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GENETIC STRUCTURE OF A COLLECTION OF SOYBEAN ACCESSIONS OF DIFFERENT GEOGRAPHICAL ORIGIN BASED ON SSR LOCI POLYMORPHISM

Pavlo Chernyshenko [1], ***Halyna Chernyshenko*** [2], ***Olga Bezugla*** [1], ***Tetiana Shelyakina*** [1]

[1] *Yuriev Plant Production Institute of NAAS, 142 Heroiv Kharkova Ave., Kharkiv 61100, Ukraine*

[2] *Testing Laboratory of Agrogen Novo LLC, 2-V Shishkivska St., Kharkiv 61070, Ukraine*

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Background. Assessment of genetic diversity and genetic structure is essential for effective soybean breeding and conservation of genetic resources. The present study aimed to evaluate the genetic variability of 29 soybean accessions originating from six countries using nine microsatellite (SSR) loci.

Materials and Methods. The collection included cultivars developed in Ukraine and introduced genotypes from Canada, Austria, France, Germany, and Serbia. Each accession was represented by a single bulk DNA sample composed of embryonic root segments from 30 seeds. Nine SSR markers recommended by the NY/T 2595-2014 standard were amplified. Genetic diversity parameters were estimated using Nei's genetic diversity index, and genetic grouping was inferred using Bayesian clustering implemented in STRUCTURE software.

Results and Discussion. Eight of the nine loci were polymorphic, yielding a total of 18 alleles with an average of 2.0 alleles per locus. Nei's genetic diversity index (He) ranged from 0.07 to 0.51 (mean 0.26). Pairwise genetic distances varied from 0.0002 to 0.0499. No identical multilocus SSR profiles were detected among the loci analyzed. Bayesian clustering analysis revealed three major genetic groups (K = 3). Twenty-five accessions were assigned to the "pure" group (Q ≥ 0.80), whereas four exhibited admixed genetic structure (Q < 0.80).

Conclusions. The results confirm the suitability of SSR markers for cultivar differentiation, assessment of genetic diversity, and analysis of genetic grouping within soybean collections. Partial correspondence between clusters and geographical origin suggests shared breeding history and germplasm exchange. The obtained data may support breeding program optimization and conservation of soybean genetic resources.

Keywords: soybean, SSR markers, genetic diversity, genetic distance, STRUCTURE, polymorphism

INTRODUCTION

Climate change, particularly the increase in average annual temperatures and the growing frequency of extreme weather events, poses serious challenges to sustainable soybean production worldwide. The development of new cultivars capable of maintaining stable yields under abiotic and biotic stress conditions has therefore become a priority in modern breeding programs. Although the agro-climatic conditions of Ukraine are generally favorable for soybean cultivation, adaptation to rapidly changing environmental conditions requires the use of a broader and genetically diverse breeding base (Kyrychenko *et al*., 2016). This highlights the importance of comprehensive characterization of soybean germplasm originating from different geographical regions.

Microsatellite (SSR) markers are widely used for assessing intraspecific genetic variability and elucidating genetic relationships among genotypes. SSR loci are characterized by high polymorphism, codominant inheritance, reproducibility, and broad distribution throughout the genome, making them effective tools in molecular genetic research and variety identification (Volkova, 2015). Numerous studies have demonstrated substantial polymorphism in soybean collections using SSR markers (Meesang *et al*., 2001; Tantasawat *et al.*, 2011; Prysiazhniuk *et al*., 2017; Kumar *et al*., 2022; Komariah *et al*., 2023; Pardeshi *et al*., 2023; Rani *et al*., 2023; Zatybekov *et al*., 2023; Achina *et al*., 2025).

In 2014, a Chinese national standard (Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2025) was developed for soybean variety identification based on the polymorphism of 36 SSR loci, emphasizing the practical relevance of SSR markers for assessing genetic purity and differentiation of cultivars.

Despite extensive application of SSR markers in soybean research, comparative analysis of accessions representing diverse geographical origins remains important for understanding genetic structure and breeding relationships.

Therefore, the aim of this study was to characterize the genetic structure and diversity of 29 soybean accessions of different geographical origin based on the analysis of nine SSR loci.

MATERIALS AND METHODS

Plant material. The study included 29 soybean accessions representing six countries (**Table 1**).

