RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
54 (1) 67-78, 2022
http://doi.org/10.54910/sabrao2022.54.1.7
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

ASSESSMENT OF SUGAR BEET GENETIC DIVERSITY IN THE REPUBLIC OF KAZAKHSTAN BY USING RAPD MARKERS AND AGROMORPHOLOGICAL TRAITS

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SUMMARY

Sugar beet is a highly valuable and profitable crop in the Republic of Kazakhstan. It is the only source of raw materials for the production of crystalline sugar and incidentals (tops, bagasse, and molasses). This study aimed to determine the genetic diversity of 53 sugar beet samples, 19 parental lines, and 34 hybrids from Kazakhstan by using random amplified polymorphic DNA (RAPD) markers, agromorphological traits, root mass weight, and sugar content at the Kazakh Research Institute of Agriculture and Plant Growing, Almalybak, Republic of Kazakhstan. The experimental conditions were optimized for the 14 RAPD primers used in this study. The polymorphism index contents varied from 0.24 to 0.46, and all 14 primers were classified as moderately informative. The cluster analysis of RAPD data divided the sugar beet samples into seven groups. The greatest distance (D = 1.4) was noted among the male sterile lines 'MS-1611', 'MS-1631', 'MS-97', and 'MS-2113' and the pollinator lines 'VP-44' and 'VP-23'. The samples were divided into six groups on the basis of root mass weight and sugar content via cluster analysis. The hybrids 'RMS-90', 'RMS-134', 'RMS-133', 'RMS-136', and 'Ramnes' were grouped in a cluster that showed the highest values of root mass weight, which ranged from 610 g to 680 g. However, the samples with high sugar content (18.2–18.5), i.e., 'Shecker', '2198', 'H-22', and '1005', were grouped into a cluster with a distance of D = 0.8. Lines located at a large genetic distance from each other were recommended for hybridization when creating highly productive hybrids. These findings can be applied in the development of new productive and stable sugar beet hybrids in Kazakhstan.

Keywords: Genetic diversity, markers, RAPD, root mass weight, sugar content, sugar beet

Key findings: This investigation showed the results of 53 samples of sugar beet lines and hybrids from the collection at the Kazakh Research Institute of Agriculture and Plant Growing. These results were obtained by using 14 RAPD primers, root mass weight, and sugar content. The results of polymorphism and cluster analyses may be used to create sugar beet crossing schemes.

Communicating Editor: Prof. Dr. Clara R. Azzam
Manuscript received: October 20, 2021; Accepted: December 23, 2021.

To cite this manuscript: Abekova AM, Yerzhebayeva RS, Bastaubayeva SO, Konusbekov K, Bazylova TA, Babissekova DI, Amangeldiyeva AA (2022). Assessment of sugar beet genetic diversity in the Republic of Kazakhstan by using RAPDmarkers and agromorphological traits. *SABRAO J. Breed. Genet.* 54(1): 67-78. http://doi.org/10.54910/sabrao2022.54.1.7

INTRODUCTION

Sugar, the common name for sucrose. is obtained from only two crops: cane and beet. Cane sugar has been produced in large quantities in tropical regions for many centuries and continues to dominate the world's supply of sugar. By contrast, sugar beet (*Beta vulgaris* L.) is a relatively new crop that appeared in temperate regions in the 19th century and spread widely only in the 20th century (Draycott, 2008, Wang *et al*., 2019). Sugar beet is currently cultivated in approximately 50 countries and supplies approximately a quarter of the 140 million tonnes of sugar currently used annually.

Sugar beet is an economically important crop in the temperate zones of the world and is grown mostly for sugar production (Ellerton, 1980). Only 25% of the world's sugar is produced from sugar beet (Draycott, 2008; Bezhin 2019). However, sugar beet is the main source of sugar production in the RK. In Kazakhstan, sugar beet cultivation is one of the priority areas for agricultural development, and the main sugar beet-growing regions are Almaty and Zhambyl (Urazaliev *et al*., 2013; Yerzhebayeva *et al*., 2019). In Kazakhstan, breeding research, including studies on the crossing, selection, and seed production of sugar beet lines, is carried out at the Kazakh Research Institute of Agriculture and Plant Growing (KRIAPG), which is located in Almalybak Village in the Almaty region, Kazakhstan. The main objective of sugar beet breeding in Kazakhstan is to create highly productive hybrids with high sugar content (Kornienko *et al*., 2011).

The highland climate of the Almaty region is favorable for sugar beet cultivation and hybrid development, as well as for the assessment of the field performance of different sugar beet genotypes under existing climatic conditions. Thus far, 15 sugar beet hybrids have been developed by KRIAPG, and eight have been approved for cultivation in Kazakhstan. Genetic diversity studies play an important role in the selection of parental lines for the best cross combinations and increasing the chances of combining the desirable traits into a single superior hybrid (Taški-Ajduković *et al*., 2017). However, the sugar beet breeding studies at KRIAPG are generally very limited due to narrow genetic diversity that is caused by the small population of the crop (Kornienko *et al*., 2014) and the lack of the availability of sugar beet germplasm in the genetic bank of the institute.

Many kinds of DNA markers have been used for the genetic analysis of sugar beet populations. These markers include simple sequence repeats (SSRs) (Li *et al*., 2010; Simko *et al*., 2012; Fugate *et al*., 2014; Ribeiro *et al*., 2016), amplified fragment length polymorphisms (AFLPs) (Schondelmaier *et al*., 1996), restriction fragment length polymorphisms (RFLPs) (Barzen *et al*., 1992), randomly amplified polymorphic DNA (RAPD) (Uphoff and Wricke, 1995; Budak *et al*., 2004; Izzatullayeva *et al*., 2014; De Lucchi *et al*., 2021), intersimple sequence repeats (ISSRs) (Budak *et al*., 2004; El-Mouhamady *et al*., 2021), and single nucleotide polymorphisms (SNPs) (Simko *et al*., 2012; Stevanato *et al*., 2014; Ries *et al*., 2016).

