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ASSESSMENT OF THE GENETIC DIVERSITY IN SUGAR BEET (*BETA VULGARIS* L.) USING SSR MARKERS

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SUMMARY

The genetic diversity and homogeneity of sugar beet (*Beta vulgaris* L.) source lines underwent analysis to select the promising parental pairs for hybridization. The 420 individual plants from 21 sugar beet lines served as research materials. A result of the study of polymorphism with nine SSR markers obtained 22 alleles, with an average of 2.4 alleles per marker. The Bvv155 marker emerged as the most useful for detecting the genetic diversity of sugar beet lines and predicting heterosis. Identifying the FDSB1002, FDSB1007, and FDSB957 markers as polymorphic determined the intra-linear heterogeneity of the source material. The analysis of molecular variance showed that in the studied sugar beet samples, the highest variation was prominent among the populations (48%), while heterogeneity within the population was 21%. The genetic distances between pollinator lines and lines with cytoplasmic male sterility ranged by 1.4–3.5 (Euclidean distances) and 0.12–1.0 (Nei's distances). Distinguishing seven parent pairs of sugar beet attained endorsement for crosses having Nei's genetic distance of D = 0.81 will create highly productive hybrids. The presented results may play a vital role in developing heterotic hybrids in sugar beet through a practical breeding program.

Keywords: Sugar beet (*Beta vulgaris* L.), parental forms and their hybrids, genetic diversity, cluster analysis, genetic distance, intra-linear heterogeneity, microsatellite markers

Key findings: The genetic diversity and homogeneity of sugar beet (*Beta vulgaris* L.) source lines' analysis helped select the promising parental pairs for hybridization. As a result of the polymorphism study with nine SSR markers, 22 alleles emerged, with an average of 2.4 alleles per marker.

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INTRODUCTION

Nowadays, methods that allow the usage of heterosis, i.e., the effect of hybrid vigor or outbreeding enhancement, are widely used in sugar beet (*Beta vulgaris* L.) breeding (Hallahan *et al*., 2018). The primary attention of breeders focuses on developing new highly productive interlinear sugar beet hybrids based on cytoplasmic male sterility (Cheng *et al.*, 2009; Richardson, 2010; Karakotov *et al.*, 2021). In obtaining such heterotic crosses, selecting initial hybridization components was crucial. Therefore, the breeding aimed to acquire constant source lines (CMS lines, sterility fixers, and pollinators) and study their combining ability (Bastaubayeva *et al*., 2022; Bogomolov and Vostrikova, 2022; Nemeata-Alla and Helmy, 2022).

Developing these sugar beet lines and their conservation and maintenance based on economically valuable traits requires profound theoretical and practical development and the use of molecular markers (Moritani *et al.,* 2013; Bogomolov and Vostrikova, 2022). Traditionally aligned lines of sterility fixers and sugar beet pollinators were obtainable by repeated selection of self-pollinated pedigrees based on valuable traits. Annual scrutiny of separation, uniformity, and line sterility has continued in past studies (Zhuzhzhalova *et al.,* 2012; Fedorova *et al.,* 2019).

Classical breeding of sugar beet lines and hybrids based on phenotypic traits is extensive and long-term. Difficulties in breeding and maintaining the genetic uniformity of linear material emerged from the two-year cycle of sugar beet development, inbreeding depression, and the phenomenon of self- and cross-incompatibility (McGrath and Pannella, 2018; Taguchi *et al.,* 2019; Zhuzhzhalova *et al.,* 2020). Parental lines with the best combining ability incurred selection based on productivity, sugar content and harvesting, and disease resistance (Richardson, 2010; Bogomolov and Vostrikova, 2022).

The possibility of using phenotypic traits has limitations with their number, time, and clarity of the genetic expression, which largely depends on the growing conditions and the plant development stages (Cheng *et al.,* 2009; Hallahan *et al.,* 2018). The majority of scientists point out that in addition to assessing phenotypic traits, it was necessary to use molecular markers. With the help of molecular markers, it was possible to reduce the complexity of determining suitable parental lines for crossing (McGrath, 2010; Abbasi *et al.,* 2014; Shilov *et al.,* 2020). The use of molecular marker technology makes it possible to shorten the breeding process and make a reliable assessment of the authenticity of the source lines, their genetic distance, and homogeneity (Bogacheva *et al.,* 2019; Nalbandyan *et al.,* 2020).

An efficient method for studying and exploring genetic diversity is using microsatellite markers. Since these microsatellite markers were evenly distributed in the plant genome and characterized by a specific location on the chromosomes, high variability, accuracy in reproducing results, and a co-dominant type of inheritance, making it possible to detect the homozygous and heterozygous state of the loci (Nachimuthu *et al.,* 2015). This method has previously shown successful use both for studying the genetic diversity of sugar beet and related species and marking the loci associated with economically valuable traits (Li *et al*., 2010; Taški-Ajduković *et al*., 2017; Nalbandyan *et al*., 2020). The latest research aims to assess the genetic diversity and alignment of sugar beet (*Beta vulgaris* L.) lines used as components of hybrids by simple sequence repeat (SSR) analysis.

