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ANALYSIS OF GENETIC DIVERSITY AMONG DIFFERENT TOMATO GENOTYPES USING ISSR DNA MARKER

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Inter simple sequence repeat (ISSR) markers were used for variation analysis among 41 tomato accessions. A total of 50 scorable bands were obtained, where 32 were polymorphic, representing 63.3% of all the amplified loci. Polymorphism percentage ranged from 50 to 90% and an average number of polymorphic bands of 4.0 were observed. An average genetic diversity index was 0.61. Primer UBC860 and UBC825 generated the greatest diversity index with a value of 0.89 and 0.85 respectively. The smallest diversity identified by primer UBC808, with an index of 0.34. The genetic similarity among studied genotypes ranged from 0.52 to 0.98. The cluster analysis based on Jaccard's similarity coefficient divided genotypes into 6 distinct clusters on a value of 0.74. The lowest genetic distance was found between 'Gronastiy' and 'AG1224' (0.52), 'Orange' and 'AG1224' (0.54), and 'Evgeniya' and 'AG1224' (0.55) accessions. The highest similarity of 0.98 was determined between 'Zafar' and 'Azerbaijan-94', 'Khachmaz-1' and 'Azerbaijan-94', 'Khachmaz-1' and 'Severyanka', and 'Shakar' and 'Absheron-1' accessions.

Key words: allele, genetic diversity, markers, tomato

INTRODUCTION

Cultivated tomato (*Solanum lycopersicum* L.) is a significant vegetable crop of economic importance and widely grown around the world (HE *et al.,* 2003; WANG *et. al*., 2005; ELHAM *et al*., 2010). The tomato core collection of the European Solanaceae database is consisted of about 7000 domesticated (*S. lycopersicum* L.) lines, along with many representatives of wild species provided by international gene banks and by donations from private collections (https://www.eu-sol.wur.nl/).

The cultivated tomato has limited variability mainly due to population bottlenecks occurred though domestication and evolution of modern cultivars (FOOLAD *et al*., 2007). Therefore, sufficient

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information on the genetic diversity among tomato genotypes conserved in the gene banks is necessary for the development of effective breeding strategies. On the other hand, knowledge about genetic diversity is a prerequisite for elimination of suspected duplicates for effective management in the germplasm collections.

Nowadays, numerous markers are being widely used for description of genetic resources (SCHLÖTTERER, 2004). Exploring of genetic diversity using DNA markers is very important strategy and much more cost effective than traditional or phenotypic approach. A numbers of DNA markers, including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeats (ISSR), simple sequence repeats (SSRs), cleaved amplified polymorphic sequence (CAPS), sequence-tagged site (STS), sequenced characterized amplified region (SCAR) and single nucleotide polymorphism (SNP), markers that are being widely used for genetic diversity analysis of germplasm collections (SEMAGN *et al.,* 2006). Despite of using different molecular markers to study genetic diversity in cultivated tomatoes, many of them identify limited level of polymorphism (MILLER and TANKSLEY*,* 1990; ALVAREZ *et al.,* 2001; ARCHAK *et al.,* 2002; KOCHEIVA *et al.,* 2002; TIKUNOV *et al.,* 2003; PARK *et al.,* 2004; FRARY *et al.,* 2005; RUIZ *et al.,* 2005; BOJINOV and DANAILOV, 2008; TERZOPOULOS and BEBELI, 2008). Therefore, identification of more polymorphic molecular markers is important for tomato research.

ISSR markers are considered very useful in studies of genetic diversity, phylogeny, genomics and evolutionary biology (REDDY *et al.,* 2002; HAVLICKOVÁ *et al.,* 2014). The aim of this study was to evaluate the genetic diversity among the tomato accessions conserved at the Gene Bank of Genetic Resources Institute (AGRI) of Azerbaijan National Academy of Sciences (ANAS) by using ISSR markers.

MATERIALS AND METHODS

Research material consisted of 34 tomato genotypes conserved at the gene bank and 7 new obtained accessions from markets (Table 1).