The analysis of genetic diversity and the certification of valuable hybrids and cultivars are the prerequisites for the successful conservation and commercial utilization of various crop plants. Therefore, research on this area is also crucial for protecting breeding achievements and breeders; rights. A protected environment encourages breeders to create new and improved genotypes of various crop species, which eventually contribute to food security (Liu and Muse, 2005). DNA based-markers are more informative, stable, and reliable than pedigree and morphological markers (DeLaporta *et al*., 1983) and are mainly used to study the genetic diversity of different crop species, including sugar beet (Yu, 1992; Lörz and Wenzel, 2005).

RAPD markers have been used for genetic diversity studies on sugar beet and cultivar development (Zhang *et al*., 2016; Monteiro *et al*., 2018; Norouzi *et al*., 2018). This technique is based on the amplification of genomic DNA segments via the polymerase chain reaction (PCR) by using primers with arbitrary sequences (Marić *et al*., 2008; Limanskaya *et al*., 2017; Moritani *et al*., 2013). It is considerably faster and simpler than other molecular genotyping techniques that are widely used for crop genetic diversity studies (Amini *et al*., 2008). The informative value of RAPD profiles in assessing the genetic diversity of sugar beet populations has been extensively reported (Uphoff and Wricke, 1995; Ghasemi *et al*., 2014; Izzatullayeva *et al*., 2014; Bogacheva *et al*., 2020). However, in Kazakhstan, only a few reports are available on genetic diversity studies on sugar beet using DNA markers (Abekova *et al*., 2017). This study aims to assess the genetic polymorphism of sugar beet line material and hybrids of

Table 1. Sugar beet genotypes with their origin, sugar content, and root mass weight.

No.	Art. number	Origin/name	Hybrid/ line	Ploidy	Country of origin	Root mass weight (g)	Sugar content (%)
1	2137	(BC MS × VP -23) RC / Aisholpan	hybrid	diploid	Kazakhstan- Ukraine	640 ± 52.3	18.2 ± 0.28
2	2120	KazMSF1 × VP-24 RC	hybrid	diploid	Kazakhstan	490 ± 48.4	16.7 ± 0.22
3	2201	(SOAN-38 × SOAN98) YAN-10	hybrid	diploid	Kazakhstan	550 ± 49.7	16.9 ± 0.19
4	2210	Lenuron × VP44	hybrid	diploid	Kazakhstan	610 ± 46.4	17.1 ± 0.23
5	2229	(Kaz MS F1× H-22) SINT-1	hybrid	diploid	Kazakhstan	620 ± 38.3	18 ± 0.22
6	2247	MS Denok × SOAN-22	hybrid	diploid	Germany	660 ± 44.3	17.6 ± 0.21
7	2256	(KazMSF1 × SOAN-22) SINT-1	hybrid	diploid	Kazakhstan	450 ± 42.2	17.5 ± 0.24
8	2280	Uman MS × VP-24 / Sheker	hybrid	diploid	Kazakhstan	480 ± 46.7	18.5 ± 0.20
9	2232	MS09 F1	hybrid	diploid	Kazakhstan	475 ± 47.9	17.4 ± 0.24
10	1014	KazMS F1 SOAN 22. D × SOAN 22 / KazSib14	hybrid	diploid	Kazakhstan	480 ± 42.3	17.9 ± 0.25
11	2245	(MS F1 × H22) SOAN – 98 / Aksu	hybrid	diploid	Kazakhstan	590 ± 58.4	18.3 ± 0.27
12	1005	MS F × 916 -Yalt 740S × B24	hybrid	diploid	Kazakhstan	490 ± 51.5	18.2 ± 0.26
13	2198	KazMS F1 × SOAN-22	hybrid	diploid	Kazakhstan	460 ± 56.7	18.4 ± 0.27
14	2227	Iris A-1	line	triploid	Kazakhstan	320 ± 38.7	18.5 ± 0.22
15	2287	Lenora A2	line	triploid	Kazakhstan	470 ± 44.7	17.5 ± 0.21
16	2251	Roksan A2	line	diploid	Kazakhstan	400 ± 49.6	16.9 ± 0.22
17	2235	P09-20-06	line	diploid	Kazakhstan	550 ± 48.7	17.6 ± 0.24
18	2216	SOAN-5	line	diploid	Russia	670 ± 42.3	17.5 ± 0.29
19	2221	SEM	line	diploid	Russia	490 ± 41.4	17.0 ± 0.27
20	2318	Po-117	hybrid	diploid	Russia	560 ± 49.3	18.2 ± 0.23
21	2317	RMS-60	hybrid	diploid	Russia	380 ± 58.3	17.1 ± 0.27
22	2320	RMS-90	hybrid	diploid	Russia	680 ± 48.3	16.3 ± 0.26
23	2319	RMS-133	hybrid	diploid	Russia	640 ± 54.2	15.3 ± 0.25
24	2321	RMS-134	hybrid	diploid	Russia	670 ± 58.6	16.5 ± 0.19
25	2330	RMS-135	hybrid	diploid	Russia	620 ± 47.2	17.5 ± 0.18
26	2331	RMS-136	hybrid	diploid	Russia	610 ± 47.3	15.7 ± 0.17
27	2322	Ramnes	hybrid	diploid	Russia	630 ± 58.8	15.2 ± 0.14
28	2324	Final	hybrid	diploid	Russia	400 ± 41.6	16.5 ± 0.19
29	2325	Smena	hybrid	diploid	Russia	390 ± 51.3	17.7 ± 0.18
30	2327	Kubanskiy MS – 95	hybrid	diploid	Russia	450 ± 41.5	18.0 ± 0.21
31	2328	Uspekh	hybrid	diploid	Russia	430 ± 42.9	16.6 ± 0.17
32	-	MS-2113 × GO MM (14044+15676) /Ruslan	hybrid	diploid	Russia	490 ± 48.4	16.3 ± 0.19
33	2332	Barskiy	hybrid	triploid	Russia	640 ± 49.7	18.0 ± 0.20
34	-	OP GO MM (14044+15676)	line	diploid	Russia	580 ± 38.6	18.3 ± 0.22
35	2329	MS 2113	hybrid	diploid	Russia	630 ± 48.3	17.5 ± 0.21
36	2335	MS-7	hybrid	diploid	Russia	550 ± 41.3	17.6 ± 0.22
37	2336	MS-1949	hybrid	diploid	Russia	530 ± 45.1	17.3 ± 0.22
38	2291	MS-97	hybrid	diploid	Ukraine	380 ± 46.2	17.5 ± 0.24
39	2333	MS-1611	hybrid	diploid	Ukraine	580 ± 37.3	17.7 ± 0.25
40	2334	MS-1631	hybrid	diploid	Ukraine	540 ± 44.7	18.8 ± 0.24
41	2339	MS-1633	hybrid	diploid	Ukraine	600 ± 46.2	18.3 ± 0.23
42	2340	MS-1638	hybrid	diploid	Ukraine	380 ± 48.3	17.5 ± 0.20
43	2165	VP-23	hybrid	diploid	Ukraine - Kazakhstan	560 ± 38.4	17.2 ± 0.28
44	2243	VP-44	hybrid	diploid	Ukraine - Kazakhstan	480 ± 58.2	17.7 ± 0.29
45	-	VP-29	hybrid	diploid	Ukraine - Kazakhstan	-	-
46	2223	H-22	hybrid	diploid	Ukraine - Kazakhstan	480 ± 38.3	18.2 ± 0.24
47	2289	Aleksandria	hybrid	triploid	Ukraine	560 ± 47.7	17.6 ± 0.27
48	2298	Olzhich	hybrid	triploid	Ukraine	540 ± 48.6	17.3 ± 0.28
49	2280-1	Zluka	hybrid	triploid	Ukraine	490 ± 38.4	17.6 ± 0.29
50	2309	Kozak	hybrid	diploid	Ukraine	520 ± 58.3	17.6 ± 0.22
51	2307	Patriot	hybrid	diploid	Ukraine	480 ± 42.6	17.6 ± 0.24
52	KK-71	Svetlana	hybrid	diploid	Kirghizia	495 ± 45.5	17.9 ± 0.27
53	2342	Roksan	hybrid	diploid	France	510 ± 44.3	17.6 ± 0.21
	$\bar{x} \pm \sigma$					17.5±0.8	

