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Analysis of soybean (*Glycine max* L.) breeding material using SSR markers

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Purpose. To determine the genetic diversity in soybean breeding material and select an effective set of SSR markers for hybridization assessment. **Methods.** Breeding (selection, reciprocal crosses), molecular (PCR, agarose gel electrophoresis), and statistical (hierarchical clustering, principal component analysis) methods were applied. Fifteen soybean breeding samples were analyzed using 10 SSR markers to identify polymorphic loci and evaluate genetic differentiation among parental and hybrid genotypes. **Results.** PCR analysis revealed from one to three alleles per locus. Seven markers (AW277661, Satt691, Satt349, Satt680, Satt545, Satt277, and Satt177) were polymorphic and effectively distinguished parental forms and hybrid combinations. Markers Satt152, Satt115, and Satt229 showed no polymorphism (allele frequency = 1.00), indicating their limited applicability for hybridization efficiency assessment. Hybrid combinations derived from parental forms No. 1 × No. 11, No. 1 × No. 15, and No. 1 × No. 17 showed two alleles at loci Satt349 and Satt691, confirming heterozygosity. Jaccard's similarity coefficients (0–0.75) indicated the formation of two major cluster groups and one separate cluster represented by parental form No. 11. Clustering and principal component analysis (PCA) results were consistent, with the first two components (PC1 – 38.687%, PC2 – 27.432%) explaining 96.838% of the total variance. The highest variability was associated with markers AW277661, Satt691, Satt349, Satt680, Satt545, and Satt277, demonstrating their high informativeness in reflecting genetic differentiation among genotypes. **Conclusions.** The results confirm the effectiveness of SSR markers for identifying soybean hybrid combinations and assessing genetic similarity among parental lines. Seven polymorphic markers are recommended for evaluating hybridization efficiency, while Satt177 is considered potentially informative for further use. The consistency between clustering and PCA results supports the reliability of the genetic structure obtained. To enhance heterosis expression and broaden the genetic base of breeding programs, crosses between genotypes from different clusters are advisable. The use of a comprehensive set of informative SSR markers can improve the accuracy of parental selection and accelerate the development of high-yielding soybean varieties.

Keywords: *similarity coefficients; principal components; DNA analysis; allele; hybridization efficiency.*

Introduction

The soybean (*Glycine max* L.) is one of the cheapest sources of protein and oil, accounting for around half of global oilseed production. It has been cultivated for thousands of years, initially in China and East Asia before becoming the world's most widely grown oilseed crop [1]. Ukraine is the leading producer of soybeans among European countries and the eighth largest producer worldwide. There is significant potential for further growth in production and for the crop to become a major export for the country [2].

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Since soybeans are a self-pollinating crop with a cross-pollination rate of less than one percent, breeders face a narrowing genetic base. Consequently, they rely on a limited number of selected genotypes as parental forms [3].

Breeders conduct various types of crosses between varieties or gene pool lines to alter the frequency of genes in the breeding population by promoting gene recombination. Proper management of the crossing scheme and breeding population is essential for achieving more genetically productive material [4].

A significant number of breeding programmes are operating in European countries with the aim of creating high-yielding soybean varieties to meet the needs of the rapidly growing market for protein raw materials. One of the key challenges for these programmes is the low morphological variability and limited or absent pedigree information of the source material, which makes the selection of genetically distant parental forms for hybridisation significantly more difficult [5]. Furthermore, relying solely on morphological characteristics can considerably slow down the breeding process, since a full vegetation cycle is usually necessary for evaluation. The genetic basis of modern soybean varieties can be broadened by examining molecular characteristics using DNA markers, which are more informative, stable and reliable than the morphological markers traditionally employed [5]. Recent studies have also shown that DNA markers are effective in predicting phenotype based on genotype using artificial intelligence tools [6].

Although SNP (Single Nucleotide Polymorphism) markers, particularly KASP (Kompetitive allele specific PCR), are currently among the most popular for breeding purposes, SSR analysis remains an effective and reliable method for assessing genetic diversity, determining hybrid formulas and identifying varieties.

Simple sequence repeats (SSRs) are an effective tool for assessing genetic diversity and determining the relatedness of genotypes. These markers have an advantage over other types of molecular markers in genetic research due to the high reproducibility of results. Using them does not require significant initial financial investment and ensures high genotyping efficiency due to the small amount of DNA required. Furthermore, the genotyping process using SSR markers can be fully or partially automated, significantly increasing the productivity and reliability of the analysis [7].