Pedigree information was provided for cultivars for which such data were available in official breeding records or publicly accessible sources. For most foreign commercial cultivars, detailed pedigree information was not publicly available, as it is often confidential within breeding companies.

Table 1. **Soybean accessions included in the study**

No	Variety/ line name	Pedigree	Country (origin of variety/line)	Originator
1	Volonterka	Kharkivska 80/ Fiskeby (SE)	Ukraine	Yuriev Plant Production Institute of NAAS
2	Svitlytsia	Sprytna/No 4106–04	Ukraine	Yuriev Plant Production Institute of NAAS
3	Pysanka	Horyzont/ Evans (US)	Ukraine	Yuriev Plant Production Institute of NAAS
4	Rizdviana	Romantyka/ Nordia (PL)	Ukraine	Yuriev Plant Production Institute of NAAS
5	Fortetsia	Mriia/L 148–20	Ukraine	Yuriev Plant Production Institute of NAAS
6	line 1–1	Podiaka/Kyivska 27	Ukraine	Yuriev Plant Production Institute of NAAS
7	line 1–2	Medeia/ mutant 82–205	Ukraine	Yuriev Plant Production Institute of NAAS
8	line 2–2	Estafeta/Amsoy 71	Ukraine	Yuriev Plant Production Institute of NAAS
9	Cordoba	Publicly unavailable	Canada	AgRELIANT GENETICS Inc.
10	Lenka	Publicly unavailable	Canada	Semenses Prograin INC.
11	Azura	Publicly unavailable	Canada	Semenses Prograin INC.
12	Niagara	Publicly unavailable	Canada	Sevita Genetics
13	Alaska	Publicly unavailable	Canada	Semenses Prograin INC.
14	Vatson	Publicly unavailable	Canada	North Star Genetics
15	Mahony	Publicly unavailable	Canada	SeCan
16	Kofu	Publicly unavailable	Canada	Semenses Prograin INC.
17	Canzas	Publicly unavailable	Canada	Sertis Holding Group
18	S0009-M2	Publicly unavailable	Canada	NK Seeds
19	Aurelina	Publicly unavailable	Austria	SAATBAU LINZ eGen
20	Acardia	Publicly unavailable	Austria	tycht Ges.m.b.H & Co KG
21	Atacama	Publicly unavailable	Austria	Probstdorfer Saatzucht Ges.m.b.H & Co KG
22	Abaca	Publicly unavailable	Austria	SAATBAU LINZ eGen
23	Adessa	Publicly unavailable	Austria	SAATBAU LINZ eGen
24	Adelfia	C107xAdams	Austria	SAATBAU LINZ eGen
25	ES Albator	Publicly unavailable	France	Euralis Semences
26	Suedina	Publicly unavailable	France	RAGT 2n
27	Mozart	Publicly unavailable	Germany	Deutsche Saatveredelung AG
28	Windsor	Publicly unavailable	Germany	Deutsche Saatveredelung AG
29	Rubin	Publicly unavailable	Republic of Serbia	Institut za ratarstvo i povrtarstvo

DNA extraction and PCR amplification. Genomic DNA was extracted using a sorbent-based spin column method according to the manufacturer's protocol. Each accession was represented by a single bulk DNA sample composed of embryonic root tissues collected from 30 seeds. Each accession was analyzed as a single multilocus profile (variety-as-individual). For all loci scored, bulk samples consistently produced

a single clear allele per accession; if more than one allele had been detected, single-seed genotyping would have been performed to resolve within-accession variation.

The variability of nine microsatellite (SSR) loci (Satt197, Satt429, Sat_112, Sat380, Sat239, Sat588, Satt300, Sat_084, and Sat005), selected according to the NY/T 2595-2025 standard and previous studies as informative in soybean, was evaluated (**Table 2**).