various origins on the basis of RAPD profiles, root mass weight, and sugar content levels and explore the possible applications of these data.

MATERIALS AND METHODS

Breeding material

This study on sugar beet was carried out at the Kazakh Research Institute of Agriculture and Plant Growing, Almalybak, Republic of Kazakhstan. Fifty-three sugar beet samples were provided by the KRIAPG gene bank for this study. This collection included 15 samples of Kazakhstani breeds, 33 samples from various foreign collections, and five samples of Kazakhstani–Ukrainian breeds. The Kazakhstani samples included 11 diploid hybrids, two diploid lines, and two triploid lines. The samples from foreign collections comprised 18 diploid hybrids, four triploid hybrids, and 11 diploid lines. Four of the Kazakhstani–Ukrainian samples were hybrids and one was a line. Both the hybrids and the line were diploid. The detailed list is presented in Table 1. Most of the foreign samples were provided by Russian and Ukrainian Research

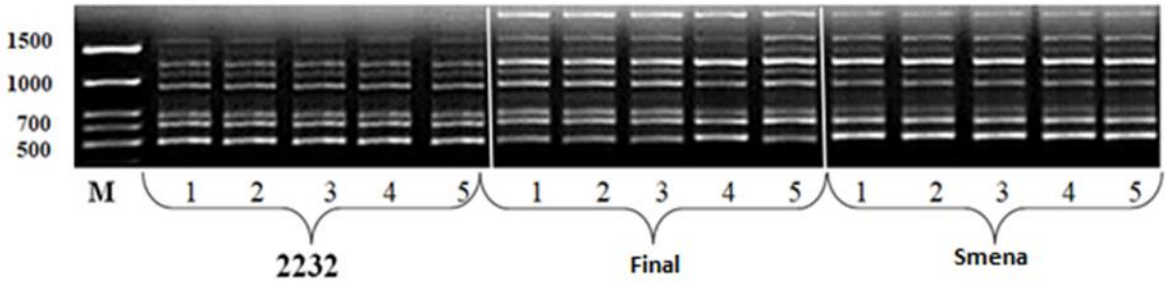

Figure 1. Electrophoretic profile of PCR products obtained by using the RAPD marker OPC-06 for five individual plants of three sugar beet hybrids.

Institutions, specifically by the Federal State Budgetary Scientific Institution "All-Russian Scientific Research Institute of Plant Protection" (St. Petersburg–Pushkin, Russia), Federal State Budgetary Scientific Institution "All-Russian Scientific Research Institute of Sugar beet and Sugar Named after A.L. Mazlumov" (Voronezh Region, Russia), Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia), and Institute of Bioenergy Crops and Sugar Beet (Kyiv, Ukraine).

DNA isolation

Genomic DNA was isolated from sugar beet seedlings after the emergence of the first set of true leaves (DeLaporta *et al*., 1983). DNA was isolated from the leaves of a pool of 10 plants with minimal intravarietal variation. Hybrid DNA was represented by a mixture of DNA isolated from the leaves of five plants isolated on the basis of homogeneity after studying the intravarietal variation of 15 plants (Figure 1).