MATERIALS AND METHODS

Plant material

The study of the genetic diversity and intra-linear homogeneity used 21 source lines and potential components of hybrids of the sugar beet (*Beta vulgaris* L.), i.e., 11 cytoplasmic male sterile (CMS) lines, including introgressive alloplasmic lines with the nuclear genome of sterility fixers, five sterile cytoplasms of wild species of the genus *Beta* L. multi-seeded pollinators, and five maintainer

Table 1. Detailed information of nine microsatellite loci in sugar beet.

Micro-satellite markers	Forward primer (5'-3') Reverse primer (5'-3')	Annealing Temp.	Allele Size (bp)	References
Bvv21	TTGGAGTCGAAGTAGTAGTGTTAT GTTTATTCAGGGGTGGTGTTTG	*53*	250-285	Smulders *et al.* (2010)
Bvv53	CATGTCGAGGAGTGAGTTCAGGAA GTTTCAACTATAGGTGCATCTTTTAC	53	185-200	Smulders *et al.* (2010)
Bvv155	TGCTGACCTTGCAGTTAATAAGTT GTTTCATGTGATGGCTTGCTTTCTAA	53	200-298	Smulders *et al.* (2010)
FDSB957	TCAATCCATCTCTATTCTCTCCG GTCATGGTTGGTCGATCCTT	58	126-158	Laurent *et al.* (2007)
FDSB1001	ACTTCAACCACTATCACAAAGTGAG ATCTTATGCTGCCATGACCA	50-55	308–348	Laurent *et al.* (2007)
FDSB1002	GAAAACGGAGTTCAGTCAGGGA CCTTAAACCTAAAAACGCCAGC	58	143–177	Laurent *et al.* (2007)
FDSB1007	ATTAGAATAGCATCAATTGTGG CCTTATAGTTGGAATTGAGAAA	55	280-296	Laurent *et al.* (2007)
BvGTT1	CAAAAGCTCCCTAGGCTT ACTAGCTCGCAGAGTAATCG	58	120	Viard *et al.* (2002)
SB04	ACCGATCACCAATTCACCAT GTTTTGTTTTGGGCGAAATG	55	192-208	Richards *et al.* (2004)

line for Owen cytoplasmic male sterility (O-type). All lines served as a working collection of the Kazakh Research Institute of Agriculture and Plant Growing LLP. The material has representative samples obtained from the Institute of Bioenergetic Crops and Sugar Beet (Ukraine), Belotserkovskaya experimental station (Ukraine), Kutnovskiy's Sugar Beet Breeding Station (Kutnowska Hodowla Buraka Cukrowego Sp. Z.O.O.) (Poland), All-Russian Research Institute of Sugar Beet and Sugar named after A.L. Mazlumov (Russia) and breeding samples of Kazakh Research Institute of Agriculture and Plant Growing. The study transpired in 2021–2022 within the framework of the Young Scientists grant No. APP09057999.

Research methodology

Genomic DNA isolation from sugar beet seedlings occurred in the phase of the first pair of true leaves using the CTAB (Cetyltrimethylammonium bromide) technique (Murray and Thompson, 1980). The DNA extraction of 20 individual plants from each line ensued, and the total number of plants was 420. PCR analysis utilized an Eppendorf Mastercycler pro amplifier (Germany). In this study, markers of simple repeat sequences (SSR) underwent synthesis by Biolabmix LLC, Novosibirsk, Russia (Table 1). The reaction medium for PCR amplification consisted of 2 µl (50 ng) of test DNA, 2 µl reaction buffer (10 × *TagBuffer* with $[NH_4]_2SO_4$), 1 µl dNTP (4 mM) mixture of four dNTPs, 250 µM of primer, 2 µl (25 mM) $MgCl_2$, 0.3 µl (5u/µl) of *Taq Polymerase* (Biosan LLC, Novosibirsk, Russia), 11.7 µl of sterile, and nuclease-free water (Biotechnology Grade, USA).

Separating amplification products progressed in 8% polyacrylamide gel (Sigma Life Science, USA) stained with ethidium bromide. Visualization of amplification products continued in a gel chamber (Quantum ST 4, France). The DNA marker "Step50" plus (LLC 'Biolabmix,' Novosibirsk, Russia) served as a marker of molecular weights. Identification of the size (bp) of PCR fragments proceeded in the Quantum–ST4 gel-documentation system (France). Analysis of molecular dispersion (AMOVA - Analysis of molecular variance) of components within and between test populations (Peakall and Smouse, 2006), observed (Na) and the effective number of alleles (Ne) (Kimura and Crow, 1964), observed (Ho) and expected heterozygosity (He) (Nei, 1973), Shannon index (I) (Lewontin, 1972), and the Nei's genetic distance (Nei, 1972, 1978) of these SSR markers of sugar

Table 2. Statistics of genetic diversity of SSR markers for sugar beet lines.

