The objects of this study were 288 soybean accessions from different countries, including 36 released cultivars and prospective lines from Kazakhstan (Zatybekov et al., 2018). The world collection was represented by the soybean accessions originated from 5 geographic regions, including Eastern Europe (n = 122), Western Europe (n = 23), East Asia (n = 57), North America (n = 50), and Kazakhstan (n = 36). Cv. 'Zhansaya' was used as a check cultivar Almaty Region. Field trials were conducted on the experimental plots of the Kazakh Research Institute of Agronomy and Plant Industry (KRIAPI) in 2018 and 2019 (Zatybekov et al., 2018). The soybean collection was grown in three randomized replicates on one-meter plots. The resistance to FLS, FRR, and PSS was studied under natural infection. A nine-point scale was used, where point 1 denoted high resistance (no symptoms); 3 – resistance (5 – 19% affected); 5 – partial resistance (20–49% affected); 7 – susceptibility (50–79% affected); and 9 – high susceptibility (up to 80% affected) (Hnetkovsky et al., 1996). The data were used to study the relationships between the disease resistance of the collection accessions and the following 8 major agronomic traits: plant height (PH), the height of lowest pod (HLP), number of lateral branches (NLB), number of fertile nodes (NFN), number of seeds per plant (NSP), thousand seeds weight (TSW), yield per plant (YP), and yield per plot (YpP). The field trials were conducted in 2018, and 2019; the analyzed traits were evaluated according to Korsakov et al. (1968).

DNA extraction and SSR genotyping

Total DNA was isolated from seedlings of all soybean accessions, according to Dellaporta et al. (1983). The soybean collection was genotyped using nine SSR markers, with appropriate optimization of polymerase chain reaction (PCR) conditions for specific primer pairs (Cregan et al., 1999). PCR was conducted in a 20 µl total volume containing 20 ng of genomic DNA, 1U Taq DNA polymerase, 0.2 mM of each dNTP, 10 pmol of each primer, 1.5 mM MgCl2 in the 1× Taq Buffer. The list of SSR markers used for the analysis is presented in Table 1.

Table 1. The list of simple sequence repeat (SSR) markers used for screening the soybean collection

Таблица 1. Список SSR-маркеров, использованных для скрининга коллекции сои

SSR marker	Disease	Chromosome	Linkage group	Forward sequence 5' – 3'	Reverse sequence 5' – 3'	Motif
Satt565	FLS	4	C1	GCGCCCGGAACTTGTAATAACCTAAT	GCGCTCTCTTATGATGTTCATAATAA	(AAT) 19
Satt371	FRR	6	C2	TGCAAACTAACTGGATTCACTCA	GAGATCCCGAAATTTTAGTGTAACA	(TAA) 11
Satt244	FLS	16	J	GCGCCCCATATGTTTAAATTATATGGAG	GCGATGGGGATATTTTCTTTATTATCAG	(AAT) 27
Satt529	FLS	16	J	GCGCATTAAGGCATAAAAAAGGATA	GCACAATGACAATCACATACA	(ATT) 13
Sat_308	PSS	18	G	GCGTTGGCAATTCAGGATATATTTAAGATTT	GCGGCTGCGTTTTTATTCAAACTTGTT	(AT) 19
Satt038	FRR	18	G	GGGAATCTTTTTTTCTTTCTATTAAGTT	GGGCATTGAAATGGTTTTAGTCA	(ATA) 17
Satt115	PSS	18	G	GGTTCGTTTTTTATTGATG	ACGACGAAATTGATGATAA	(TAT) 18
Satt309	FRR	18	G	GCGCCTTCAAATTGGCGTCTT	GCGCCTTAAATAAAACCCGAAACT	(ATA) 13
Satt570	FRR	18	G	CTCATGTGGTCCTACCCAGACTCA	CGCTATCCCTTTGTATTTTCTTTTGC	(TAT) 11

The markers Satt244, Satt529 and Satt565 are known to be associated with FLS resistance (Rouf Mian et al., 1999); Satt038, Satt309, Satt371 and Satt570 with FRR resistance (Iqbal et al., 2001); Sat_308 and Satt115 with resistance to PSS (Jackson et al., 2008). PCR, including preliminary denaturation of total DNA at 94°C for 1 min, subsequent 30–40 cycles (94°C – 1 min, 50–65°C – 30–60 s, 72°C – 1 min) and elongation at 72°C – 7 min, was carried out using a Veriti™ Thermal Cycler (Thermo Fisher Scientific, USA). PCR products were separated on 6% polyacrylamide gels (Amresco, Solon, OH) run in 0.5× TBE buffer, pH 8.0 at 250 V for 1.5 h. Gels were stained with ethidium bromide, and the images were recorded with a Bio-Rad Image System (Bio-Rad, Hercules, CA). Allele sizes were estimated in comparison with the DNA molecular weight marker (100 bp ladder, Fermentas).