The quality of the isolated DNA was determined through electrophoresis on 1% agarose gel and staining with ethidium bromide. DNA concentration was measured via a spectrophotometric method based on the ratio of wavelengths at the maximum photometric absorption of nucleic acids at 260 nm and that of proteins at 280 nm.

PCR and RAPD analysis

PCR was performed with an Eppendorf Mastercycler pro-amplifier (Germany). The sequences of the primers (synthesized by LLC Biolabmiks, Russia, Novosibirsk) used for RAPD analysis and the PCR conditions are presented in Table 2.

Table 2. RAPD primers used in the study

Markers	Nucleotide sequence	PCR conditions
OPA-09	GGGTAACGCC	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C- 40 s, 72 °C- 40 s), 72 °C-5 min
OPA-10	GTGATCGCAG	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C- 40 s, 72 °C- 40 s), 72 °C-5 min
OPA-19	CAAACGTCGG	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C- 40 s, 72 °C- 40 s), 72 °C-5 min
OPB-18	CCACAGCAGT	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C- 40 s, 72 °C- 40 s), 72 °C-5 min
OPC-06	GAACGGACTC	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C- 40 s, 72 °C- 40 s), 72 °C-5 min
OPD-03	GTCGCCGTCA	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C- 40 s, 72 °C- 40 s), 72 °C-5 min
OPE-01	CCCAAGGTCC	94 °C-3 min, 35 cycles (94 °C-40s, 50 °C- 40 s, 72 °C- 40 s), 72 °C-5 min
OPE-12	TTATCGCCCC	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C- 40 s, 72 °C- 40 s), 72 °C-5 min
OPP-17	TGACCCGCCT	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C- 40 s, 72 °C- 40 s), 72 °C-5 min
OPP-18	GGCTTGGCCT	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C- 40 s, 72 °C- 40 s), 72 °C-5 min
PAWS 5	AACGAGGGGTTCGAGGCC	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C-40 s, 72 °C-40 s), 72 °C-10 min
PAWS 6	GAGTGTCAAACCCAACGA	94 °C-3 min, 40 cycles (94 °C-1 min, 48 °C-1 min, 72 °C-1 min), 72 °C-4 min
PAWS 16	ACCTCTGCGCTTGGAGGC	94 °C-3 min, 35 cycles (94 °C- 40 s, 50 °C-40 s, 72 °C-401 c), 72 °C-5 min
PAWS 17	CTACACGGACTGGGTCCG	94 °C-3 min, 35 cycles (94 °C-40 s, 50 °C- 20 s, 72 °C-1 min), 72 °C- 3 min

The PCR reaction mix consisted of 2 µl (50 ng) of the isolated DNA, 2 µl of the reaction buffer (10× TagBuffer c $(NH_4)_2SO_4$), 1 µl of a mixture of four dNTPs (4 mM, Biosan, Novosibirsk, Russia), 250 µM primer (Biosan, Novosibirsk, Russia), 2 µl of 25 mM $MgCl_2$, 0.3 µl (5u/µl) of *Taq*-polymerase (Biosan, Novosibirsk, Russia), and 11.7 µl of sterile nuclease-free water. Amplification products were separated on 1.2%–1.5% agarose gels (Sigma Life Science, USA) stained with ethidium bromide and visualized in a Quantum ST4 gel documenting chamber (Vilber, Lourmat, Collégien, France). The DNA markers Step50 plus, Step100 (Biolabmix, Russia, Novosibirsk), and 3000 bp O'GeneRuler Ultra Low Range (Thermo Scientific, USA) were used for the molecular weight determination of the PCR fragments.

On the basis of molecular genetic analysis, binary matrixes were compiled for each primer, in which the presence (1) or absence (0) of fragments with the same molecular weight on the electrophoregram was noted. Each RAPD fragment was considered as a separate genetic locus. The polymorphism level for each primer was determined by using the fraction of polymorphic loci out of the total number of loci per primer expressed as a percentage.

The polymorphism information content (PIC) of RAPD markers was calculated by using the formula:

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

where PIC_i is the polymorphic information content of the "i" marker, f_i is the frequency of the amplified allele (the band is present), and (1 − fi) is the frequency of the null allele (Rold´an-Ruiz *et al*., 2000).

Root mass weight and sugar content

For the determination of root mass weight and sugar content, the sugar beet plants were grown in a field with light chestnut-type soil at the Field Station of KRIAPG from 2018 to 2020. The station is located in the foothill zone of Zailiyskiy Alatau (43° N, 77° E, 740 m above sea level). Three irrigations were carried out at an interval of 30–38 days during the growing seasons (1000–1250 m^3/ha).

Each sugar beet sample was sown in a replicated plot with an area of approximately 3.6 m^2. Harvesting was carried out manually in mid-October after loosening roots with a plow. Root mass weight was assessed in accordance with the method of Apasov *et al*. (2018). Sugar content (%) was determined by using a hand-held ATAGO PAL-1 refractometer (Saitama, Japan). The mean ($\bar{x}$) and standard deviation (σ) values shown in Table 1 were calculated by using Excel.

Dendrogram analysis

The dendrogram was built in R (software version 4.0.3), using dist () and hclust () functions of the stats package. Similarity matrices were constructed using the dist () function's "binary" distance measure for the RAPD analysis data and Euclidean distance measure for the sugar content (%) and root mass weight (g) data. Based on these matrices, clusters were calculated using the "ward.D2" algorithm of the hclust () function.

RESULTS

RAPD analysis

A total of 114 amplified DNA fragments were observed in 53 sugar beet genotypes by using 14 RAPD primers (Table 3). The sizes of these amplified fragments mainly varied between 200 and 2000 bp (Figure 2). Among the 114 fragments, 86 were monomorphic and 28 were polymorphic. The average number of evaluated bands per primer was 8.1, and the average number of polymorphic fragments per primer was 2.0. The PIC values reflecting marker polymorphism were in the range of 0.24–0.46 (Table 3).