Numerous studies have investigated soybean genetic diversity using SSR markers. These studies have demonstrated the effectiveness of assessing genetic relationships, determining the origin of breeding material and identifying economically valuable traits [8–11].

The key criterion for the effectiveness of a breeding method is the degree of genetic variation. Breeders therefore strive to maximise genetic dispersion in the early stages of the breeding process by selecting genetically distant parental forms. In subsequent stages, they use selection methods that minimise the probability of the same genotype being included in subsequent generations. To objectively assess the effectiveness of these methods, it is advisable to use molecular markers, particularly SSR markers, to evaluate the genetic diversity preserved within populations bred using different methods [4].

The research aimed to determine the degree of genetic diversity in soybean breeding material, and to select an effective SSR marker system for assessing hybridisation.

Materials and methods

The research was conducted in 2024 and 2025. Fifteen soybean breeding samples from the Ukrainian Scientific Institute of Plant Breeding (Kyiv, Ukraine) were studied: four parental forms and 11 hybrid combinations. Hybridisation was carried out at the Ukrainian Scientific Institute of Plant Breeding using the reciprocal crossing method, and molecular genetic analysis was performed at the Ukrainian Institute for Plant Variety Examination (Kyiv, Ukraine).

DNA was extracted from 25 mg of soybean leaf material in two replicates using the CTAB method [13]. The genetic diversity of the soybean breeding material was studied using ten SSR markers recommended by the protocol for determining the molecular characteristics of new soybean varieties using microsatellites during examination for distinctness, uniformity and stability in Italy (see Table 1).

The reaction mixture (volume 10 µl) contained: 50 ng of DNA, 1.5×PCR buffer (10 mM Tris-HCl, pH 9.0; 50 mM KCl; 0.01% Triton X-100; 1.8 mM $MgCl_2$); 200 µM deoxynucleotide triphosphates (dNTPs), 0.50 µM of each primer, and 1 unit of Taq polymerase. PCR was performed on a SureCycle G8800A thermocycler (Agilent, USA) under the following temperature and time parameters: initial denaturation 94 °C for 2 min, Touchdown mode (10 cycles): denaturation 94 °C for 45 s; primer hybridization – from 60 °C to 50 °C, 45 s; elongation – 72 °C,

Table 1

Characteristics and nucleotide sequences of primers used in the study

No.	SSR	Primer sequence 5′→3′	Chromosome	Motif	Expected allele size, bp
1	Satt152	F* – CGCTATTCCTATCACAACAC R** – GGGTTGTCACTGTTTTGTTC	03	$(ATA)_{21}$	215–242
2	AW277661	F – GGTGCAATTTTCTTGTTCAG R – AGTAAGACCCCGAAAGAAAG	04	$(TAT)_{23}$	209–227
3	Satt545	F – AGGAATCTTCATCAGGACAA R – GGAAACACAAAGGAGTTGAA	05	$(TTA)_{24}$	190–205
4	Satt277	F – GCGGGTTACTATTACTGCTG R – ACTACCACGCTTCAGTTGAT	06	$(TTA)_{13}$	169–238
5	Satt680	F – GGGATATCGTGAGCATAGTT R – CCGATTTTGGTTTCTCA	07	$(ATT)_{48}$	349–400
6	Satt177	F – CGTTTCATTCCCATGCCAATA R – CCCGCATCTTTTTCAACCAC	08	3bp	106–115
7	Satt349	F – AACGACCAACAACAGCTAAT R – TGCTTAACAAGTGTCTCGAA	09	$(AAT)_{10}$	195–213
8	Satt691	F – AAGATAAAAAGTAGATTGAAAGAA R – ACACTCCACACCACACTACA	15	$(ATT)_{17}$	177–207
9	Satt115	F – GGTTCGTTTTTTATTGATG R – ACGACGAAATTGATGATAA	18	$(TAT)_{18}$	132–153
10	Satt229	F – CACACCTGCTAAGGGAATAA R – CAACTACACTAGCATTGCATCT	19	$(AAT)_{22}$	295–298

*F – forward primer; **R – reverse primer.

45 s; normal mode (35 cycles): denaturation – 94 °C, 1 min; primer hybridization – 55 °C, 1 min; elongation – 72 °C, 1 min; final elongation – 72 °C, 7 min.