Statistical analysis

Statistical analyses of field data were done using SPSS 22.0 and STATISTICA 13.5 software.

The effective number of alleles per locus was determined using the GenAlex, ver.6.5 software (Peakall et al., 2012). The values of the PIC index (polymorphism information content) suggested the effectiveness of the markers used, given that markers with a value of PIC > 0.5 were considered as highly informative; 0.5 > PIC > 0.25 as informative; and PIC ≤ 0.25 as marginally informative (Botstein et al., 1980). Genetic diversity was assessed on the basis of Nei's genetic diversity index and Shannon Information Index, using the GenAlex, ver.6.5 program (Peakall et al., 2012). The resulting similarity matrix was further analyzed using the unweighted pair-group method with arithmetic mean (UPGMA) clustering algorithm for the construction of the dendrogram. Variation among populations was studied using Principal Coordinate Analysis (PCoA) with the software GenAlex, ver.6.5 (Peakall et al., 2012).

Analysis of the population structure was carried out using STRUCTURE (v2.3.4.) software with a Bayesian Markov Chain Monte Carlo (MCMC) approach, based on the admixture and correlated frequency models (Evanno et al., 2005). The output from STRUCTURE was analyzed for the delta K value (ΔK) in STRUCTURE HARVESTER (Earl et al., 2012). The genetic map was drawn using MapChart v.2.3 software (Voorrips, 2002).

Results

Phenotypic variation of the soybean resistance to fungal diseases

Results of the two-year field trials of 288 soybean accessions revealed variations in their resistance to FLS, FRR, and PSS caused by three fungal pathogens – *Cercospora sojina*, *Fusarium solani*, and *Cercospora kikuchii* respectively (Table 2).

In 2018, symptoms of infection by the FRR causative agent were not observed. More than 90% of the analyzed soybean collection showed high resistance to the studied fungal diseases (Table 2). The Pearson correlation analysis based on the average values of 2018 and 2019 field trials helped to reveal

Table 2. Evaluation of 288 soybean accessions for resistance to frogeye leaf spot, fusarium root rot, and purple seed stain

Таблица 2. Оценка 288 образцов сои по устойчивости к церкоспорозу, корневой гнили и пурпурному церкоспорозу

Resistance type	Accessions resistant to fungal diseases, %					
	2018			2019		
	FLS	FRR	PSS	FLS	FRR	PSS
R	97.2	0	91.7	98.6	98.9	98.9
MR	1.4	0	6.9	1.4	1.1	0.7
S	1.4	0	1.4	0	0	0.4

Note: FLS – frogeye leaf spot, FRR – fusarium root rot, PSS – purple seed stain; R – resistance, MR – moderate resistance, S – susceptibility

Примечание: FLS – церкоспороз, FRR – корневая гниль, PSS – пурпурный церкоспороз; R – устойчивый, MR – среднеустойчивый, S – восприимчивый

a highly positive relationship between morphological traits and yield components ($P < 0.01$) (Fig. 1). The disease resistance traits had negative correlations with agronomic traits. Most of them were observed in the relations between FLS resistance and major agronomic traits. The resistance to FRR had negative correlations with TSW and PH, while resistance to PSS was negatively correlated with YpP (Fig. 1).

Microsatellite analysis of soybean accessions

Soybean collection was studied using 9 SSR markers associated with resistances to frogeye leaf spot, fusarium root rot, and purple seed stain. Figures 2–4 present the results of electrophoresis of PCR products with primers Satt244, Satt309 and Sat_308. The Satt244 marker is known to be associated with resistance to FLS (Rouf Mian et al., 1999). The Satt244-154 allele linked to the *Rcs3* gene on chromosome 4 (linkage group C1) was detected for 50 accessions (Fig. 2).

According to Ding et al. (2012), the Satt565 marker is closely linked to the gene *Rcs1* controlling the resistance to FLS. In our study, the resistance-associated allele Satt565-208 was identified in 32 soybean accessions. Analysis of the studied collection using SSR markers associated with the resistance to FRR made it possible to identify 48 accessions with the Satt309-165 allele (Fig. 3) described by Iqbal et al. (2001).

