AMEA-nın Xəbərləri (biologiya və tibb elmləri), cild 68, №1, səh. 104-108 (2013)

Comparative Assessment Of Genetic Polymorphism Of Sugar Beet Genotypes Based On RAPD And ISSR Analysis

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The genetic diversity of 42 sugar beet accessions of diverse origin was studied using RAPD and ISSR markers. RAPD and ISSR DNA profiles revealed high polymorphism degree of DNA fragments among tested genotypes. Genetic similarity and distance among accessions was calculated according to Jaccard and Nei coefficients. The similarity and distance coefficients were then used to construct a dendrogram using the UPGMA cluster analysis. Correlation between two matrices was determined based on Mantel test, correlation coefficient (r) was found as r=0.1902.

Key words: *sugar beet, PCR analysis , RAPD and İSSR markers*

INTRODUCTION

In recent years, breeders in their practical work use such a powerful tool as molecular markers. The development of molecular biology has opened entirely new opportunities for geneticists. The advantage of the new technology is the ability to select valuable genotypes not by phenotypic evaluation of the adult plant, but on the basis of direct genetic information in any stages of plant development. This reduces the time of selection and saves material resources and labor costs. Molecular markers, generated by PCR, can evaluate the genetic diversity of the initial material, classify breeding forms, mark genes of economically important traits and can be used in mapping genomes (Kochieva, 1999). Currently, there are over 15 different types of markers used for molecular genetic analysis of the plant genome. The most popular molecular markers include RFLP-, CAPS-, STS-, SSR-, SNP-, RAPD-, SCAR-, AFLP-, ISSR-markers (Khlestkin and Salina, 2006). At the present, it is important to examine the possibility of application of a set of molecular genetic markers based on RAPD-, ISSR-analyzes that allows to group studied material according to the degree of genetic relationship, to identify genetic differences between the contrasting qualitative and quantitative traits of plants. ISSR markers were more useful in the study of genetic diversity within and among populations (ISSR-fingerprinting). This method which related to the methods of multilocus molecular analysis allows to simultaneously evaluate the polymorphism of tens of loci (30 loci and more) and to use for assessment of the degree of genetic diversity, identification and certification on inter-and intraspecific level in a wide range of crop species. Widespread use of such markers is due to a number of advantages over other marker systems, which certainly makes it very promising for scientific practice (Zietkiewicz, 1994).According to the literature, RAPD markers are also of interest and are used when it is necessary to distinguish between organisms similar in genetic sequence. One of the advantages of RAPD method - the ability for genotyping many loci simultaneously localized in different parts of the genome, which is especially important (Lanying, 2008). A number of examples of effective use of DNA markers in molecular genetic studies for breeding of cereals, legumes, pulses and vegetable crops are known. Studies on the development of molecular markers and their use in breeding of sugar beet began to appear only in the last few years. Sugar beet in terms of genetic markers remains poorly understood practically, so researches aimed at studying the genetic diversity of sugar beet based on RAPD and İSSR markers are highly relevant. The purpose of this paper is a comparative analysis of the genetic diversity of genotypes of sugar beet using inter microsatellite ISSR and RAPD analyzes, as well as search for ISSR and RAPD markers promising for use in the study of the genetic polymorphism of sugar beet.

MATERIALS AND METHODS

Plant material: 42 genotypes of sugar beet (*Beta vulgaris* L.) of different origins with different phenotypic characteristics were served as a material for molecular genetic studies. The name and the origin of the samples were shown in Table 1.

DNA extraction:Genomic DNA was extracted from the germinated seeds according to CTAB protocol. The quality of the DNA was checked in 1%