The PCR products were visualised by electrophoresis in a 2% agarose gel in 0.5× TBE (Tris-borate buffer), using the standard method with ethidium bromide. Electrophoresis was performed for 1.5 hours at a field strength of 5 V/cm. The size of the obtained fragments was determined relative to the molecular weight marker using the TotalLab Tl120 computer program (trial version).

Statistical data processing

The presence or absence of a specific allele in the soybean breeding material samples was marked as 1 or 0 based on the obtained data. The samples were then grouped into clusters using the unweighted pair group method with arithmetic averages. Principal component analysis was applied using the XLSTAT computer program (trial version) to determine the variability of the soybean breeding material samples by SSR markers [16, 17].

Research results

PCR analysis of 15 soybean breeding samples using 10 SSR markers identified one to three alleles for each marker. Seven of the ten SSR markers used in the study were found to be polymorphic, enabling the identification of hybrid combinations resulting from the crossing of four parental forms. The allele frequencies obtained in the hybrid combinations ranged from 0.18 to 0.86 (see Table 2).

Table 2

Characteristics of the alleles identified in 11 hybrid combinations of the studied soybean samples

No.	SSR	Number of alleles, pcs.	Allele size, bp	Allele frequency
1	Satt152	1	230	1.00
2	AW277661	3	213–233	0.32–0.36
3	Satt545	2	180–207	0.14–0.86
4	Satt277	2	167–235	0.14–0.86
5	Satt680	2	376–400	0.18–0.82
6	Satt177	2	122–145	0.36–0.64
7	Satt349	2	200–215	0.18–0.82
8	Satt691	2	183–207	0.50
9	Satt115	1	155	1.00
10	Satt229	1	292	1.00

The obtained data show that the SSR markers Satt152, Satt115 and Satt229 identified alleles of the same size in the soybean samples' parental forms. This makes it impossible to use them to determine the effectiveness of hybridization, as evidenced by the frequency of alleles found in hybrid combinations – 1.00. Polymorphism was detected in at least two parental forms for the other seven SSR markers, which made it possible to identify heterozygotes in the obtained hybrid combinations (Figs. 1–2).

Based on PCR results using Satt349 SSR primers, it was determined that parental forms No. 1, No. 15 and No. 17 contain a 215 bp allele, while parental form No. 11 contains a 200 bp allele. Therefore, in the hybrid combinations resulting from crossing parental forms No. 1 and No. 11, two alleles measuring 200 and 215 bp were identified. In other hybrid combinations of the studied soybean samples, only one 215 bp allele was identified.

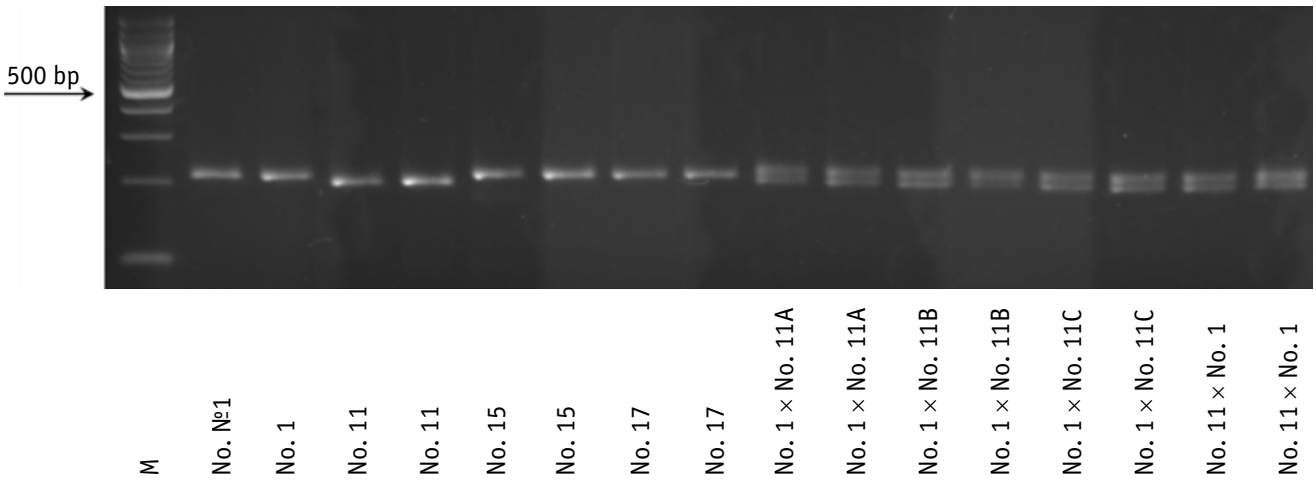

Fig. 1. Electrophoregram of soybean breeding material samples using the Satt349 marker: M – molecular weight marker 100 bp DNA Ladder (ThermoFisher Scientific, USA); No. 1, No. 11, No. 15, and No. 17 – parental forms of soybean breeding samples; No. 1 × No. 11A, No. 1 × No. 11B, No. 1 × No. 11C, and No. 11 × No. 1 – hybrid combinations