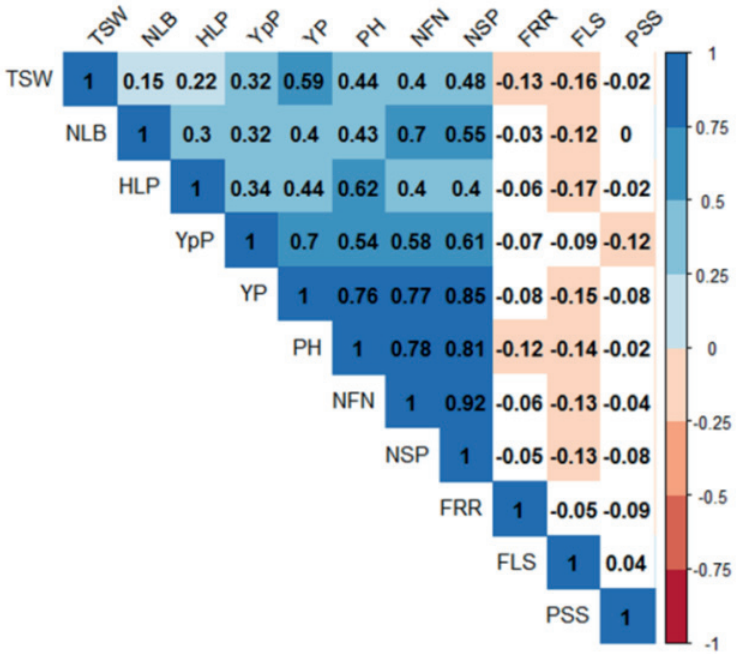

Fig. 1. The Pearson correlation analysis of the two-year average values (2018–2019) in disease resistance: frogeye leaf spot (FLS), fusarium root rot (FRR), and purple seed stain (PSS); morphological characters: plant height (PH), the height of lowest pod (HLP), number of lateral branches (NLB), and number of fertile nodes (NFN); yield components (number of seeds per plant (NSP), thousand seeds weight (TSW), yield per plant (YP), yield per plot (YpP)

Рис. 1. Корреляционный анализ Пирсона по средним значениям двухлетних данных (2018–2019): по устойчивости к болезням: церкоспороз (FLS), корневая гниль (FRR), пурпурный церкоспороз (PSS); морфологическим признакам: высота растения (PH), высота прикрепления нижнего боба (HLP), число боковых ветвей (NLB), число продуктивных узлов (NFN); компонентам урожайности: количество семян с растения (NSP), масса тысячи семян (TSW), урожайность с растения (YP), урожайность с делянки (YpP)

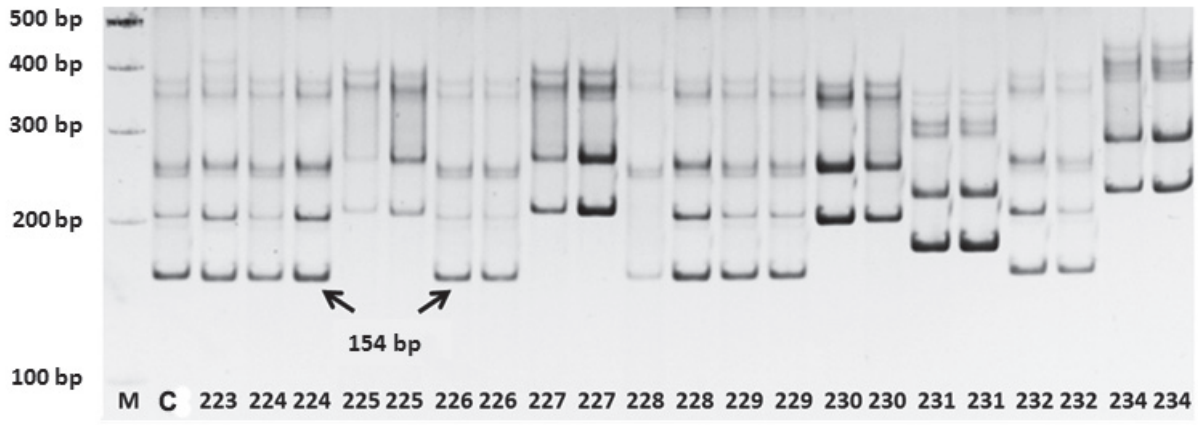

Fig. 2. Electrophoregrams of PCR products amplified with primers Satt244:
M – DNA molecular weight marker (100 bp ladder, Fermentas), C – check cultivar Zhansaya, 223–234 – soybean cultivars: 223 – Ken Fen 20; 224 – 551; 225 – Dong Dow 29; 226 – Jing Xin 2; 227 – Dong Dow 339; 228 – Hei Hye 47; 229 – Hay Fen 50; 230 – Dong Dou 027; 231 – May Fen 18; 232 – Xu Xiong 1; 234 – Klaxxon.
The arrow indicates marker fragment Satt244-154, associated with resistance to frogeye leaf spot