Three markers, namely, OPP-18, OPB-18, and OPC-06, produced the largest number of amplicons at 10 for each primer, and the numbers of their RAPD profiles were equal to 4, 2, and 4, respectively. Thus, the highest polymorphism rate was recorded for these primers: OPP-18÷ - 40%, OPC-06÷ - 40%, and OPA-19÷ - 37.5% (Table 3).

The difference between the dendrograms of the markers with low and high levels of polymorphism is shown in Figure 3. Two markers in the current study (OPE-12 and OPB-18) demonstrated different levels of polymorphism. Figure 3 (a) shows a dendrogram of the OPE-12 marker with a low level of polymorphism in 53 sugar beet samples. Marker OPB-18 demonstrated a high level of polymorphism with the same samples as can be seen in Figure 3 (b). The remaining markers exhibited a sufficient level of polymorphism.

Table 3. Analysis of 53 sugar beet genotype samples using RAPD markers.

Primers	PCR fragment size (bp)	Number of amplified fragments	Number of polymorphic fragments	Polymorphism level (%)	PIC
OPA-09	317–1684	8	2	25	0.35
OPA-10	287–2156	9	2	22	0.37
OPA-19	439–1843	8	3	37.5	0.37
OPB-18	229–1761	10	2	20	0.39
OPC-06	374–2183	10	4	40	0.42
OPD-03	200–1683	9	1	11	0.44
OPE-01	116–2152	8	2	25	0.41
OPE-12	395–1514	3	0	0	0.26
OPP-17	264–1716	8	1	12.5	0.25
OPP-18	200–2163	10	4	40	0.46
PAWS 5	125–1838	8	2	25	0.35
PAWS 6	228–2595	8	2	25	0.34
PAWS 16	369–2220	6	2	33	0.38
PAWS 17	177–1396	9	1	11	0.24
Average	-	8.1	2.0	21.9	0.4

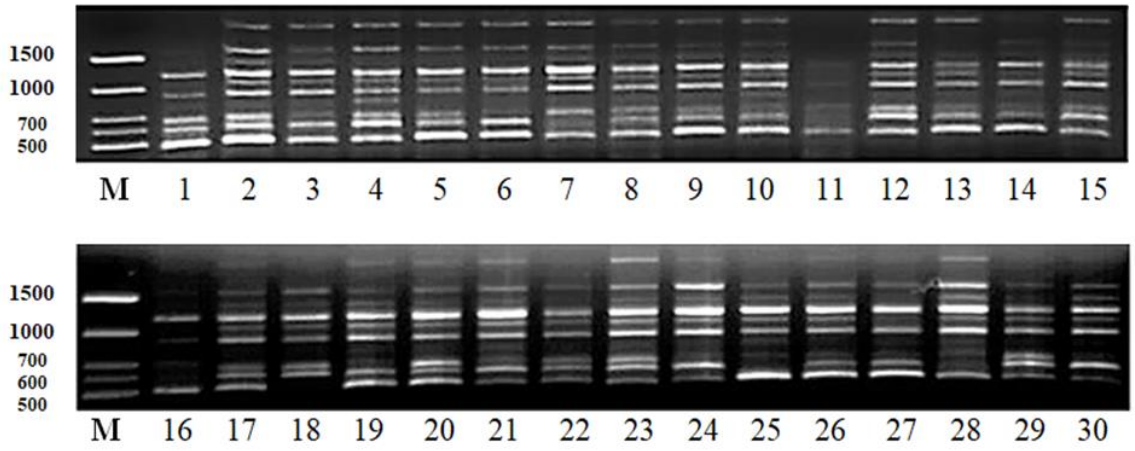

Figure 2. Electrophoretic profile of PCR products obtained by using the RAPD marker OPC-06. The samples were loaded in the following sequence: Marker–step 50. 1. MS-7, 2. MS-1949, 3. Aksu, 4. 2120, 5. 1005, 6. 2198, 7. COAH-5, 8. RMS 90, 9. RMS 134, 10. Roksan, 11. VP 29, 12. H-22, 13. Patriot, 14. KazSib 14, 15. Kozak, 16. Svetlana, 17. Olchizh, 18. Zluka, 19. Ro117, 20. RMS 133, 21. Ramnes, 22. 2232, 23. Final, 24. Smena, 25. Kubanskiy MS-95, 26. Uspekh, 27. MS 2113, 28. GO MM (14044+15676), 29. Ruslan, 30. RMS 135.

The polymorphism of primers OPP-18 and OPC-06 in sugar beet has been reported previously (Ghasemi *et al*., 2014). In accordance with the classification of Botstein *et al*. (1980), highly informative primers included those with PIC ≥ 0.5, medium informative ones having a PIC value within in the range of 0.5–0.25, and lowly informative ones had PIC ≤ 0.25. According to the present research, all the markers used in the genetic analysis were medium informative.

Clustering analysis of RAPD data

Clustering analysis was carried out, and a dendrogram was constructed on the basis of the results of RAPD analysis. The dendrogram showed the similarity of 53 sugar beet genotypes. In accordance with the binary similarity measure, the sugar beet samples were divided into seven clusters at a distance of 0.7 (Figure 4).