Table 1. List of tomato genotypes used for genetic diversity analysis

No	**[1]NI code**	**Institute code**	**Accession number**	**Accession name**	**Country of origin**	**[*]Biol Stat.**	**[**]Coll. source**	**[#]Storage**
1	AZE	AZE015	AzGR-4675	Shalala	AZE	500	40	12
2	AZE	AZE015	AzGR-9468	Krasnodar	RUS	500	40	12
3	AZE	AZE015	[n]AzGR	Atol	POL	999	23	-
4	AZE	AZE015	[n]AzGR	Krakus	POL	999	23	-
5	AZE	AZE015	AzGR-9474	Shakar	AZE	500	40	12
6	AZE	AZE015	AzGR-10264	Zafar	AZE	500	40	12
7	AZE	AZE015	AzGR-7651	AG-1222	AZE	999	30	12
8	AZE	AZE015	AzGR-7653	AG-1224	AZE	999	30	12
9	AZE	AZE015	AzGR-8635	Saatly	AZE	990	30	12
10	AZE	AZE015	AzGR-9934	Absheron-3	AZE	999	30	12
11	AZE	AZE015	AzGR-4679	Yablinka rozi	UKR	500	40	12
12	AZE	AZE015	AzGR-4677	Fakel	RUS	500	40	12

13	AZE	AZE015	AzGR-3893	Zarrabi	AZE	500	40	12
14	AZE	AZE015	AzGR-3968	Garatag	AZE	300	40	12
15	AZE	AZE015	AzGR-9937	Absheron-1	AZE	999	30	12
16	AZE	AZE015	AzGR-9936	Absheron-2	AZE	999	30	12
17	AZE	AZE015	AzGR-8636	Sabirabad	AZE	999	30	12
18	AZE	AZE015	AzGR-10840	AG-2695	AZE	999	23	12
19	AZE	AZE015	AzGR-11333	Azerbaijan-94	AZE	500	40	12
20	AZE	AZE015	[n]AzGR	Khachmaz-1	AZE	999	23	-
21	AZE	AZE015	[n]AzGR	Khachmaz-2	AZE	999	23	-
22	AZE	AZE015	AzGR-7652	AG-1223	AZE	999	30	12
23	AZE	AZE015	AzGR-9931	Severyanka	RUS	500	40	12
24	AZE	AZE015	AzGR-8159	Vkusniy-3	RUS	500	40	12
25	AZE	AZE015	AzGR-9935	Charodey	RUS	500	40	12
26	AZE	AZE015	AzGR-8628	Gurman	AZE	500	40	12
27	AZE	AZE015	AzGR-3889	Volgograd 5/95	RUS	500	40	12
28	AZE	AZE015	AzGR-9929	Evgeniya	RUS	500	40	12
29	AZE	AZE015	[n]AzGR	Gronastiy	POL	999	23	-
30	AZE	AZE015	AzGR-9928	Chernomor	RUS	500	40	12
31	AZE	AZE015	AzGR-8631	Leningradskiy krupniy rozoviy	RUS	500	40	12
32	AZE	AZE015	AzGR-8157	Ronita	FRA	500	40	12
33	AZE	AZE015	AzGR-8158	Podarok	KGZ	500	40	12
34	AZE	AZE015	AzGR-8632	Tigris	RUS	500	40	12
35	AZE	AZE015	AzGR-8633	Banan krasniy	RUS	500	40	12
36	AZE	AZE015	AzGR-8634	Neoskiy	RUS	500	40	12
37	AZE	AZE015	[n]AzGR	Orange	POL	999	23	-
38	AZE	AZE015	[n]AzGR	Cherry	POL	999	23	-
39	AZE	AZE015	AzGR-3899	Leyla	AZE	500	40	12
40	AZE	AZE015	AzGR-3898	Ilkin	AZE	500	40	12
41	AZE	AZE015	AzGR-3967	Elnur	AZE	500	40	12

(The descriptors are numbered according to the FAO/IPGRI multi-crop passport descriptors (http://www.ecpgr.cgiar.org))

[1]**NI code – National Inventory Code**:

*** Biol. Stat. (Biological status of accession)**

100 - Wild; 300 - Traditional cultivar/landrace; 500 - Advanced/improved cultivar; 999 - (Others): unknown.