Table 1. The name and the origin of the samples

№	Accessions	Origin	№	Accessions	Origin
1	Sugar VNIISP-1	Russia	22	9648	Iran
2	G.R.N.	France	23	7901	Iran
3	A.m№ 1	United Kingdom	24	9586	Iran
4	C. Turzii 34	Romania	25	9296	Iran
5	P.P. 34 mm	Russia	26	9625	Iran
6	G.W.H.	Bulgaria	27	9588	Iran
7	Zwanesse 3	Netherlands	28	9634	Iran
8	16126- MOS	Germany	29	8155	Iran
9	Burakicukrove	Poland	30	9606	Iran
10	Ramonskaya 125	Russia	31	9623	Iran
11	Uladovsky 120	Ukraine	32	9583	Iran
12	Monomarcom	France	33	9656	Iran
13	Ramonskaya odn. 9	Russia	34	9641	Iran
14	Non-Uki 400	Japan	35	9665	Iran
15	Verhnyachskayaodn 36	Ukraine	36	8148	Iran
16	Verhnyachskaya 126	Ukraine	37	9565	Iran
17	Ivanovskaya odn.31	Ukraine	38	9621	Iran
18	M-2	Russia	39	9585	Iran
19	HelleshoqPoliploid	England	40	9597	Iran
20	Cukorrepa	Hungary	41	9597-P-26	Iran
21	Ximona	Germany	42	9569-P-26	Iran

agarose gel. The concentration and purity of the DNA was determined by spectrophotometer.

PCR analysis: The reaction mixture containing PCR buffer, mixture dNTP, $MgCl_2$, primers, Taq-polymerase enzyme and DNA samples was prepared under sterile conditions. Polymerase chain reaction with RAPD-primers was performed in a thermocycler as follows: pre-denaturation at 94°C for 4 minutes followed by 35 cycles of denaturation 94°C - 1 min, annealing temperature depending on the primers used - 1 min, synthesis 3 minutes at 72°C, a final cycle of elongation at 72°C for 10 minutes. Amplification with ISSR primers was performed under the following conditions: initial denaturation - 7 min 94ºC, followed by 35 cycles of denaturation - 1 min 94ºC, annealing - 1 min 54 ºC, elongation - 3 min 72ºC and a final cycle elongation at 72ºC for 10 minutes. For multilocus ISSR analysis were tested 12 polymorphic ISSR primers, 18 nucleotides in length. A set of random primers with length of 10 nucleotides was used for RAPD-analysis (Table 2).

Agarose gel electrophoresis: Electrophoresis of PCR products was performed on 2% agarose gel by addition of ethidium bromide and visualized under ultraviolet light. The presence of amplified DNA fragments in gels was determined based on color intensity.

Statistical data processing: To analyze the level of the polymorphism of markers among genotypes got data were presented in matrix form of the binary attributes, in which "presence" or "absence" of the PCR fragments with the same molecular weight was considered as the state 1 and 0 respectively. The number and percentage of polymorphic loci and Nei's genetic diversity index were then determined. The coefficient of genetic diversity was calculated according to Weir:

$$H = 1 - \sum_i^n p_i^2$$

where, H - index of genetic diversity, p_i - frequency of alleles.

Using the method of UPGMA (Unweighted Pair-Group Method with Arithmetical average) cluster analysis was carried out and the dendrogram was constructed on the basis of Nei's genetic distance index and genetic similarity index of Jaccard. To assess the validity of the correlation between the matrices Mantel test was used (Mantel, 1967).

Table 2. The nucleotide sequence of RAPD - ISSR primers used for amplification of DNA (Y=(C or T), R= (A or G))

№	RAPD primers	Sequence (5'-3')	ISSR primers	Sequence (5'-3')
1	OP-S4	CACCCCCTTG	UBC864	ATGATGATGATGATGATG
2	OP-G6	GTGCCTAACC	UBC859	TGTGTGTGTGTGTGTGRC
3	OP-B10	CTGCTGGGAC	UBC857	ACACACACACACACACYG
4	OP-F15	CCAGTACTCC	UBC814	CTCTCTCTCTCTCTCTA
5	A04	AATCGGGCTG	UBC855	ACACACACACACACACYT
6	A05	AGGGGTCTTG	UBC853	CTCTCTCTCTCTCTCRT
7	A07	GAAACGGGTG	UBC851	GTGTGTGTGTGTGTGTYG
8	A08	GTGACGTAGG	UBC848	CACACACACACACACARG
9	A09	GGGTAACGCC	UBC847	CACACACACACACACARC
10	A14	TCTGTGCTGG	UBC845	CTCTCTCTCTCTCTCTRG
11	A19	CAAACGTCGG	UBC842	GAGAGAGAGAGAGAGAT
12	A20	GTTGCGATGC	UBC840	GAGAGAGAGAGAGAGAYT