Рис. 2. Электрофореграммы продуктов ПЦР с праймерами Satt244:
М – маркер молекулярного веса ДНК (100 bp ladder, Fermentas), С – сорт-стандарт Жансая, 223–234 – сорта сои: 223 – Ken Fen 20; 224 – 551; 225 – Dong Dow 29; 226 – Jing Xin 2; 227 – Dong Dow 339; 228 – Hei Hye 47; 229 – Hay Fen 50; 230 – Dong Dou 027; 231 – May Fen 18; 232 – Xu Xiong 1; 234 – Klaxxon.
Стрелкой отмечен маркерный фрагмент Satt244-154, ассоциированный с устойчивостью к церкоспорозу

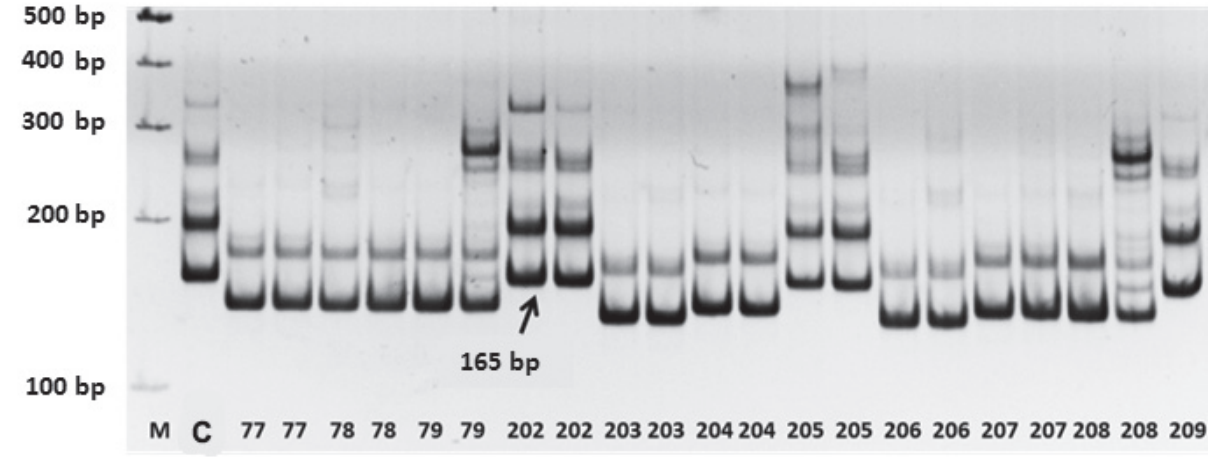

Fig. 3. Electrophoregrams of PCR products amplified with primers Satt309:
M – DNA molecular weight marker (100 bp **ladder**, Fermentas), C – check cultivar Zhansaya, 77–209 – soybean cultivars and lines: 77 – Prikarpatska 81; 78 – Chernovickaya 7; 79 – Spritna; 202 – 1082; 203 – 1028; 204 – 1055; 205 – 1095; 206 – 1026; 207 – 1071; 208 – 1003; 209 – 1022.
The arrow indicates marker fragment Satt309-165, associated with resistance to fusarium root rot

Рис. 3. Электрофореграммы продуктов ПЦР с праймерами Satt309:
М – маркер молекулярного веса ДНК (100 bp ladder, Fermentas), С – сорт-стандарт Жансая, 77–209 – сорта и линии сои: 77 – Прикарпатска 81; 78 – Черновицкая 7; 79 – Spritna; 202 – 1082; 203 – 1028; 204 – 1055; 205 – 1095; 206 – 1026; 207 – 1071; 208 – 1003; 209 – 1022.
Стрелкой отмечен маркерный фрагмент Satt309-165, ассоциированный с устойчивостью к корневой гнили

Also, soybean collection was analyzed on Satt570 (Iqbal et al., 2001), the marker closely linked to the *Rfs* gene that controls resistance to FRR. Seventy accessions with the Satt570-110 allele were identified. The analysis of the studied soybean collection using Sat_308 (Fig. 4) associated with resistance to PSS revealed 28 accessions carrying the Sat_308-310 allele, previously described by Jackson et al. (2008).