Table 2. **Characteristics of SSR loci and primers used for amplification**

No	Loci	Molecular linkage group	Expected size of amplification products, bp (according to NY/T 2595-2014)	Primer sequence (forward/reverse)	Annealing temperature (Tm), °C	Multiplex
1	Satt197	B1	133-202	Forward: CACTGCTTTTCCCCTCTCT Reverse: AAGATACCCCCAACATTATTTGTAA	55	Multiplex 1
2	Satt429	A2	243-275	Forward: GCGACCATCATCTAATCACAATCTACTA Reverse: TCCCCATCATTTATCGAAAATAATAATT	55	
3	Sat_112	E	310-345	Forward: TGTGACAGTATACCGACATAATA Reverse: CTACAAATAACATGAAATATAAGAAATA	55	
4	Sat380	J	122-135	Forward: GCGAGTAACGGTCTTCTAACAAGGAAAG Reverse: GCGTGCCCTTACTCTCAAAAAAAAA	60	Multiplex 2
5	Sat239	I	172-193	Forward: GCGCCAAAAAATGAATCACAAT Reverse: GCGAACACAATCAACATCCTTGAAC	60	
6	Sat588	K	114-169	Forward: GCTGCATATCCACTCTCATTGACT Reverse: GAGCCAAAACCAAAGTGAAGAAC	60	Multiplex 3
7	Satt300	A1	238-262	Forward: GCGCCCACACAACCTTTAATCTT Reverse: GCGGCGACTGTTAACGTGTC	60	
8	Sat005	D1b	132-170	Forward: TATCCTAGAGAAGAACTAAAAAA Reverse: GTCGATTAGGCTTGAAATA	55	Multiplex 4
9	Sat_084	N	132-153	Forward: AAAAAAGTATCCATGAAACAA Reverse: TTGGGACCTTAGAAGCTA	52	

PCR reactions were performed in multiplex groups as indicated in **Table 2**. PCR amplification was performed in a final reaction volume of 20 μL containing 20 ng of genomic DNA. In multiplex reactions, each primer was used at a final concentration of 0.25 μM. The total primer concentration within a multiplex reaction depended on the number of loci included. Primer concentrations were adjusted during preliminary optimization to ensure balanced amplification of all loci within each multiplex set. Primers combined within each multiplex set had compatible annealing temperatures and non-overlapping expected fragment size ranges. The amplification protocol consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at the locus-specific temperature (**Table 2**) for 45 s, elongation at 72 °C for 45 s, and a final extension at 72 °C for 7 min.

Electrophoresis and fragment analysis. PCR products were separated on 2% agarose gels prepared in low-ionic-strength borate buffer (Brody & Kern, 2004). The low-conductivity buffer system permits electrophoresis at higher voltage with reduced heat generation, resulting in improved resolution of small DNA fragments compared to conventional TAE/TBE systems. Within the analyzed fragment size range (120–374 bp), allele size differences were ≥10 bp for all polymorphic loci, allowing reliable discrimination under the applied electrophoretic conditions. No ambiguous or merged bands were observed during allele scoring. Gels were stained with ethidium bromide and visualized under UV illumination. Fragment sizes were estimated using Totallab 120 software.

Genetic diversity analysis. Genetic diversity was estimated using Nei's genetic diversity index (He), calculated as:

$$\mathrm{He} = 1 - \sum p_i^2,$$

where p_i – represents the frequency of the *i*-th allele at a given locus.

Pairwise genetic distances among soybean accessions were calculated using the Nei and Li coefficient implemented in PHYLIP software:

$$D_{ij} = 1 - S_{ij} - \frac{2a}{2a + b + c},$$

where *a* is the number of shared alleles between two accessions, while *b* and *c* represent the numbers of alleles unique to each accession.

Population structure analysis. Population structure was analyzed using the Bayesian clustering algorithm implemented in STRUCTURE v2.3.4 (Pritchard *et al*., 2000) under the admixture model with correlated allele frequencies. The LOCPRIOR model was not applied.

The number of genetic clusters (K) was tested from K = 1 to K = 10. For each K value, 20 independent runs were performed using a burn-in period of 100,000 iterations followed by 500,000 Markov Chain Monte Carlo (MCMC) repetitions.

SSR genotypes were treated as codominant markers and encoded based on allele sizes in base pairs.

Mean log-likelihood values [LnP(K)] and their standard deviations were calculated across independent runs using StructureSelector. The optimal number of clusters was determined using the ΔK method of (Evanno *et al*. 2005).

RESULTS AND DISCUSSION

Polymorphism of SSR loci. Of the nine SSR markers analyzed (Satt197, Satt429, Sat_112, Sat380, Sat239, Sat588, Satt300, Sat005, and Sat_084), eight were polymorphic, yielding a total of 18 alleles. The number of alleles per locus ranged from 1 to 3, with an average of 2.0 ± 0.5. The highest allelic variability (three alleles) was observed at locus Sat239. Locus Sat_084 was monomorphic, whereas the remaining loci displayed two alleles each.