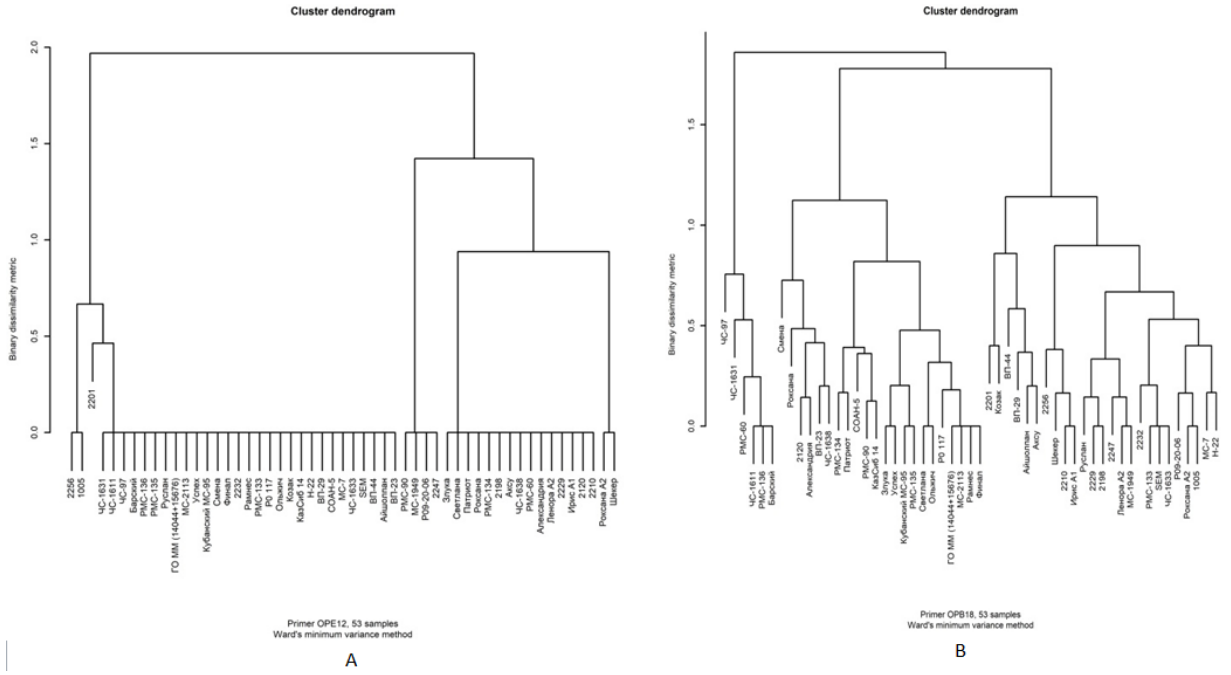

Figure 3. Dendrogram of RAPD analysis for individual markers. a. marker OPE-12, b. marker OPB-18. The comparison shows the difference in the clustering of markers with a low level of polymorphism (e.g., OPE-12) and markers with a high level of polymorphism (e.g., OPB-18).

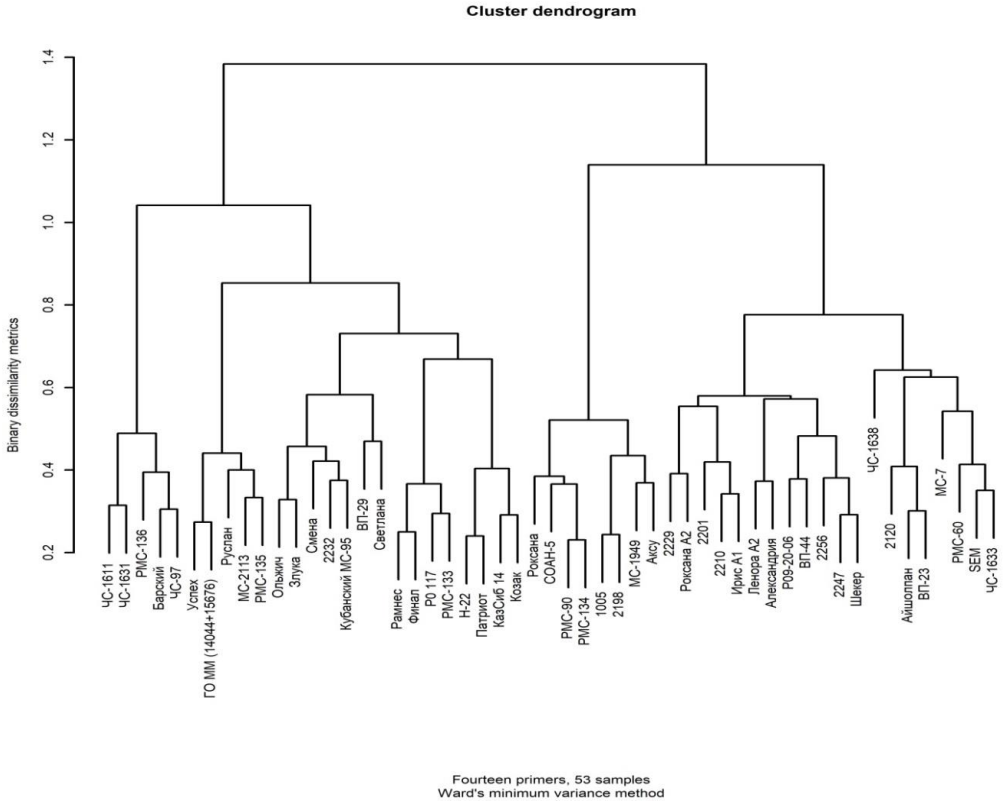

Figure 4. Dendrogram showing the dissimilarity among 53 sugar beet genotypes based on RAPD analysis.

Clustering based on RAPD data has shown its informativeness and applicability in assessing the kinship and similarity of sugar beet samples. The hybrid 'Aisholpan' (RK-Ukraine) with its component pollinator 'VP-23' was located in one cluster at a distance of 0.3. According to the dendrogram, the hybrid 'Ruslan' and its parent forms 'OP MM' (14044 + 15676) and 'MS 2113' were found in one cluster at a distance of 0.45. The male-sterile lines 'MS 1611,' 'MS 1631,' and 'MS 97' obtained from the Institute of Bioenergy Crops and Sugar Beet, Kyev, Ukraine, combined into one cluster at a distance of 0.48 (Figure 4).