The frequencies for the alleles of SSR loci known to be related to disease resistance were detected for each group of soybean accessions (Table 3).

The results based on using 9 SSR polymorphic markers associated with resistance to FLS, FRR, and PSS revealed 40 alleles, with the average number of 4.42 alleles per marker (Table 4). The number of alleles per locus was from

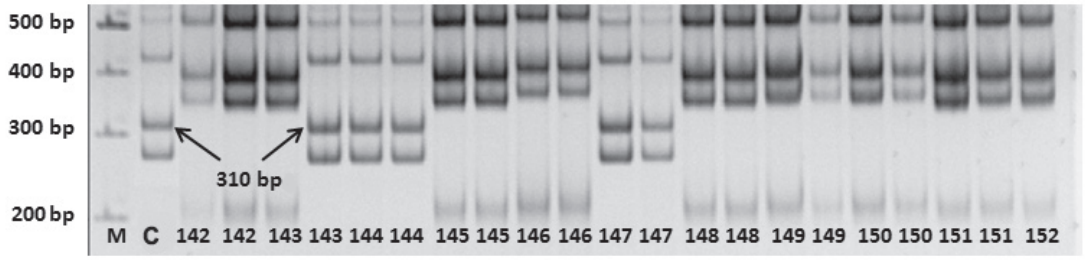

Fig. 4. Electrophoregrams of PCR products amplified with primers Sat_308:
M – DNA molecular weight marker (100 bp ladder, Fermentas), C – check cultivar Zhansaya, 142–152 – soybean cultivars: 142 – Sava; 143 – Venera; 144 – Zen; 145 – Protina; 146 – Sponsor; 147 – Isidor; 148 – Shama; 149 – Safrfna; 150 – Santana; 151 – Lada; 152 – Lira.
The arrow indicates marker fragment Sat_308-310 associated with resistance to purple seed stain

Рис. 4. Электрофореграммы продуктов ПЦР с праймерами Sat_308:
M – маркер молекулярного веса ДНК (100 bp ladder, Fermentas), C – сорт-стандарт Жансая, 142–152 – soybean cultivars: 142 – Sava; 143 – Venera; 144 – Zen; 145 – Protina; 146 – Sponsor; 147 – Isidor; 148 – Shama; 149 – Safrfna; 150 – Santana; 151 – Lada; 152 – Lira.
Стрелкой отмечен маркерный фрагмент Sat_308-310, ассоциированный с устойчивостью к пурпурному церкоспорозу

Table 3. The number of accessions carrying the alleles of SSR loci associated to three fungal diseases resistance, pcs

Таблица 3. Число образцов сои, несущих аллели SSR-локусов, ассоциированные с устойчивостью к трем грибным болезням, шт.

Origin group	Satt244-154	Satt565-208	Satt038-182	Satt570-110	Satt309-165	Satt371-272	Sat_308-310
Eastern Europe	12	21	9	21	17	23	11
Western Europe	3	1	7	4	5	2	4
East Asia	9	3	7	11	7	5	7
North America	3	5	9	20	7	10	1
Kazakhstan	23	2	1	14	12	10	5
Total	50	32	33	70	48	50	28

Table 4. Assessment of the genetic diversity level of SSR loci associated with resistance to FLS, FRR and PSS in the global soybean collection

Таблица 4. Оценка уровня генетического разнообразия SSR-локусов, связанных с устойчивостью к FLS, FRR и PSS в мировой коллекции сои

Diseases	Marker	na	ne	Nei	PIC
FLS	Satt244	5.2	3.72	0.64	0.83
	Satt565	5.0	3.16	0.68	0.78
	Satt529	4.0	2.59	0.57	0.76
FRR	Satt038	5.0	3.09	0.66	0.79
	Satt570	5.0	2.68	0.61	0.74
	Satt309	3.4	2.05	0.46	0.55
	Satt371	5.4	3.29	0.63	0.83
PSS	Sat_308	3.0	1.38	0.27	0.32
	Satt115	3.8	1.42	0.29	0.34
Mean		4.42	2.6	0.54	0.66
SE		0.44	0.34	0.06	0.07