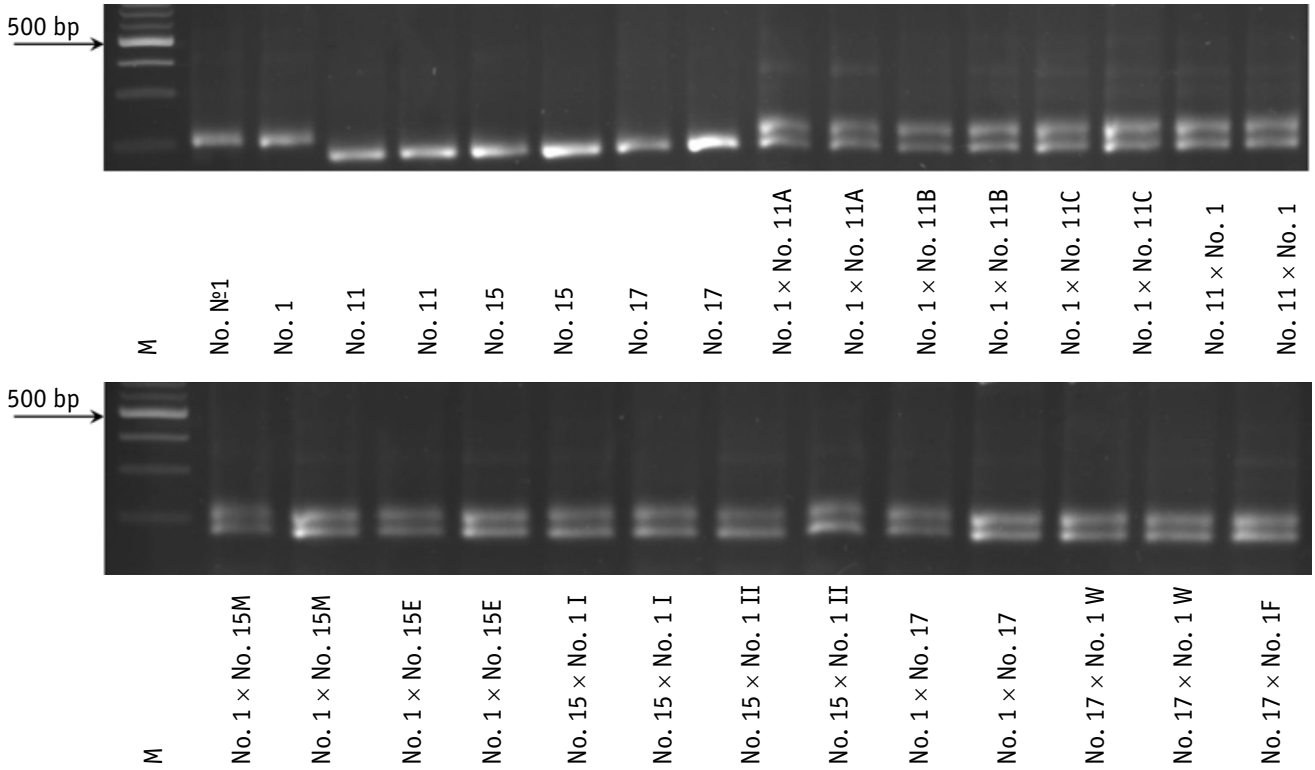

Fig. 2. Electrophoregram of soybean breeding material samples using the Satt691 marker: M – molecular weight marker 100 bp DNA Ladder (ThermoFisher Scientific, USA); No. 1, No. 11, No. 15, and No. 17 – parental forms of soybean breeding samples; No. 1 × No. 11A, No. 1 × No. 11B, No. 1 × No. 11C, No. 11 × No. 1, No. 1 × No. 15M, No. 1 × No. 15E, No. 15 × No. 1I, No. 15 × No. 1II, No. 1 × No. 17, No. 17 × No. 1W, and No. 17 × No. 1F – hybrid combinations

Hybrid combinations obtained by crossing parental forms No. 1 and No. 11, No. 1 and No. 15, and No. 1 and No. 17, and using the Satt691 marker, demonstrated the presence of 183 and 207 bp alleles.