The sizes of amplified fragments ranged from 135 to 374 bp (**Fig. 1**). Comparable fragment size ranges for these loci have been reported previously in soybean germplasm (Chernyshenko *et al*., 2024).

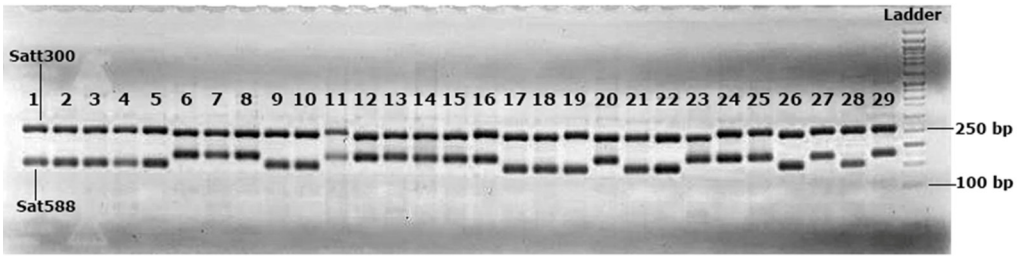

Fig. 1. Electrophoretic profile of multiplex 3 (SSR loci Sat_588 and Satt300) in 29 soybean accessions. Lanes 1–29 correspond to the accessions listed in Table 1

The highest frequency of the major allele was observed at Sat_112 (0.96), while the lowest was recorded at Sat588 (0.52). Nei's genetic diversity index (He) varied from 0.07 (Sat_112) to 0.51 (Sat588, Satt300), with the mean value of 0.26 ± 0.06 (**Fig. 2**).

Genetic distances. Pairwise genetic distances among the 29 soybean accessions ranged from 0.0002 to 0.0499, as calculated using Nei's genetic distance. No identical multilocus SSR profiles were detected among the loci analyzed, as all pairwise distances were greater than zero.

The greatest genetic divergence (0.0499) was observed between line 1–1 (Ukraine) and Suedina (France), followed by 0.0495 between Kofu (Canada) and Aurelina (Austria). In contrast, the smallest genetic distance (0.0002) was recorded between Rizdviana (Ukraine) and Adessa (Austria). The mean genetic distance across all pairwise comparisons was 0.0249.

Population structure. Bayesian clustering analysis revealed the highest ΔK value at K = 3 (ΔK = 14.47), indicating the presence of three major genetic groups within the analyzed soybean collection (**Fig. 3**).

The mean log-likelihood value [LnP(K)] at K = 3 was −140.58 with a low standard deviation (SD = 1.08) across 20 independent runs, demonstrating stable clustering results under extended MCMC settings (burn-in = 100,000; MCMC = 500,000).

At K = 3, the first cluster comprised eight accessions: Volonterka, Svitlytsia, Pysanka, Rizdviana, Fortetsia (Ukraine), Lenka and Canzas (Canada), and Suedina (France).

The second cluster included eleven accessions: line 1–1 and line 2–2 (Ukraine), Cordoba, Niagara, Vatson and Mahony (Canada), Aurelia, Atacama, Abaca and Adessa (Austria), and Windsor (Germany).