Note: na – the number of alleles per locus; ne – the effective number of alleles; I – Shannon information index; Nei – Nei's diversity index; PIC – polymorphic information content
Примечание: na – количество аллелей на локус; ne – эффективное количество аллелей; I – индекс информативности Шеннона; Nei – индекс разнообразия Нея; PIC – индекс полиморфизма

3 (Sat_308) to 7 (Satt244). Each of the four microsatellite loci (Satt038, Satt371, Satt565, and Satt570) was represented by 6 alleles. The average number of alleles ranged from 3.0 for Sat_308 to 5.4 for Satt371 (Table 4). The effective number of alleles varied from 1.38 to 3.72, with a mean value of 2.6. Nei's genetic diversity index averaged 0.54 (Table 4). The average value of polymorphism information content (PIC) was 0.66, ranging from 0.32 for Sat_308 to 0.83 for Satt371.

The PCoA analysis based on the variability data for nine microsatellite markers in soybean populations demonstrated that genotypes from Western Europe are genetically more distant from the other four groups of origin. The PCoA2 coordinate effectively separated groups of North America and Eastern Europe from groups of East Asia and Kazakhstan. The local soybean accessions were close to the East Asian genotypes (Fig. 5).

ern Europe and East Asia. Most of the local genotypes were grouped in the Population 3 (Table 5).

Associations with alleles linked to disease resistance genes

Seven out of nine SSR loci were located close to the known resistance genes that controlled immune response to three studied fungal diseases (Fig. 7). The polymorphism observed at Satt244 and Satt565 associated with resistance to FLS showed that 78 accessions had at least one allele related to disease resistance. The analysis of polymorphism at four SSR loci (Satt038, Satt309, Satt371, and Satt570) related to FRR resistance helped to identify 154 accessions with at least one allele. There were no accessions carrying alleles of all four SSR marker loci related to disease resistance. The screening suggested that 38 out of 154 accessions had at least two alleles, and 4 accessions had three alleles associated with disease re-

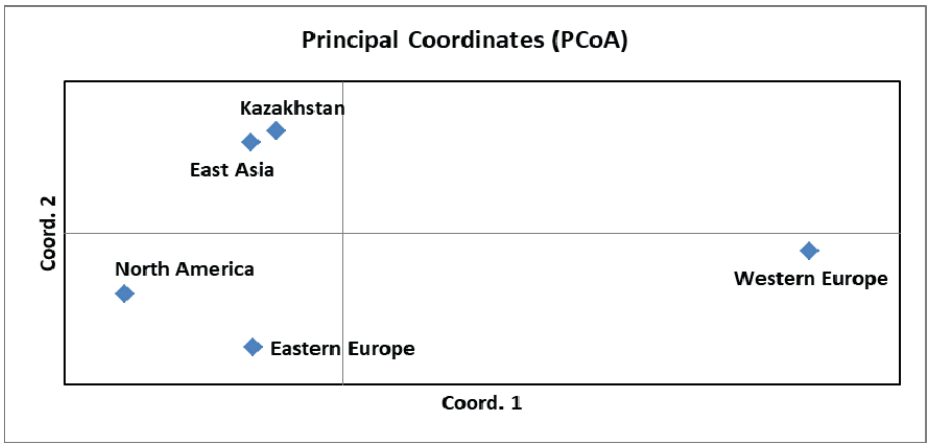

Fig. 5. Principal coordinate analysis of 288 soybean accessions divided into 5 groups based on 9 SSR markers associated with the resistance to fungal diseases

Рис. 5. Результаты анализа 288 образцов сои, разделенных методом главных координат на 5 групп, на основе данных скрининга с использованием 9 SSR-маркеров, связанных с устойчивостью к грибным болезням

The analysis based on using the STRUCTURE HARVESTER led to the development of Delta K (ΔK), which showed that the collection of 288 soybean accessions had an optimal number of populations equal to 3 (Fig. 6).

sistance, respectively. The Sat_308-310 allele associated with the resistance to PSS was found in 28 soybean accessions.