The analysis revealed heterozygous forms for all hybrid combinations that contained the two alleles identified in the parental forms by the Satt691 marker. The effectiveness of SSR markers in assessing hybridisation between parental forms has been confirmed by other scientists. Thus, the authors identified polymorphic SSR loci in the parental lines and forms that enable the effectiveness of hybridisation to be assessed, significantly accelerating the breeding process [3, 18, 19]. Therefore, when using SSR markers to evaluate hybrid combinations, it is important to select markers that are characterised by a high level of polymorphism to enable accurate differentiation of alleles inherited from each parent.

It was revealed that the genotypes were divided into two cluster groups and one separate cluster according to Jacquard's similarity coefficients of the studied soybean breeding samples (Fig. 3).

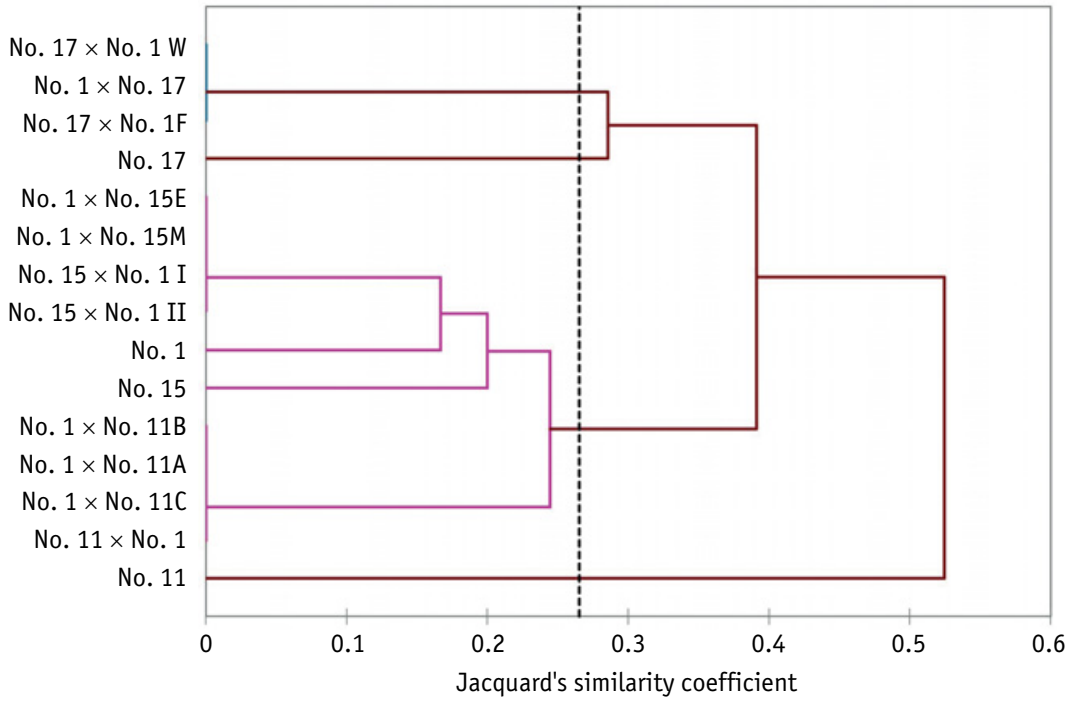

Fig. 3. Dendrogram of relationships between soybean breeding material samples based on 10 SSR markers (2024–2025)

The first cluster group consisted of genotypes obtained from the hybridisation of parental forms No. 1 and No. 17, as well as parental form No. 17 itself. The second cluster group included parental forms No. 1 and No. 15, their hybrid combinations and those resulting from crossing No. 1 with No. 11. Based on the alleles determined by 10 SSR markers, it should be noted that parental form No. 11 is distributed in a separate cluster.

According to Jacquard, the obtained values of similarity coefficients indicate that parental forms No. 1 and No. 15 are the most genetically similar, with a coefficient of 0.33. According to the 10 SSR markers studied, the most distinct parental forms are No. 11 and No. 17, with a similarity coefficient of 0.75. Parental forms No. 1 and No. 11 showed a fairly high level of difference, as evidenced by a Jacquard similarity coefficient of 0.67.