SSR markers	N	Na	Ne	I	Ho	He	uHe	F	PIC
Bvv21	420	2	2.00	0.69	0.50	0.50	0.50	0.01	0.28
Bvv53	420	3	3.00	1.10	0.68	0.67	0.67	-0.02	0.14
Bvv155	420	3	3.00	1.10	0.72	0.68	0.67	-0.06	0.23
FDSB957	420	3	3.00	1.10	0.69	0.67	0.67	-0.03	0.40
FDSB1001	420	2	2.00	0.69	0.49	0.50	0.50	0.02	0.41
FDSB1002	420	2	2.00	0.69	0.52	0.50	0.50	-0.04	0.49
FDSB1007	420	2	1.98	0.69	0.47	0.50	0.50	0.05	0.49
BvGTT1	420	2	2.00	0.69	0.49	0.50	0.50	0.01	0.41
SB04	420	3	3.00	1.10	0.67	0.67	0.67	0.00	0.34
Average		2.4	2.44	0.87	0.58	0.57	0.57	-0.01	0.35

N – number of samples; Na – observed number of alleles; Ne – effective number of alleles; I – Shannon's information index; Ho – observed heterozygosity; He – expected heterozygosity; uHe– unbiased expected heterozygosity; F – fixation index; PIC – polymorphic information content.

beet used GenAlEx 6.51b2 software for estimates (Genetic Analysis in Excel) (Peakall and Smouse, 2012). Calculating the index of informativeness of markers PIC (polymorphism information content) employed the formula 1 (Riek *et al.,* 2001).

$$PIC_i = 2f_i (1 - f_i) \qquad (1)$$

Where:
PICi is the polymorphic information content of the marker "I," fi is the frequency of the amplified allele (band is present), and (1-fi) is the frequency of the null allele (band is absent).

The work continued with the percentage of uniformity calculation based on the identity of amplified fragments in 20 individual plants using nine SSR markers. The data used on the lengths of fragments typical of the studied line (occurrence of more than 60%) led to the assessment of the genetic diversity of the observed lines. The results are available in Table 2. Construction of the dendrogram ensued in the R software version 4.1.2 (2021—11-01) "Bird Hippie." Similarity matrices construction used the Euclidean metric. Based on the matrices, calculating clusters utilized the UPGMA method (unweighted pair group method with arithmetic mean) (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Assessment of uniformity

Genetic homogeneity of hybrid components is a valuable feature, as their use allows for obtaining reproducible results – heterotic hybrids. Given the peculiarity of sugar beet as a cross-pollinated crop, parental lines may not be homogeneous and represent a mixture of genotypes, and F_1 hybrids will consist of the composition of plants from different parental combinations. It caused several difficulties in testing and registering sugar beet hybrids (Riek *et al.,* 2001). Genetic uniformity can only be a success by breeding in strict isolation or by vegetative propagation (microclonal reproduction). In this regard, first, our research tasked us to study the genetic homogeneity of the lines planned for use as hybrid components. When assessing the genetic homogeneity of 21 lines (420 plants) using nine markers, we identified 22 loci, with six polymorphic. The quantity of alleles varied from two to three, with an average of 2.4 per locus (Table 2). This polymorphism of markers was significantly lower than in the past findings that also had correlated to the less diverse sugar beet material (Viard *et al.*, 2002; Richards *et al.*, 2004; Laurent *et al.*, 2007; Smulders *et al.*, 2010).

Analysis of each plant showed that heterozygous plants were present among the studied samples. Statistical analysis of SSR marker values in the analysis of 420 individual plants revealed that the expected heterozygosity for SSR markers varied from 0.47 to 0.72 with an average value of 0.58, and the observed heterozygosity varied from 0.50 to 0.68, with an average of 0.57 (Table 2). The average rate of observed heterozygosity and the nine markers were within the same limits as that of 0.54 observed in past studies on sugar beet pollinating lines (Taški-Ajduković *et al.*, 2017; Bastaubayeva *et al*., 2022; Nemeata-Alla and Helmy, 2022). The Shannon index ranged from 0.69 to 1.10 (Table 2).