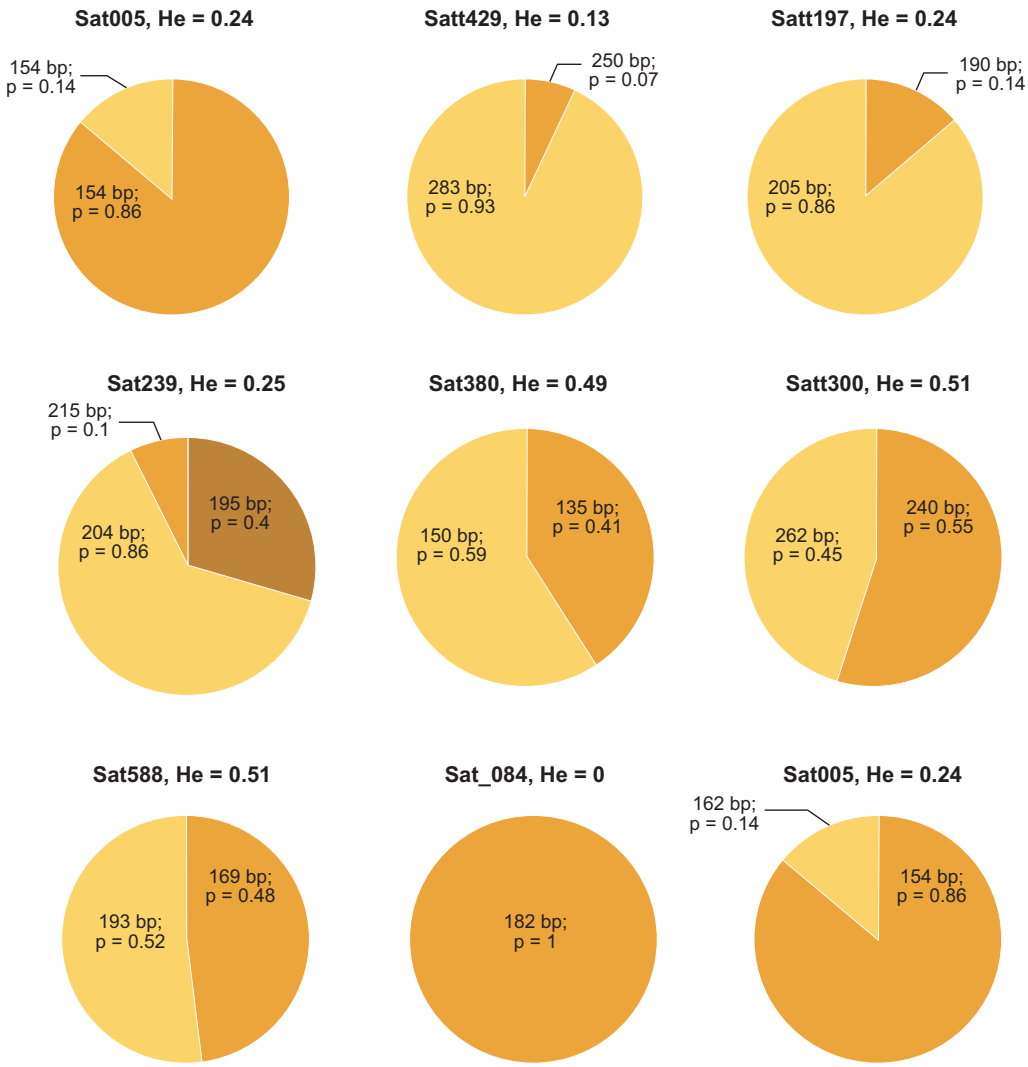

Fig. 2. Genetic diversity (He) values of nine SSR loci analyzed in 29 soybean accessions. Nei's genetic diversity index (He) was calculated for each locus based on allele frequencies. The number of alleles per locus ranged from 1 to 3. The mean He value across loci was 0.26 ± 0.06

The third cluster consisted of six accessions: Azura and Kofu (Canada), Acardia (Austria), ES Albator (France), Mozart (Germany), and Rubin (Serbia) (**Fig. 4**).

Four accessions (line 1–2, Alaska, S0009-M2, and Adelfia) showed admixed ancestry ($Q < 0.80$). In total, 25 accessions were classified as genetically homogeneous ("pure"), while four exhibited mixed membership patterns.

The clustering pattern partially corresponded to geographical origin; however, the shared breeding history and germplasm exchange likely contributed to the observed genetic structure.