SSR markers are widely used to assess genetic diversity in the study of the soybean gene pool. According to the results of study [20], 14 polymorphic SSR markers were identified to assess 29 soybean genotypes of different origins. These formed two main cluster groups and four subgroups based on the obtained alleles. The authors demonstrated that the genotypes were distributed according to their origin and the similarity of the parental forms used in the breeding programme. Our research also showed that the 15 soybean breeding samples formed cluster groups according to the genetic profile of the parental forms used to create hybrid combinations. The genetic diversity of the soybean breeding samples was studied based on SSR markers [11]. Jacquard similarity coefficients, obtained based on 367 alleles from 56 soybean genotypes, showed a level of genetic similarity ranging from 0.29 to 0.95 between genotypes. In our studies, the similarity coefficients ranged from 0 to 0.75. The zero Jacquard similarity coefficients obtained in our studies indicate the absence of polymorphism for three SSR markers in the soybean parental forms. Close genetic links (0.17–0.27) may indicate high similarity between parental forms and low marker polymorphism. However, the data obtained indicates that genotypes from two different cluster groups can be used as potential candidates for hybridisation to enhance the heterosis effect.

The study [21] demonstrates the effectiveness of using SSR markers to evaluate the hybridisation results of soybean parental forms. Thanks to the codominant properties of SSR markers, the authors were able to identify hybrid forms based on 11 cross combinations. Our work also shows that the difference between the sizes of identified alleles for seven SSR markers in parental genotypes allowed us to

identify hybrid forms of the studied soybean breeding samples.

Principal component analysis was used to study the dataset's multidimensional relationships, reflecting the proportion of genetic variation based on similarity indices for the studied SSR markers. To study the proportion of genetic variation reflected in the dataset, a principal component analysis based on similarity indices for the SSR markers was performed. The results showed that five of the six components had a weight greater than 1.0, together explaining 96.838% of the variability in the genotypes studied (see Table 3).

Table 3

The significance of the main components of soybean breeding material samples according to SSR markers

Principal components (PC)	Weight of principal component	Variability, %	Cumulative dispersion, %
PC1	5.803	38.687	38.687
PC2	4.115	27.432	66.120
PC3	1.953	13.020	79.140
PC4	1.526	10.173	89.313
PC5	1.129	7.526	96.838
PC6	0.474	3.162	100.000

Using 10 SSR markers, it has been established that PC1 and PC2 determine 38.687% and 27.432%, respectively, of the variability of soybean breeding material samples. PC1 is associated with the presence of the following alleles: 233 bp for the AW277661 marker; 180 bp for the Satt545 marker; and 167 bp for the Satt277 marker. Meanwhile, PC2 is associated with the presence of the following alleles: 227 bp for AW277661, 207 bp for Satt691, 215 bp for Satt349, and 376 bp for Satt680 (see Table 4).

According to the obtained data, the greatest variability among soybean breeding material samples is associated with the following alleles: AW277661, Satt691, Satt349, Satt680, Satt545 and Satt277. The values of the principal component eigenvectors for the Satt115, Satt229 and Satt152 markers are zero, which indicates an absence of polymorphism for these markers among the studied samples.

The results of the PCA enabled us to identify two distinct groups: Group I and Group II, as well as two separate genotypes, No. 11 and No. 17, which were used as parental forms during hybridisation (see Fig. 4). Group I comprised samples of breeding material resulting from the hybridisation of parental forms No. 1, No. 11 and No. 15 in various combinations. Group II consisted exclusively of samples resulting from various combinations of the hybridisation of parental forms No. 1 and No. 17.

Table 4

Principal component eigenvectors for soybean breeding material samples based on SSR markers

SSR	Alleles, bp	PC1	PC2	PC3	PC4	PC5	PC6
AW277661	213	−0.315	−0.100	**0.422**	0.053	−0.123	−0.168
	227	−0.022	**0.473**	−0.005	0.122	0.114	−0.294
	233	**0.356**	−0.119	0.188	0.292	0.059	−0.078
Satt177	122	−0.350	0.057	−0.038	−0.239	**0.388**	0.170
	145	0.163	0.295	**0.375**	−0.133	−0.186	**0.556**
Satt691	183	−0.031	−0.103	0.261	0.066	**0.839**	0.184
	207	0.000	**0.427**	0.217	0.176	−0.125	**0.439**
Satt349	200	−0.315	−0.100	**0.422**	0.053	−0.123	−0.168
	215	0.191	**0.361**	0.157	−0.299	0.102	−0.346
Satt680	376	0.191	**0.361**	0.157	−0.299	0.102	−0.346
	400	−0.315	−0.100	**0.422**	0.053	−0.123	−0.168
Satt115	155	0.000	0.000	0.000	0.000	0.000	0.000
Satt229	292	0.000	0.000	0.000	0.000	0.000	0.000
Satt152	230	0.000	0.000	0.000	0.000	0.000	0.000
Satt545	180	**0.356**	−0.119	0.188	0.292	0.059	−0.078
	207	−0.221	0.283	−0.163	**0.464**	0.053	−0.055
Satt277	167	**0.356**	−0.119	0.188	0.292	0.059	−0.078
	235	−0.221	0.283	−0.163	**0.464**	0.053	−0.055