In total, the study made it possible to identify four genotypes which had at least one allele associated with resis-

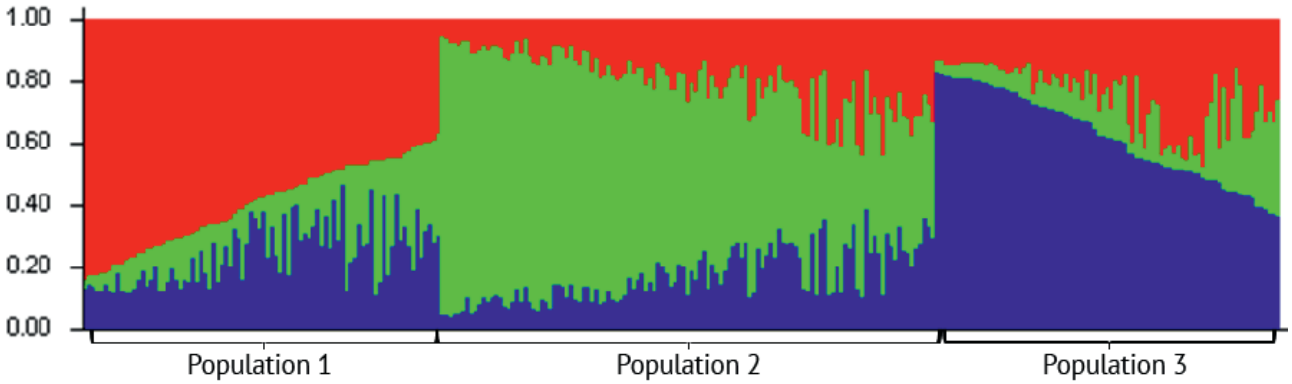

Fig. 6. Population analysis of 288 soybean accessions using STRUCTURE software

Рис. 6. Популяционный анализ 288 образцов сои с использованием программного обеспечения STRUCTURE

Three generated populations were represented by genotypes from all five groups with different origins. Population 2 had a largest number of accessions, which mainly included genotypes from Eastern Europe and North America. Population 1 included the majority of accessions from East-

tance to one of the three studied fungal diseases. The application of the *t-test* did not reveal statistically significant differences between accessions with positive and negative alleles of SSR markers associated with resistance to three diseases under field conditions.

Table 5. **Distribution of accessions from 5 groups of origin among the obtained populations, pcs**

Таблица 5. **Распределение образцов по популяциям из 5 групп происхождения, шт.**

Origin group	Population 1	Population 2	Population 3
Eastern Europe	43	51	28
Western Europe	7	10	6
East Asia	21	19	17
North America	5	37	8
Kazakhstan	11	2	23

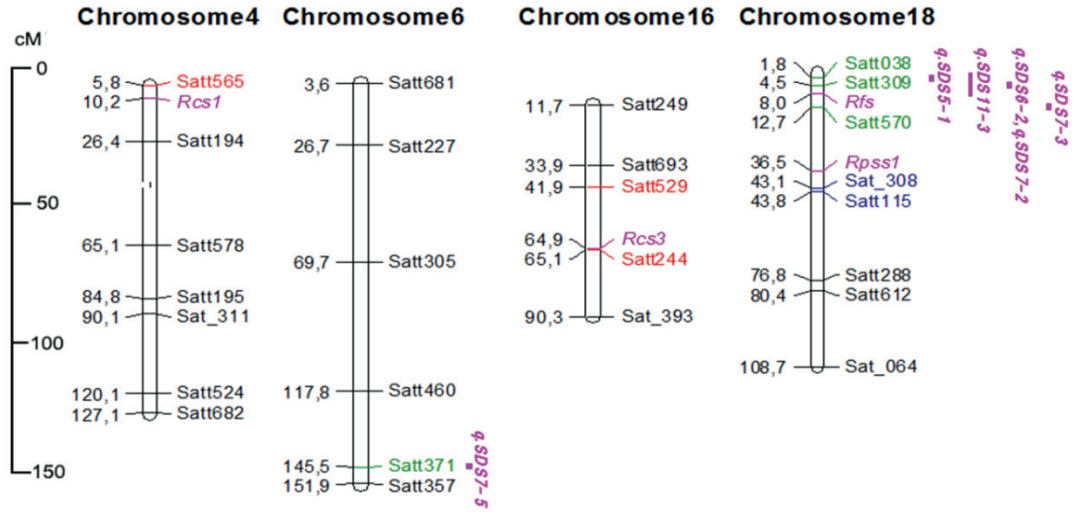

Fig. 7. **Localization of 9 SSR markers associated with resistance to three studied fungal diseases**
(the SSR markers associated with the resistance to frogeye leaf spot are shown in red, to fusarium root rot in green, and to purple seed stain in blue color, respectively. The positions of known soybean genes and quantitative traits loci (QTL) controlling disease resistance are shown in pink according to Soybase.org database)