The observed heterozygosity and Shannon index were the highest for the Bvv155 marker (0.72 and 1.10, respectively). Two polymorphic fragments out of three appeared for this marker. This marker bore highlights as more informative for assessing genetic diversity. An electropherogram of three sugar beet lines using Bvv155 is visible in Figure 1. The PIC value ranged from 0.14 to 0.49, with an average of 0.35. The recorded highest PIC came for FDSB1002 (0.49%), FDSB1007 (0.49%), and FDSB957 (0.40%) markers. These polymorphic markers allowed us to detect heterogeneity within the studied lines for the FDSB1002 marker, on average, up to 72.6%, SB04 up to 79%, FDSB1007 up to 84.5%, and FDSB957 up to 84.3% (Table 4). An electropherogram of five sugar beet lines **(**FMS 1 Rh 184, OP-17232, CMS-16952, CMS-16954-2, and FMS Rh 167) using Bvv155 and FDSB1002 markers appears in Figure 1.

Statistical analysis of molecular variance started using the AMOVA program based on data from 21 sugar beet lines (420 individual plants). The AMOVA procedure provides a general framework for analyzing the genetic structure of a population based on the calculation of a distance matrix. The study used Euclidean distances between individual plant values. The analysis showed that the highest variation in the studied samples was evident among the populations (48%), while the heterogeneity within the population was 21% (Table 3). The difference of individual plants within the samples was 31%. In the presented study, the intra-population variation was lower than in similar studies of pollinators and CMS lines (77.3%) (Taški-Ajduković *et al.,* 2017) and 68% (Abbasi *et al.,* 2014). It indicates a higher alignment of the tested sugar beet lines. The value of Fst = 0.209, according to the Wright classification (1946, 1951, 1965), ranges from 0.15 to 0.25 and indicates a moderate heterogeneity of the studied populations (Table 3).

The estimated overall assessment of the degree of intra-linear homogeneity of the baselines for an average of nine markers in percent (the frequency of occurrence of a typical fragment) was, on average, ranging from 73.3%–95.6%. The average value for all lines was about 84.8% (Table 4). The highest uniformity recording occurred on lines FMS 1 Rh 184 (95.6%), OP-RK (91.7%), OP-17232 (90.6%), OP-17231 (90.6%), and FMS Cr 183 (90%). The FMS 1 Rh 184 line showed high uniformity at 90%–100% level for eight markers, and the FMS Rh 167 and OP-17232 lines for seven (Figure 1a, b). Analysis of the results using AMOVA allows us to detail the values within each population by the sum of squares (Table 3). According to past research, such values can be an estimate of the intra-linear homogeneity of the sugar beet samples (Riek *et al.*, 2001). Ranking the values of the sum of squares was from the minimum to the maximum. In the first place, in terms of uniformity and the percentage assessment, the line of the Polish selection FMS 1 Rh 184 arose, whereas in the last post was the most non-homogeneous line O-type 16955-3. However, the values do not fully match. The result of SSR analysis of intra-linear homogeneity using nine markers for each line, and each marker sets a typical profile – fragments with a high frequency of occurrence.

Assessment of genetic diversity

In the study of homogeneity and identification of typical fragments of SSR loci, compiling a genetic passport materialized. Genetic analysis data allowed us to determine the degree of similarity of the studied samples by determining the genetic distances between