*Red indicates principal component eigenvectors with absolute values greater than 0.300.

The results of the PCA are comparable with the cluster analysis data. The latter showed the difference between the parental forms No. 11 and No. 17. It also allowed us to identify two cluster groups formed by hybrid combinations of the parental forms No. 1, No. 11 and No. 15. There was a separate group formed by the hybridisation of the parental forms No. 1 and No. 17.

The study [22] presents the results of an analysis of soybean samples from various origins, conducted using SSR markers. The authors identified patterns in the distribution of the samples according to their origin, as determined by principal component analysis. This was also confirmed by the genetic distances

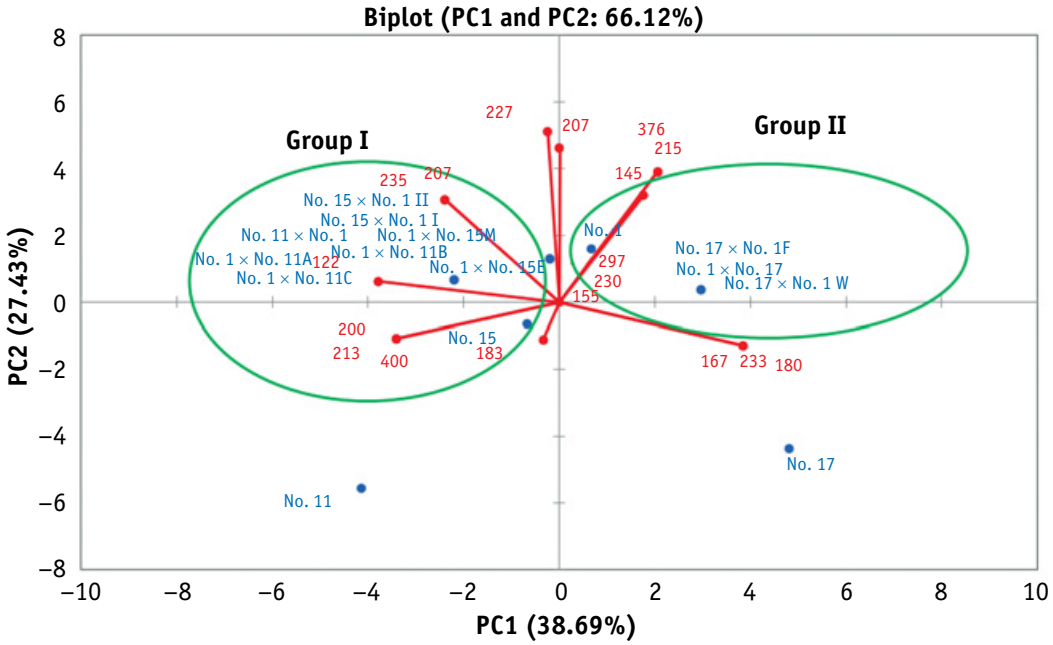

Fig. 4. Results of the PCA of soybean breeding material samples (2024–2025)

between the samples, according to which they were distributed into corresponding cluster groups. In our study, the soybean genotypes examined also formed two groups based on the results of the PCA and the genetic distances obtained by Jacquart, indicating their genetic differences. According to the results obtained [3], the soybean genotypes under study were divided into three groups based on the alleles identified by SSR markers. The authors demonstrated that 28.9% of the variability in the soybean samples studied using SSR markers was explained by the first two principal components. In our study, PC1 and PC2 explained 96.838% of the variability in the genotypes, indicating the high informativeness of the selected molecular markers and the significant effectiveness of the analysis in reflecting genetic differentiation between samples. The results obtained from clustering and PCA are consistent with each other. This contributes to a deeper understanding of the genetic structure of the breeding material, which is important for improving the efficiency with which selection, refinement and cross-planning are carried out in future breeding programmes. The optimal approach is to carry out crosses between representatives of different clusters or groups to expand and maintain genetic diversity.