Рис. 7. **Локализация 9 SSR-маркеров, связанных с устойчивостью к трем изученным грибным болезням**
(SSR-маркеры, связанные с устойчивостью к церкоспорозу, выделены красным, к корневой гнили – зеленым и к пурпурному церкоспорозу – синим цветом соответственно. Локализация известных генов устойчивости к болезням и локусов количественных признаков (QTL) показаны розовым цветом на основе базы данных Soybase.org)

Discussion

The results of field trials of the global soybean collection in the environments of Southeastern Kazakhstan showed high levels of resistance in a majority of the accessions to common fungal diseases in the tested environments. The two-year study of the resistance to FLS, FRR and PSS suggested that more than 97% of the collection were resistant to these diseases. The correlation analysis revealed significant positive correlations between agronomic characters and their negative correlations with resistance to fungal diseases. The soybean collection consisting of 288 accessions from different parts of the world was studied using nine microsatellite markers related to the genes of resistance to three fungal diseases, FLS, FRR and PSS. The SSR markers used in this study were highly polymorphic and informative, with the average PIC equal to 0.66. Two markers linked to PSS, Sat_308 and Satt115, showed moderate PIC values: 0.32 and 0.34, respectively (Table 4). At the same time, PIC for the entire soybean collection of 288 accessions, using the other seven SSR markers (Satt244, Satt565, Satt529, Satt038, Satt570, Satt309 and Satt371) related to FLS and FRR resistances, was higher (0.75), confirming high informativeness of these markers. The SSR loci with more than five alleles and PIC over 0.6 would be informative for genetic structure analysis and marker-assisted selection.

The PCoA analysis using nine SSR markers showed that local genotypes were genetically closer to East Asian cultivars, compared with accessions from other regions. The result confirms the field test data, suggesting that they have higher adaptability to the environments of Southeastern Kazakhstan.

Screening of the global soybean collection with nine polymorphic SSR markers made it possible to identify genotypes carrying 7 marker alleles associated with resistance to three fungal diseases. They were Satt244-154 and Satt565-208 associated with resistance to FLS; Satt 038-182, Satt309-165, Satt371-272 and Satt570-110 associated with resistance to FRR; and Sat_308-310 associated with the response PSS.

The accessions Slaviya, Amantai, OO533, and 10991 had the alleles Satt244-154 and Satt565-208 associated with FLS resistance. Satt244 was localized almost at the same position

(64.9 cM, linkage is less than 1 cM) on chromosome 16 (linkage group J) with the dominant resistance gene *Rcs3* (Fig. 7) governing the resistance to all known races of *C. sojina* (Rouf Mian et al., 1999). The soybean accessions carrying all four alleles related to FRR resistance were not revealed in this collection. At the same time, the screening identified four genotypes (L315/07, #98, Semu315, and Iskra) carrying three alleles associated with FRR resistance. Satt309 (4.53 cM) and Satt570 (12.74 cM) were localized close to the *Rfs* gene (Fig. 7) which controls the response of soybean to *F. solani* infection (Iqbal et al., 2001). Earlier it was reported that Sat_308 connected with PSS resistance was mapped in 6.6 cM from the *Rpss1* gene on chromosome 18 (Fig. 7) (Jackson et al., 2008). In our study, 28 soybean accessions from the studied collection carried the allele Sat_308-310. In previous studies, several disease resistance genes were identified on linkage group G (chromosome 18), which indicated the location of the cluster of resistance genes (Wang et al., 2001). The results can be used to effectively discriminate soybean accessions of Kazakhstan and strengthen breeding programs for the studied disease resistances.

Conclusion

Field trials of the soybean collection consisting of 288 accessions from different parts of the world suggested that over 97% of the collection was resistant to FLS, FRR and PSS fungal diseases in the studied period. Still, the correlation analysis showed that FLS and FRR had a negative effect on TSW, while FRR and PSS negatively correlated with yield. The evaluation of accessions using nine SSR markers associated with the resistance to FLS, FRR and PSS differentiated local samples from soybean accessions from other regions and indicated that they are genetically closer to cultivars from East Asia. The usage of the *t-test* did not show significant differences between the accessions with alleles linked to resistance and those with other alleles, which could be explained by low infection spreads of the three studied diseases in 2018 and 2019. The assessment of the SSR genotyping results revealed a high level of polymorphism and suggested that a majority of the applied markers can be successfully used in DNA documentation of soybean collections and breeding programs.

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