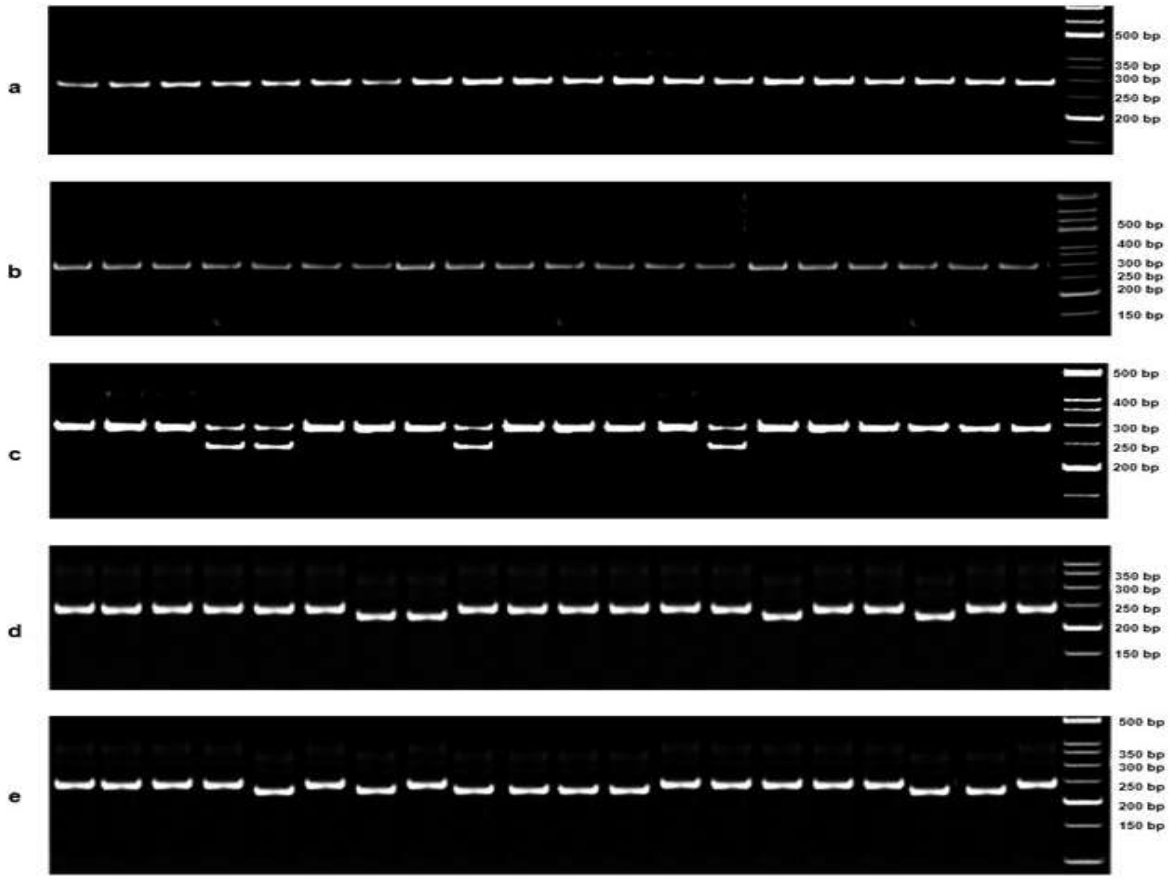

Figure 1. PCR products of 20 individual plants (1–20 plants) of the sugar beet lines using the Bvv155 and FDSB1002 markers. (a - FMS 1 Rh 184, b - OP-17232, c - CMS-16952-1 with the Bvv155 marker. d - CMS-16954-2 and e - FMS Rh 167 with the FDSB1002 marker).

Table 3. Analysis of molecular variance (AMOVA).

Source of variation	d.f.	S.S.	M.S.	Est. Var.	Percentage
Within the Population (Among Populations)	20	664.8	33.2	0.7	21%
Between Populations (Among Individuals)	399	1756.6	4.4	1.7	48%
Within Samples (Within Individuals)	420	447.2	1.1	1.1	31%
Total	839	2868.6		3 .5	100%
Fst	0.209				

Significant at $P \leq 0.001$, d.f. – Degrees of Freedom; S.S. – Sums of Squares; M.S. – Mean Sums of Squares; Est. Var. – Estimated Variation – (estimated deviation/estimated variance); Fst – Coefficient of inbreeding within the subpopulation in relation to the total number.

Table 4. The degree of intra-linear homogeneity and polymorphism of sugar beet samples (%).

Line names	Country of origin	Ploidy	SSR Markers									The average value of the occurrence of typical fragments	SSWP (*sums of squares within each population)*	Range
			Bvv21	Bvv53	Bvv155	FDSB 957	FDSB 1001	FDSB 1002	FDSB 1007	BvGTT1	SB04			
FMS 161	Poland	2n	95/5	100	100	20/80	65/35	20/80	10/90	100	60/10/30	85.6	120,873	19
FMS 162	Poland	2n	60/40	85/15	5/15/80	100	100	20/80	100	100	85/15	87.8	114,575	15
FMS Rh 167	Poland	2n	100	100	10/90/	10/90	100	40/60	45/55	100	90/10	87.2	107,250	9
FMS 173	Poland	2n	60/40	100	85/15	100	95/5	70/30	100	100	85/15	88.3	98,850	5
FMS Cr 183	Poland	2n	100	100	90/10	20/80	75/25	85/15	5/95	100	85/15	90.0	97,325	4
FMS 1 Rh 184	Poland	2n	100	100	95/5	100	85/15	90/10	100	100	90/10	95,6	65,775	1
O-type L53	USA	2n	20/80	100	50/50	100	90/10	40/60	100	100	100	86.7	114,700	16
O-type UK-A	Ukraine	2n	100	100	25/70/5	100	100	40/60	100	100	75/25	89.4	107,425	10
OP-17232	Ukraine	-	100	100	100	60/40	100	35/65	10/90	100	100	90.6	66,860	2
OP-17231	Ukraine	-	100	100	80/20	100	100	75/25	75/25	100	85/15	90.6	110,150	12
OP-GO MM 14044	Russia	2n	85/15	45/40/15	60/40	100	100	30/70	95	100	80/20	81.7	116,075	17
OP-RK	Kazakhstan	2n	100	100	85/15	100	100	90/10	70/30	100	80/20	91.7	102,550	7
OP-UK-A	Ukraine	4n	95/5	90/10	100	60/40	35/65	30/70	85/15	15/85	55/45	78.3	122,875	20
O-type 16950-1	Ukraine	2n	100	100	100	15/85	30/70	30/70	100	80/20	80/20	87.2	70,975	3
CMS-16951-1	Ukraine	2n	95/5	80/20	95/5	30/70	75/25	20/80	95/5	70/30	80/20	82.2	105,350	8
CMS-16952-1	Ukraine	2n	100	90/10	80/20	25/75	40/60	60/40	80/20	80/20	80/20	78.3	108,975	11
O-type 16953-2	Ukraine	2n	100	95/5	85/15	35/65	40/60	30/70	90/10	90/10	65/35	80.0	116,500	18
CMS-16954-2	Ukraine	2n	50/50	90/10	95/5	25/75	25/75	20/80	35/65	90/10	70/30	76.7	113,050	14
O-type 16955-3	Ukraine	2n	95/5	90/10	95/5	65/35	50/50	40/60	45/55	25/75	25/75	73.3	129,850	21
CMS-16956-3	Ukraine	2n	100	100	90/10	35/65	90/10	65/35	60/40	95/5	80/20	82.8	111,550	13
CMS-UK-A	Ukraine	2n	75/25	75/25	100	100	60/40	85/15	75/25	65/35	60/40	77.2	102,275	6
The average value of the occurrence of typical fragments			90.0	92.4	86.9	84.3	81.7	72.6	84.5	91.9	79.0	84.8		