After the agromorphological assessment of the collected samples, the lines with the highest sugar content and root mass weight were indicated. The comparison of the data on the best-performing lines with their position on the cluster tree highlighted four samples ('MS-1631', 'OP GO MM,' 'H-22', and 'MS 2113') from one large cluster and three samples ('Iris A-1,' 'SOAN-5', and 'MS-1633') from the other cluster. These large clusters joined at a binary dissimilarity measure of 1.4.

Cluster analysis results can be applied when drawing up schemes of sugar beet crosses. General recommendations suggest hybridizing lines that are genetically distant from each other for creating highly productive hybrids (Fedulova *et al*., 2016; Bogacheva *et al*., 2019; Nalbandian *et al*., 2020). The greatest distances (D = 1.4) were noted among the male-sterile lines 'MS 1611,' 'MS 1631,' 'MS 97,' and 'MS 2113' and the pollinator lines 'VP-44' and 'VP-23.'

Root mass weight and sugar content

The root mass weight of a sugar beet genotype is one of the main indicators of its productivity. Putulina (2018) stated that morphobiological traits, such as root weight and size, are important for the producibility and suitability of root crops for mechanical work. The average root weight of the sugar beet genotypes was 533 ± 88.3 g and varied from 380–680 g across different genotypes (Table 1). The top five sugar beet samples with the highest averages of root mass weight were 'RMS-90' (680 g), 'RMS-134' (670 g), 'SOAN-5' (670 g), '2247' (660 g), and 'Aisholpan' (640 g). The highest sugar content of 18.5% was found in the male sterile line 'MS-1631' (18.8%), the apozygotic line 'Iris A-1' (18.5%), the hybrid 'Sheker' (18.5%), and '2198' (18.5%) (Table 1).

Cluster analysis was carried out on all of the sugar beet genotypes by using the average values of the two productivity indicators, i.e., root mass weight and sugar content. In accordance with the Euclidean similarity metric at the distance D = 3, the sugar beet samples were divided into six clusters (Figure 5). The first cluster included the samples of sugar beet genotypes that showed relatively low values of sugar content and root mass weight (Smena, Final, SEM, Ruslan, 2120) under the environmental conditions of the Almaty region, Kazakhstan. The hybrids of the Russian selection i.e., 'RMS-90,' 'RMS-134,' 'RMS-133,' 'RMS-136,' and 'Ramnes', were allocated into a separate cluster that showed high root mass weights of 610 g to 680 g. The sugar beet genotypes with high sugar content were 'Shecker', '2198', 'H-22', and '1005'. Their sugar contents ranged from 18.2% to 18.5%. They also gathered in a single cluster at a distance of D = 0.8.

DISCUSSION

The primer OPC-06 displayed the highest polymorphism rate of 40%. The utility of this primer for the genotyping of sugar beet samples has also been demonstrated in earlier studies (Schneider *et al*., 2007; Ghasemi *et al*., 2014). In this study, almost all of the used markers were classified as medium informative on the basis of the definition of Botstein *et al*. (1980).

The sugar beet lines obtained from various sugar beet stations in Veselopodolyansk, Yaltushkovsk, and Belotserkovsk in Ukraine and from the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences have considerably contributed to the development of many hybrid cultivars used in this study. The pollinators, i.e., 'VP-23', 'VP-24', 'VP-29', and 'N-22', obtained from the Stations of Veselopodolyanskaya and Nemerchanskaya (Ukraine) have been used with the male sterile lines 'Uman MS' and 'BC MS' of Kazakhstan for the creation of hybrids. The lines 'SOAN-22', 'SOAN-38', and 'SOAN-98' belong to the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences. They have been used as stabilizers of monogermity and exhibit high sugar contents.