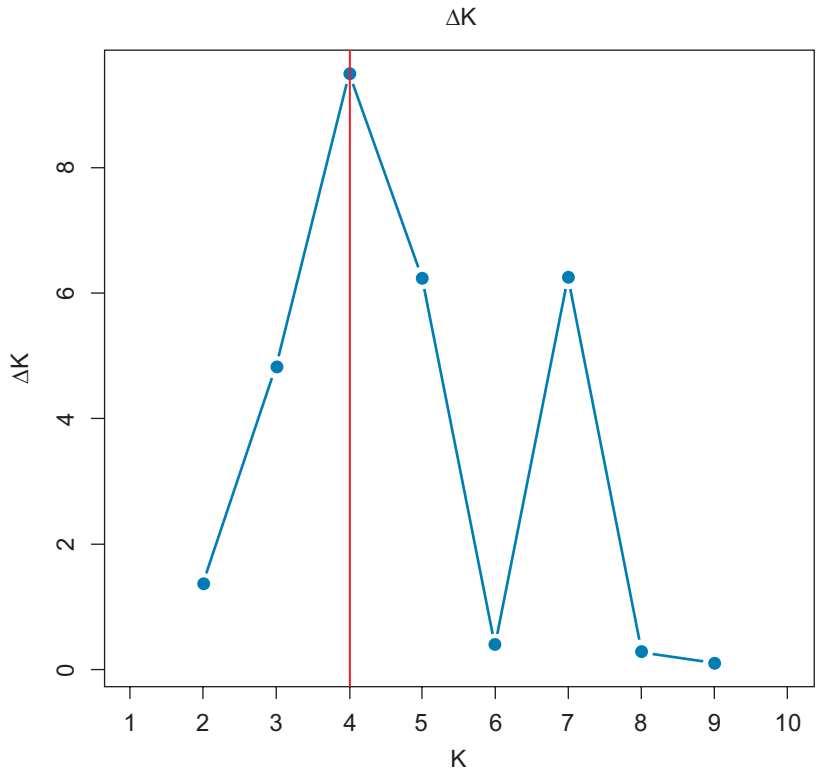

Fig. 3. Determination of the optimal number of genetic clusters (K) using the ΔK method of G. Evanno *et al*. (2005). Mean log-likelihood values [LnP(K)] were calculated across 20 independent STRUCTURE runs for each K value (K = 1–10). The highest ΔK value was observed at K = 3, indicating the most likely number of genetic groups within the analyzed soybean collection

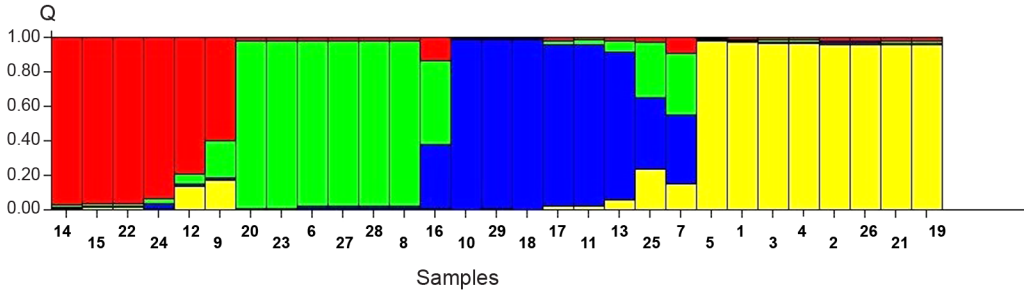

Fig. 4. Population structure of 29 soybean accessions inferred by STRUCTURE at K = 3 under the admixture model with correlated allele frequencies. Each vertical bar represents one accession. Colors indicate the proportional membership coefficient (Q value) of each accession in the inferred genetic clusters. Accessions with Q ≥ 0.80 were classified as “pure,” whereas those with Q < 0.80 were considered admixed

Thus, the present study evaluated the polymorphism of nine SSR loci in a collection of 29 soybean accessions originating from different geographical regions. A total of 18 alleles were detected, with an average of 2.0 alleles per locus. Nei’s genetic diversity index ranged from 0.07 to 0.51 (mean 0.26), and one locus (Sat_084) was monomorphic.

The observed level of genetic diversity was lower than that reported in studies employing larger marker sets or high-resolution genotyping systems (Prysiazhniuk *et al*., 2017; Kumar *et al*., 2022; Komariah *et al*., 2023; Pardeshi *et al*., 2023; Rani *et al*., 2023; Zatybekov *et al*., 2023; Achina *et al*., 2025). This comparatively moderate level of polymorphism may be attributed to the limited number of loci analyzed and the relatively narrow breeding background of modern soybean cultivars.

Despite the moderate diversity estimates, the SSR markers enabled clear differentiation among accessions. No identical multilocus SSR profiles were detected based on the nine loci examined. It should be noted, however, that the resolution of genetic similarity is limited to the marker set used in this study.