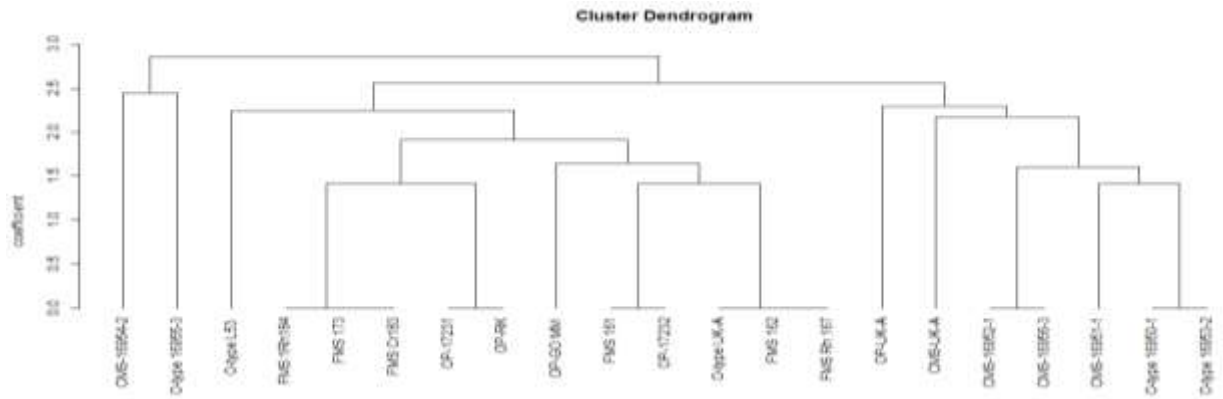

Figure 2. Dendrogram based on the results of SSR analysis of the 21 sugar beet lines.

different hybrid components. The study of genetic relationships between breeding lines helps to select the best combinations for crossing, which ensures the production of highly productive hybrids aligned in all characteristics (Altay *et al.,* 2019; Abekova *et al.,* 2022). As a result, cluster analysis of data construction in the R-program dendrogram developed, reflecting the genetic relationships among the homogeneous sugar beet lines (Figure 2).

Data on nine microsatellite loci helped construct the dendrogram. Based on the analysis of similarities and differences in genetic profiles at a distance of 2.5, the studied sugar beet lines incurred division into three separate clusters. The first cluster was more genetically isolated and included only two lines of Ukrainian origin, CMS-16954-2 and O-type 16955-3. These lines' creation transpired in the Cytogenetics Laboratory at the Institute of Bioenergetic Cultures and Sugar Beet to improve the expansion of the sugar beet culture plasmaphone. New sources of CMS obtained from the wild beet form *Beta maritima* L. originated from Greece, Turkey, and France, with the wild species *Beta patula* (Royik *et al.,* 2013; Kovalchuk *et al.,* 2019). The second cluster united all the lines of CMS of Polish breeding. In the same cluster, separate subclusters included pollinator lines of Russian and Ukrainian selection and the Kazakh pollinator line. The O-type L53 line of American origin, obtained through the N. Vavilov VIR, showed the most isolation in the second cluster. A distinct third cluster contains all other introgressive lines from Ukraine, created using the genetic potential of the wild species *Beta maritima* and *Beta patula* germplasms.