Table 3. Used ISSR and RAPD-primers and their statistical parameters

RAPD primers	Total number of bands amplified	Polymorphic bands	Percentage of polymorphism (%)	Genetic diversity index	ISSR primers	Total number of bands amplified	Polymorphic bands	Percentage of polymorphism (%)	Genetic diversity index
OP-S4	20	19	95	**0.93**	**UBC864**	14	14	100	**0.96**
OP-G6	20	18	90	**0.89**	**UBC859**	17	17	100	**0.97**
OP-B10	17	11	64.7	0.79	**UBC857**	17	13	76.47	0.72
OP-F15	18	15	83.3	**0.90**	**UBC814**	16	16	100	0.92
A04	20	20	100	0.85	**UBC855**	17	17	100	**0.97**
A05	13	12	92.3	0.84	**UBC853**	17	17	100	**0.97**
A07	11	10	90.9	0.84	**UBC851**	12	12	100	0.93
A08	14	14	100	0.77	**UBC848**	15	15	100	**0.95**
A09	19	19	100	**0.89**	**UBC847**	16	16	100	**0.95**
A14	18	18	100	**0.93**	**UBC845**	16	16	100	0.94
A19	18	18	100	**0.92**	**UBC842**	10	9	90	0.86
A20	16	16	100	0.82	**UBC840**	11	11	100	0.76
Total	204	190		-	**Total**	178	173	-	-
Mean	17	15.83	93.02	0.86	**Mean**	14.8	14.4	97.2	0.91

RESULTS AND DISCUSSION

Used RAPD-ISSR primers presented a wide range of variation in the number of amplified fragments. The main area of distribution of fragments is located in the range of 200-3000 bp (RAPD analysis) and 500-3000 bp (ISSR analysis). The resulting frequency of RAPD-ISSR fragments were used to estimate the main parameters of the genetic variability of the studied sugar beet genotypes. As a result 12 ISSR primers amplified 178 PCR fragments, while 204 fragments were amplified using 12 random RAPD primers.

According to Table 3, out of 204 RAPD fragments 190 were polymorphic, 14 - monomorphic. The average number of polymorphic fragments for each primer was 15.8. As a result of comparative electrophoretic analysis with ISSR primer it was revealed that 173 of 178 spectra were polymorphic, 5 - monomorphic. The average number of polymorphic fragments per primer was 14.4. Ten of the twelve ISSR markers - UBC864, UBC859, UBC814, UBC855, UBC853, UBC851, UBC848, UBC847, UBC84 and UBC840 revealed allelic polymorphism among the analyzed samples. The variation of the percentage of polymorphic ISSR loci depending on primer was between 76.47 and 100%, the average was 97.2.

Thus all primers, except primer ISSR UBC-857 had high levels of polymorphism. It should be noted that the analysis of the experimental data obtained by RAPD primers showed 100% of polymorphic loci only for 6 (A04, A08, A09, A14, A19 and A20) of the 12 tested primers. The frequency of the polymorphic fragments ranged from 64.7 to 100%, depending on the primer, and the average was 93.02. Thus, we can conclude that selected for the molecular genetic analysis ISSR primers were more informative. Similar high effective results with ISSR markers were also obtained in studies of other researches. Cheng and co-workers (Cheng et.al., 2012) identified 13 sugar beet samples using 6 ISSR primers and revealed 48 DNA fragments. Percentage of polymorphism was 87.5%. The index of genetic similarity ranged from 0.46 to 0.89. As a result it was recommended to use ISSR markers to determine the genetic distance of sugar beet genotypes.

In addition, the genetic diversity indices for each ISSR and RAPD primers were calculated. According to Table 3, ISSR primers compared with RAPD primers had highest value of genetic diversity index, with the average of 0.91, whereas the average value of the genetic diversity index for RAPD primers was 0.86. Among the tested ISSR markers, a high index of genetic diversity had primers UBC-864, UBC-859, UBC-814, UBC-855, UBC-853, UBC-851, UBC-848, UBC-847 and UBC-845. Experimental studies showed that these ISSR markers were sufficient polymorphic and can be used to assess genetic polymorphism. As a result of RAPD analysis following primers were differed for their high genetic diversity index: OP-S4 (93%), A14 (93%), A19 (92%), OP-F15 (90%), OP-G6 (89%) and A09 (89%). The data allow us to assume that these RAPD primers can be recommended as the most effective to study the genetic diversity of sugar beet. Nagl and co-workers (Nagl et. al., 2011.) evaluated the genetic

diversity of 12 samples of sugar beet by applying the RAPD markers. As a result of studies the level of polymorphism was 75.86%.