Bayesian clustering analysis identified three major genetic groups (K = 3), partially corresponding to geographical origin. The clustering pattern likely reflects both historical breeding practices and germplasm exchange among countries rather than strict geographic differentiation.

CONCLUSION

The analysis of nine SSR loci enabled differentiation of soybean accessions and detection of multilocus genetic variation within the studied collection. Despite the moderate level of polymorphism, the markers were sufficient to distinguish accessions and to identify three major genetic groups.

The obtained results have practical implications for soybean breeding. The identified genetic groupings may assist in the selection of genetically divergent parental forms for hybridization, thereby increasing the probability of obtaining transgressive segregants and enhancing breeding efficiency. The detected multilocus profiles can also support varietal identification and contribute to maintaining genetic purity in seed production systems.

Furthermore, the characterization of accessions of different geographical origin provides a foundation for optimizing germplasm utilization strategies and preserving genetic diversity within breeding programs.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

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AUTHOR CONTRIBUTIONS

Conceptualization, [Ch.P.; Ch.H.]; methodology, [Ch.P.; Ch.H.]; validation, [Ch.H.]; formal analysis, [Ch.H.].; investigation, [Ch.P.; Ch.H.; Sh.T.]; resources, [Sh.T.; B.O.]; data curation, [Ch.P.; Ch.H.; B.O.]; writing – original draft preparation, [Ch.H.]; writing – review and editing, [Ch.P.; B.O.]; visualization, [Ch.H.] supervision, [Ch.P.]; project administration, [Ch.P.]; funding acquisition, [Ch.P.].

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ГЕНЕТИЧНА СТРУКТУРА КОЛЕКЦІЇ ЗРАЗКІВ СОЇ РІЗНОГО ГЕОГРАФІЧНОГО ПОХОДЖЕННЯ ЗА ПОЛІМОРФІЗМОМ SSR-ЛОКУСІВ

***Павло Чернишенко*[1], *Галина Чернишенко*[2], *Ольга Безугла*[1], *Тетяна Шелякіна*[1]**

[1] Інститут рослинництва ім. В. Я. Юр'єва НААН
просп. Героїв Харкова, 142, Харків 61100, Україна

[2] Випробувальна лабораторія ТОВ "Агроген Ново"
вул. Шишківська, 2-В, Харків 61070, Україна

Вступ. Оцінка генетичного різноманіття і структури колекцій є важливою передумовою ефективної селекції сої та збереження генетичних ресурсів. Метою дослідження було визначити генетичну мінливість 29 зразків сої різного географічного походження за дев'ятьма SSR-локусами.

Матеріали та методи. До аналізу залучено сорти української селекції та інтродуковані генотипи з Канади, Австрії, Франції, Німеччини і Сербії. Кожен зразок був представлений однією сумарною пробою ДНК, виділеною з ембріональних корінців 30 насінин. Для дослідження використано дев'ять SSR-маркерів, рекомендованих стандартом NY/T 2595-2014. Генетичне різноманіття оцінювали за індексом Nei, а генетичне групування визначали за допомогою байєсівського кластерного аналізу в програмі STRUCTURE.

Результати. Вісім із дев'яти локусів були поліморфними; загалом виявлено 18 алелів, у середньому 2,0 алелі на локус. Індекс генетичного різноманіття Nei (He) варіював від 0,07 до 0,51 (середнє значення – 0,26). Генетичні дистанції між парами зразків становили від 0,0002 до 0,0499. Ідентичних багатолокусних SSR-профілів серед досліджених зразків не виявлено. Аналіз у програмі STRUCTURE визначив оптимальну кількість кластерів за K = 3. До "чистих" (Q ≥ 0,80) залучено 25 зразків, тоді як 4 продемонстрували змішану генетичну структуру (Q < 0,80).

Висновки. Отримані результати підтверджують доцільність використання SSR-маркерів для диференціації сортів, оцінки генетичного різноманіття й аналізу генетичного групування зразків сої. Часткова відповідність кластерів географічному походженню свідчить про спільне селекційне минуле або обмін генетичним матеріалом. Дані можуть бути використані для оптимізації селекційних програм і збереження генетичних ресурсів сої.

Ключові слова: соя, SSR-маркери, генетичне різноманіття, генетична дистанція, STRUCTURE, поліморфізм

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