Mathematical processing of the matrix of genetic profiles made it possible to group breeding materials according to the degree of inherited kinship and determine the pairwise genetic distances (Euclidean) of all possible combinations of crosses of the studied parental forms (Table 5) and Nei's distances (Table 6). Euclidean distances were 1.4–3.5, and Nei's were 0.12–1.00. The foremost genetic distances between pollinators and CMS forms according to the Euclidean metric (D = 3.2) occurred for the following combinations of crosses: CMS-16956-3 × OP-17232, CMS-16956-3 × OP-GO MM, CMS-UK-A × OP-GO MM, CMS-16954-2 × OP-17231, CMS-16954-2 × OP-RK, CMS-16952-1 × OP-17232, and CMS-16952-1 × OP-GO MM. Among the Polish CMS lines, genetic distances recorded within 2.8 came between the lines FMS 173, FMS Cr183, FMS 1Rh184, and the pollinator OP-UK-A. A comparative analysis of Nei's distance and Euclidean distances showed that both types of calculation of genetic distances verify each other. The use of these genetic distances and their application to the selection of parent pairs for crossing resulted in past research on sugar beet (Ćurčić *et al.,* 2017; Taški-Ajduković *et al.*, 2017). The research results are vital for sugar beets' practical breeding for developing heterotic hybrids.

Table 5. Euclidean distances between sugar beet line pairs.

Name of the pair	FMS 161	FMS 162	FMS Rh 167	FMS 173	FMS Cr183	FMS 1Rh184	O-type L53	O-type UK-A	OP-17232	OP-17231	OP-GO MM	OP-RK	OP-UK-A	O-type 16950-1	CMS-16951-1	CMS-16952-1	O-type 16953-2	CMS-16954-2	O-type 16955-3	CMS-16956-3
FMS 161	0.0																			
FMS 162	1.4	0.0																		
FMS Rh 167	1.4	0.0	0.0																	
FMS 173	2.0	1.4	1.4	0.0																
FMS Cr183	2.0	1.4	1.4	0.0	0.0															
FMS 1Rh184	2.0	1.4	1.4	0.0	0.0	0.0														
O-type L53	2.4	2.0	2.0	2.4	2.4	2.4	0.0													
O-type UK-A	1.4	0.0	0.0	1.4	1.4	1.4	2.0	0.0												
OP-17232	0.0	1.4	1.4	2.0	2.0	2.0	2.4	14	0.0											
OP-17231	2.4	2.0	2.0	1.4	1.7	1.4	2.0	2.0	2.4	0.0										
OP-GO MM	2.0	1.4	1.4	2.0	2.0	2.0	2.4	1.4	2.0	2.4	0.0									
OP-RK	2.4	2.0	2.0	1.4	1.4	1.4	2.0	2.0	2.4	0.0	2.4	0.0								
OP-UK-A	2.0	2.4	2.4	2.8	2.8	2.8	2.4	2.4	2.0	2.4	2.8	2.4	0.0							
O-type 16950-1	2.8	2.4	2.4	2.8	2.8	2.8	2.4	2.4	2.8	2.4	2.8	2.4	2.0	0.0						
CMS-16951-1	2.4	2.0	2.0	2.4	2.4	2.4	2.0	2.0	2.4	2.0	2.4	2.0	2.4	1.4	0.0					
CMS-16952-1	3.2	2.8	2.8	2.4	2.4	2.4	2.8	2.8	**3.2**	2.0	**3.2**	2.0	2.4	1.4	2.0	0.0				
O-type 16953-2	2.8	2.4	2.4	2.8	2.8	2.8	2.4	2.4	2.8	2.4	2.8	2.4	2.0	0.0	1.4	1.4	0.0			
CMS-16954-2	2.8	2.4	2.4	2.8	2.8	2.8	2.4	2.4	2.8	**3.2**	2.8	**3.2**	2.8	2.0	2.4	2.4	2.0	0.0		
O-type 16955-3	2.4	2.8	2.8	3.2	3.2	3.2	2.8	2.8	2.4	3.5	3.2	3.5	2.4	3.1	3.5	3.5	3.2	2.4	0.0	
CMS-16956-3	3.2	2.8	2.8	2.4	2.4	2.4	2.8	2.8	**3.2**	2.0	**3.2**	2.0	2.4	1.4	2.0	0.0	1.4	2.4	3.5	0.0
CMS-UK-A	2.4	2.8	2.8	2.4	2.4	2.4	2.8	2.8	2.4	2.0	**3.2**	2.0	2.4	2.4	2.0	2.0	2.4	3.2	3.5	2.0

Table 6. Nei's distances between pairs of sugar beet lines.