Using the UPGMA method on the basis of Jaccard's genetic similarity and Nei's genetic distance indices sugar beet genotypes were grouped into clusters. Cluster analysis allowed to combine 42 beet genotypes into 5 clusters based on RAPD markers and into 6 clusters based on ISSR analysis (Fig. 1,2). For each cluster samples with the lowest and the highest genetic distances were identified. The level of genetic distance (RAPD analysis) ranged from 0.03 between samples 9569-P-26 and 9597-P-26 to 0.24 between genotypes Verhnyachskaya 126 and Buraki cukrove. The level of Jaccard's genetic similarity (ISSR analysis) ranged from 0.359 between genotypes 9648 and 9586 to 0.71 between genotypes 8148 and 9565 of Iran origin.

Mantel test was used to assess the validity of the correlation between two matrices based on RAPD and ISSR marker. It has been found that the correlation between matrices was high and significant (r=0,92). Thus, it can be assumed that both matrices correspond to each other and contain the similar information.

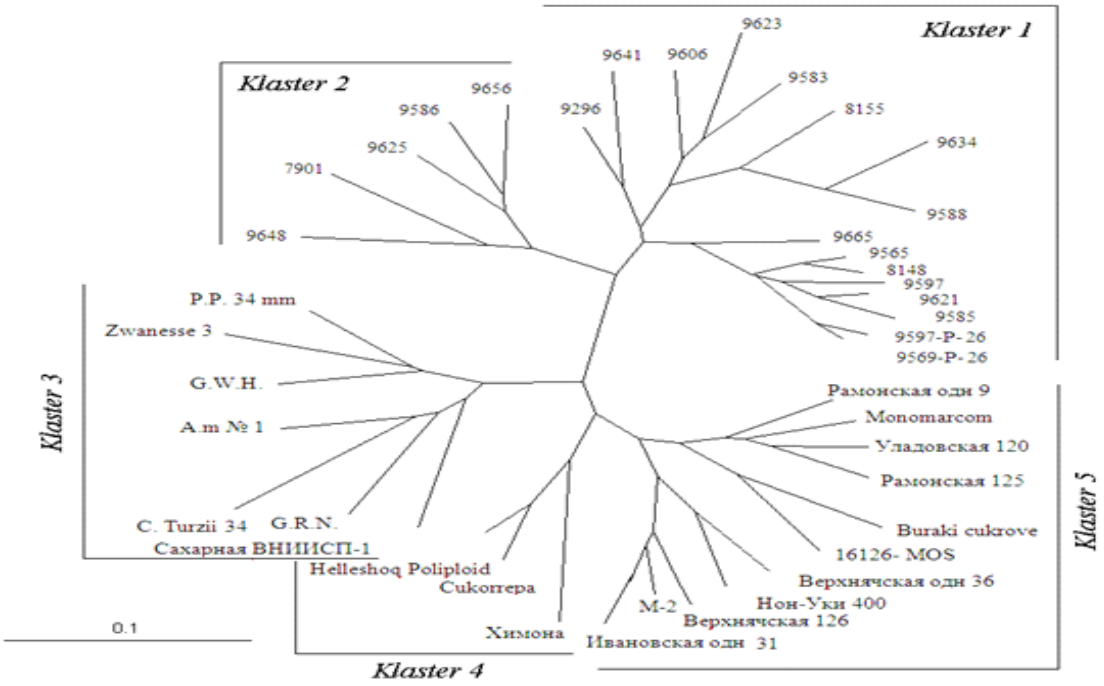

Fig. 1. Dendrogram of genetic distance of sugar beet samples based on the results of RAPD analysis.