Thus, the main components revealing 96.838% of the variability in the studied genotypes enabled six markers to be identified for evaluating the effectiveness of hybridisation in the studied soybean breeding samples: AW277661, Satt691, Satt349, Satt680, Satt545 and Satt277. Additionally, based on the absolute values of the eigenvectors of the other principal components and the allele frequencies identified by the Satt177 marker (0.36–0.64), this marker is also promising for determining hybridisation efficiency.

Conclusions

The research showed that seven out of ten SSR markers were polymorphic, enabling the effective identification of hybrid soybean combinations obtained by crossing four parental forms. The polymorphism detected for most of the markers (AW277661, Satt691, Satt349, Satt680, Satt545, Satt277 and Satt177) indicates their high level of informativeness and suitability for evaluating hybridisation. These markers have been found to be useful for establishing heterozygosity in hybrid combinations, providing reliable confirmation of successful crossings. The results confirm the feasibility of using SSR markers to control hybridity and assess genetic relatedness in soybean breeding material.

The results of PCA and genotype clustering were consistent, indicating the analysis's high accuracy and stability. Principal components 1 and 2 together explain 96.838% of the variability, demonstrating the effectiveness of the selected SSR markers in reflecting genetic differentiation between the samples under study. The formation of two main cluster groups and two separate genotypes (No. 11 and No. 17) demonstrates the clear genetic structure of the material under study. Therefore, to increase the effect of heterosis and expand genetic diversity, it is advisable to cross representatives of

the different clusters, ensuring the effectiveness of future breeding programmes.

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Мета. Визначити ступінь генетичного різноманіття селекційного матеріалу сої та дібрати ефективну систему SSR маркерів для оцінювання гібридизації. **Методи.** Селекційні (добір, реципрокні схрещування), молекулярні (ПЛР, електрофорез в агарозному гелі), статистичні (ієрархічна кластеризація, аналіз головних компонент). **Результати.** За результатами проведеного ПЛР аналізу 15 зразків селекційного матеріалу сої за 10 SSR маркерами ідентифіковано від одного до трьох алелів на локус. З них сім маркерів (AW277661, Satt691, Satt349, Satt680, Satt545, Satt277 і Satt177) виявилися поліморфними та продемонстрували здатність розрізняти батьківські форми й гібридні комбінації. У маркерів Satt152, Satt115 і Satt229 встановлено відсутність поліморфізму (частота алелів – 1,00). Це унеможливлює їх застосування для оцінювання ефективності схрещувань. У гібридних комбінаціях, отриманих від батьківських форм № 1 × № 11, № 1 × № 15 та № 1 × № 17, за маркерами Satt349 і Satt691 ідентифіковано по два алелі, що підтверджує наявність гетерозиготності. За коефіцієнтами подібності Жаккарда в діапазоні 0–0,75 досліджувані зразки сформували дві основні кластерні групи та окремий кластер батьківської форми № 11. Одержані результати кластеризації та аналізу головних компонент (ГК) узгоджуються між собою. Перші дві компоненти (ГК1 – 38,687%, ГК2 – 27,432%) пояснюють 96,838% варіабельності вивчених генотипів. Найвищу варіабельність виявлено для маркерів AW277661, Satt691, Satt349, Satt680, Satt545 і Satt277, що свідчить про їхню значну інформативність у відображенні генетичних відмінностей між зразками. **Висновки.** Отримані результати підтверджують ефективність використання SSR маркерів для ідентифікації гібридних комбінацій сої та оцінювання генетичної подібності між батьківськими формами. Визначено, що сім поліморфних маркерів можуть бути рекомендовані для контролю ефективності гібридизації. Узгодженість результатів кластеризації та аналізу головних компонент свідчить про достовірність отриманої структури генетичних взаємозв'язків. З метою посилення ефекту гетерозису та розширення генетичної бази селекційних програм доцільним є здійснення схрещувань між представниками різних кластерних груп. Застосування комплексу інформативних SSR маркерів дає змогу підвищити точність добору батьківських компонентів і прискорити селекційний процес у програмі створення високопродуктивних сортів сої.

Ключові слова: *коефіцієнти подібності; головні компоненти; ДНК-аналіз; алель; ефективність гібридизації.*

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