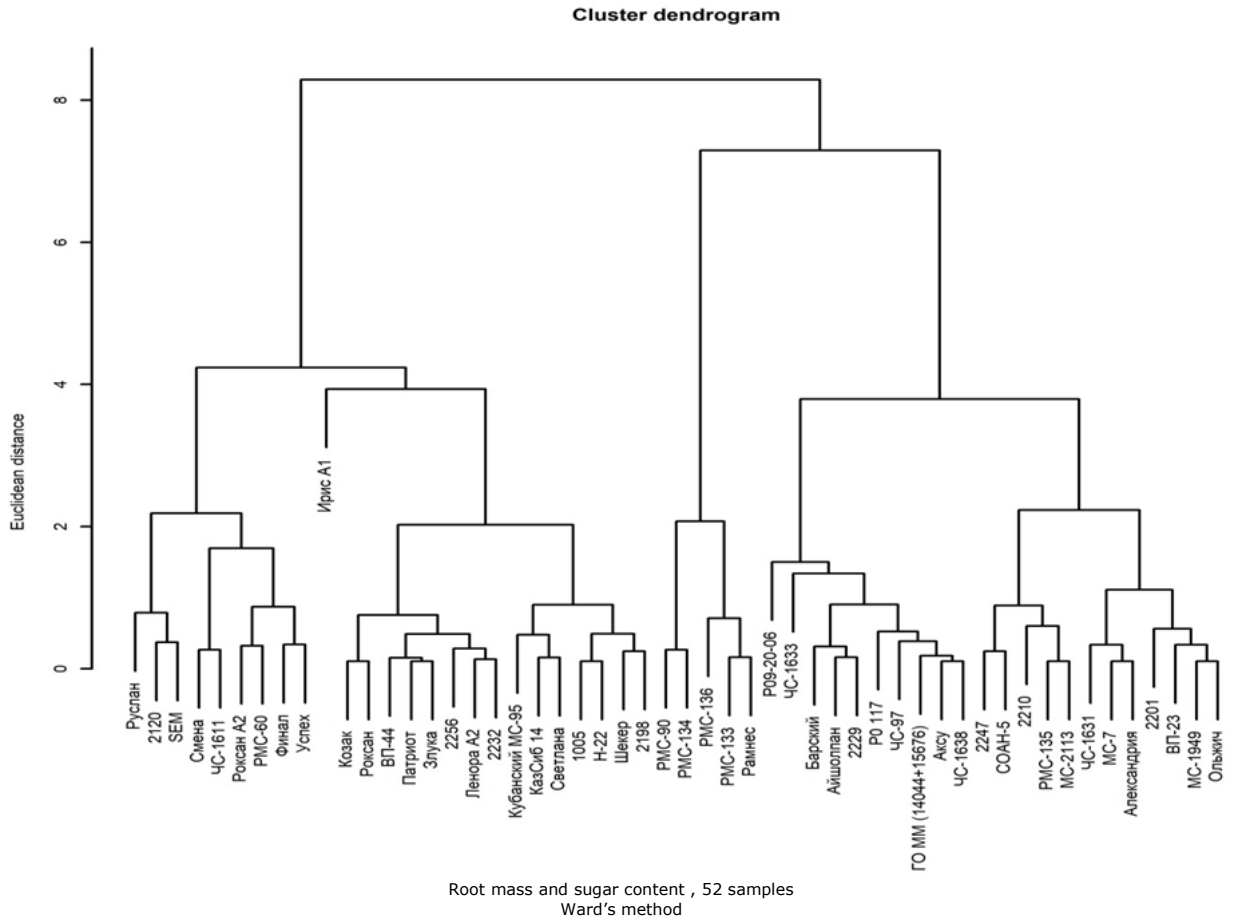

Figure 5. Dendrogram showing the relative geometric distance among 53 sugar beet genotypes based on root mass weight and sugar content.

The clustering of RAPD data analysis showed informative value and applicability for assessing the relationship among sugar beet genotypes. For example, the cluster containing the 'Aisholpan' hybrid (RK-Ukraine) also included its pollinator 'VP-23' with a distance of 0.3. Similarly, the 'Ruslan' hybrid and its parental forms 'OP GO MM' (14044 + 15676) and 'MS-2113' grouped into one cluster at a distance of 0.45. The male sterile lines 'MS-1611', 'MS-1631', and MS-97 were obtained from the Institute of Bioenergy Crops and Sugar Beet, Kiev, Ukraine, and also grouped into one cluster at the distance of 0.48. The sugar beet selection samples of VNIISS gathered in a separate cluster at the distance of 0.42 (Figure 4). The cluster analysis results can be used for the productive crossing of sugar beet lines. The sugar beet lines with large genetic distances from each other were also recommended for hybridization (McGrath *et al*., 2007; Würschum *et al*., 2013; Nalbandyan *et al*., 2020). The greatest distance (D = 1.4) was noted among the male sterile lines'MS-1611', 'MS-1631', 'MS-97', and 'MS-2113' and the pollinators 'VP-44' and 'VP-23' and thus can potentially be used for the generation of productive and stable hybrids.

In sugar beet, the sugar content is an important quality trait that varies greatly with genotypes and environmental conditions (Hoffmann *et al*., 2009). In this study, the sugar content of sugar beet genotypes ranged between 15.3% to 18.8% with an average value of 17.5% ± 0.8% (Table 1). Melentyeva *et al*. (2020) have also reported sugar contents of 16.9% to 18% in sugar beet genotypes originating from Serbia, 15.2% to 18.1% in those from the USA, 15.6% to 16.4% in those from Russia, and 16.6% to 18.2% in samples from the Republic of Belarus. The sugar content of the hybrids grown in the Tambov region of the Russian Federation ranged from 17.1% to 18.6%.

CONCLUSIONS

The polymorphism of 19 lines and 34 sugar beet hybrids was assessed by using 14 RAPD primers. For all the studied RAPD primers, a total of 114 alleles were identified with an average of 8.1 bands per marker. The PIC values of different primers varied from 0.24 (PAWS-17) to 0.46 (OPP-18) with an average of 0.4. The highest polymorphism rate was recorded for the three primers OPP-18 (40%), OPC-06 (40%), and OPA-19 (37.5%). The cluster analysis of RAPD profile data categorized the 53 sugar beet genotypes into seven clusters. These sugar beet genotypes can be used for the development of hybrids with high root mass weight and sugar content. The evaluation of similarities and differences based on RAPD analysis and agromorphological traits is important for creating positive heterosis in the hybrids. The assessment of agromorphological traits and genetic dissimilarity are important for the creation of heterotic hybrids. Our study identified two groups of lines. The first consisted of 'MS-1631', 'OP GO MM,' 'H-22', and 'MS 2113.' The second included 'Iris A-1,' 'SOAN-5', and 'MS-1633'. The samples from the first group may be genetically dissimilar to the samples of the second group. Thus, hybrids resulting from crosses between these groups may have good combining ability. We recommend that the breeders of KRIAPG include these lines into crosses to develop hybrids with high root mass weight and sugar content.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the employee of the Laboratory of Biotechnology, Physiology, Plant Biochemistry, and Assessment of Product Quality KRIAPG Kudysh Yerkezhan Muratovich for help in preparing this article. The present research was funded by the Science Committee of the Ministry of Education and Science, the Republic of Kazakhstan as part of the implementation of Grant IRN AP08956877 titled "Replenishment, enrichment of the genetic pool of sugar beet (*Beta vulgaris*) and study of its genetic diversity using molecular markers" under the budget program 217 Development of Science, subprogram 102 Grant financing of scientific research, priority Sustainable development of the agro-industrial complex and safety of agricultural products.

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