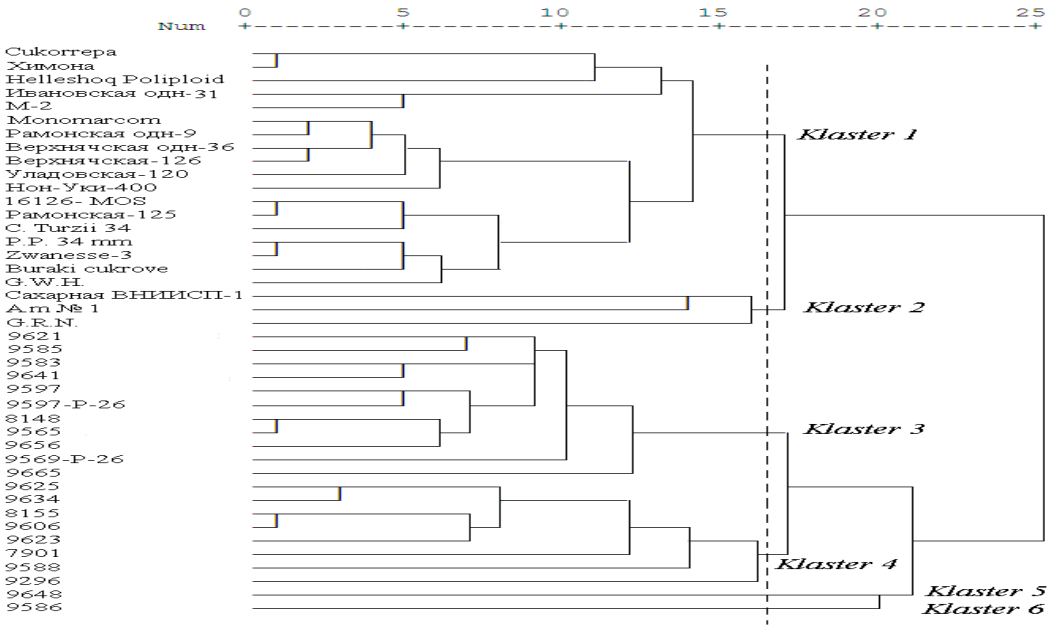

Fig. 2. Dendrogram of genetic similarity of sugar beet samples based on the results of ISSR analysis

So, a comparative analysis of 12 RAPD and 12 ISSR markers was performed. RAPD-and ISSR-primers used in our experiments showed a high level of polymorphism (an average of 93.02 and 97.2%, respectively) and fully identified studied sugar beet genotypes. As a result of RAPD analysis selected primers were distinguished for lower index of genetic diversity (0.86) compared with ISSR primer (0.91). Our results showed that the use of ISSR markers in comparison with RAPD markers is of the greatest interest for molecular genetic studies of sugar beet genotypes.

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RAPD VƏ ISSR Markerlərlə Şəkər Çuğunduru Genotiplərində Genetik Polimorfizmin Müqayisəli Qiymətləndirilməsi

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Müxtəlif mənşəli 42 şəkər çuğunduru genotipinin genetik müxtəlifliyi RAPD və ISSR markerlərdən istifadə etməklə tədqiq olunmuşdur. RAPD və ISSR DNT profilləri genotiplər arasında yüksək polimorfizm dərəcəsi olduğunu aşkar etmişdir. Nümunələr arasında genetik oxşarlıq və məsafə Cakkard və Ney indeksləri əsasında hesablanmış və UPGMA klaster analizindən istifadə etməklə dendroqram tərtib edilmişdir. Mantel testi əsasında matrislər müqayisə olunmuş və korrelayasiya əmsalı r=0.1902 təyin edilmişdir.

Сравнительная Оценка Генетического Полиморфизма Генотипов Сахарной Свеклы На Основе RAPD И ISSR-Анализов

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Генетическое разнообразие 42 генотипов сахарной свеклы было изучено с использованием RAPD и ISSR маркеров. RAPD и İSSR ДНК профили выявили высокий уровень полиморфизма среди полученных ДНК фрагментов. Генетическое сходство и расстояние среди образцов вычислялось в соответствии индексу Джаккарда (ISSR) и Нея (RAPD). С использованием кластерного анализа UPGMA и на основании индекса генетического сходства и расстояния была построена дендрограмма. Корреляция между двумя матрицами была определена на основе Мантель-теста, где коэффициент корреляции (r) составил 0.92.