Name of the pair	FMS 161	FMS 162	FMS Rh 167	FMS 173	FMS Cr183	FMS 1Rh184	O-type L53	O-type UK-A	OP-17232	OP-17231	OP-GO MM	OP-RK	OP-UK-A	O-type 16950-1	CMS-16951-1	CMS-16952-1	O-type 16953-2	CMS-16954-2	O-type 16955-3	CMS-16956-3
FMS 161	0.00																			
FMS 162	0.12	0.00																		
FMS Rh 167	0.12	0.00	0.00																	
FMS 173	0.25	0.12	0.12	0.00																
FMS Cr183	0.25	0.12	0.12	0.00	0.00															
FMS 1Rh184	0.25	0.12	0.12	0.00	0.00	0.00														
O-type L53	0.41	0.25	0.25	0.41	0.41	0.41	0.00													
O-type UK-A	0.12	0.00	0.00	0.12	0.12	0.12	0.25	0.00												
OP-17232	0.00	0.12	0.12	0.25	0.25	0.25	0.41	0.12	0.00											
OP-17231	0.41	0.25	0.25	0.12	0.12	0.12	0.25	0.25	0.41	0.00										
OP-GO MM	0.25	0.12	0.12	0.25	0.25	0.25	0.41	0.12	0.25	0.41	0.00									
OP-RK	0.41	0.25	0.25	0.12	0.12	0.12	0.25	0.25	0.41	0.00	0.41	0.00								
OP-UK-A	0.25	0.41	0.41	0.59	0.59	0.59	0.41	0.41	0.25	0.41	0.59	0.41	0.00							
O-type 16950-1	0.59	0.41	0.41	0.59	0.59	0.59	0.41	0.41	0.59	0.41	0.59	0.41	0.25	0.00						
CMS-16951-1	0.41	0.25	0.25	0.41	0.41	0.41	0.25	0.25	0.41	0.25	0.41	0.25	0.41	0.12	0.00					
CMS-16952-1	0.81	0.59	0.59	0.41	0.41	0.41	0.59	ver	0.81	0.25	0.81	0.25	0.41	0.12	0.25	0.00				
O-type 16953-2	0.59	0.41	0.41	0.59	0.59	0.59	0.41	0.41	0.59	0.41	0.59	0.41	0.25	0.00	0.12	0.12	0.00			
CMS-16954-2	0.59	0.41	0.41	0.59	0.59	0.59	0.41	0.41	0.59	0.81	0.59	0.81	0.59	0.25	0.41	0.41	0.25	0.00		
O-type 16955-3	0.41	0.59	0.59	0.81	0.81	0.81	0.59	0.59	0.41	1.00	0.81	1.00	0.41	0.81	1.00	1.00	0.81	0.41	0.00	
CMS-16956-3	0.81	0,59	0,59	0.41	0.41	0.41	0.59	0.59	0.81	0.25	0.81	0.25	0.41	0.12	0.25	0.00	0.12	0.41	1.00	0.00
CMS-UK-A	0.41	0.59	0.59	0.41	0.41	0.41	0.59	0.59	0.41	0.25	0.81	0.25	0.41	0.41	0.25	0.25	0.41	0.81	1.00	0.25

CONCLUSIONS

The genetic diversity or homogeneity of sugar beet (*Beta vulgaris* L.) source lines' analysis helped select parental pairs for hybridization. The study result of polymorphism with nine SSR markers obtained 22 alleles, with an average of 2.4 alleles per marker. The marker Bvv155, most effective in detecting the genetic diversity of sugar beet lines and forecasting heterosis, brought about its selection. The three indicators, FDSB1002, FDSB1007, and FDSB957, identified as polymorphic, determined the intra-linear heterogeneity of the source material. Based on the AMOVA, in the studied sugar beet lines, the maximum variation was evident among the populations (48%), heterogeneity within the population was 21%, and the difference of individual plants within the samples was 31%. The SSR analysis of 20 individual plants of sugar beet source lines allowed us to determine typical DNA profiles for breeding material. Identifying genetic distances and performing cluster analysis allowed the differentiation of the studied sugar beet source lines into clusters depending on their genetic relationship. The genetic distances among the pollinator lines and lines with cytoplasmic male sterility had ranges of 1.4–3.5 (Euclidean distances) and 0.12–1.00 (Nei's distances). Promising sugar beet parent pairs' identification (CMS-16956-3 × OP-17232, CMS-16956-3 × OP-GO MM, CMS-UK-A × OP-GO MM, CMS-16954-2 × OP-17231, CMS-16954-2 × OP-RK, CMS-16952-1 × OP-17232, and CMS-16952-1 × OP-GO MM) also moved for recommendation for crosses with Nei's genetic distance of D = 0.81 to create highly productive hybrids. The relevant results may play an influential role in developing sugar beet heterotic hybrids through practical breeding in the future.

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