****Coll. source (Collecting/acquisition source)**

23- Market or shop**;** 30- Backyard, kitchen or home garden (urban, peri-urban or rural)**;** 40- Institute, Experimental station, Research organization, Gene bank

[#]**Storage**

12- Medium term

[n]AzGR- these accessions have not been included gene bank yet.

Genomic DNA was extracted by PureLink DNA purification Kit (Invitrogen^{TM}). Nanodrop 2000 spectrophotometer was used for checking quality and concentration of isolated DNA. A total of 11 ISSR primers synthesized by IDT (https://www.idtdna.com) were used for PCR amplification (Table 2).

Table 2. List of ISSR primers used for diversity analysis.

Primers	Sequence 5'-3'	Tm
UBC 808	(AG)8C	48.8
ISSR 814	(CT)8TG	47.6
UBC 825	(AC)8T	51.4
UBC 834	(AG)8CT	50.6
ISSR 857	(AC)8YT	53.1
UBC 860	(TG)8RA	53.1
HB8	(GA)6GG	41.9
HB9	(GT)6GG	46.6
HB13	(CAC)3GC	44.7
HB14	(CTC)3GC	41.8
HB15	(GTG)3GC	44.0

Amplification reactions were performed in a final volume of 20 µl, containing 1x AMS PCR Buffer, 1.5 mM MgCl2, 25 mM of each deoxynucleotide (dATP, dTTP, dGTP and dCTP), 0.5 mM of primer, 0.6 units of Taq DNA polymerase enzyme and 50 ng of DNA using a Verity Thermo Cycler (Applied Biosystem). All of the mentioned reagents for PCR reactions were obtained from the company of Sinaclon (http://www.sinaclon.com/).

For ISSR amplification, after initial denaturation step for 4 min at 94°C, 38 cycles were performed each consisting of a denaturation step at 94°C for 1 min, annealing step at primer-dependent temperatures (5°C below Tm) for 1 min and extension step at 72°C for 2 min followed by a final extension at 72°C for 7 minutes.

The PCR products were separated by agarose gel electrophoresis using a 1.2% (w/v) agarose gel in TBE 1X buffer for 1.5 hrs. 100-bp DNA ladder (Sinaclon) was used for approximate fragment size calculations. Gel stained with ethidium bromide solution, visualized under UV light and recorded by “Molecular Imager® Gel DocTM XR system (Bio-Rad)”.

The generated DNA bands on agarose gels were analyzed by scoring as present (1) or absent (0) of each alleles. The genetic similarity was obtained based on Jaccard coefficient using Past3 statistic program package (HAMMER *et al.,* 2001). Genetic diversity index for each primer was calculated according to Nei (NEI, 1973).

$H = 1 - \Sigma Pi^2$

H –genetic diversity index

Pi – frequency of each pattern

RESULTS AND DISCUSSION

Tomato (*Solanum lycopersicum* L.) is the most important fruit crop in the world, as well as in Azerbaijan. Tomato plants are grown at all the vegetable producing economical regions of Azerbaijan (Figure 1). A total of 82 accessions of the cultivated tomato are maintained in the gene

bank of the AGRI. The collections comprised landraces, improved cultivar, breeding materials and unknown materials that obtained from markets or shops or collected from home-gardens and etc. Landraces and improved cultivars originated from different countries. Others accessions were called by the name of those regions that they collected and registered as unknown local populations in the gene bank, however there was no more information about their true status and origin. Several accessions with the name and origins, but lack of the biological status have bought from the markets. Although, breeders and researchers have provided descriptions based on pedigree information and morphological traits for most of local accessions, there is not enough information about the genetic diversity of whole conserved materials. In addition, only morphological characterization data does not provide accurate information necessary to distinguish different genotypes. Therefore, further comprehensive assessment of conserved germplasms at the molecular level is required. In present study we used ISSR markers for genetic diversity analysis among 41 cultivated tomato accessions belonging to our germplasm bank.

Figure 1. Main economical regions of Azerbaijan with a grown area of tomatoes (ha) (http://www.stat.gov.az)

Three out of 11 primers used (HB13, HB14 and HB15), which mainly consist of 'G' and 'C' nucleotides, did not amplified any or distinct evaluable bands. The clear, reproducible alleles amplified with the other primers were scored as 1 for presence or 0 for absence and imported into Past3 statistic program (Figure 2).

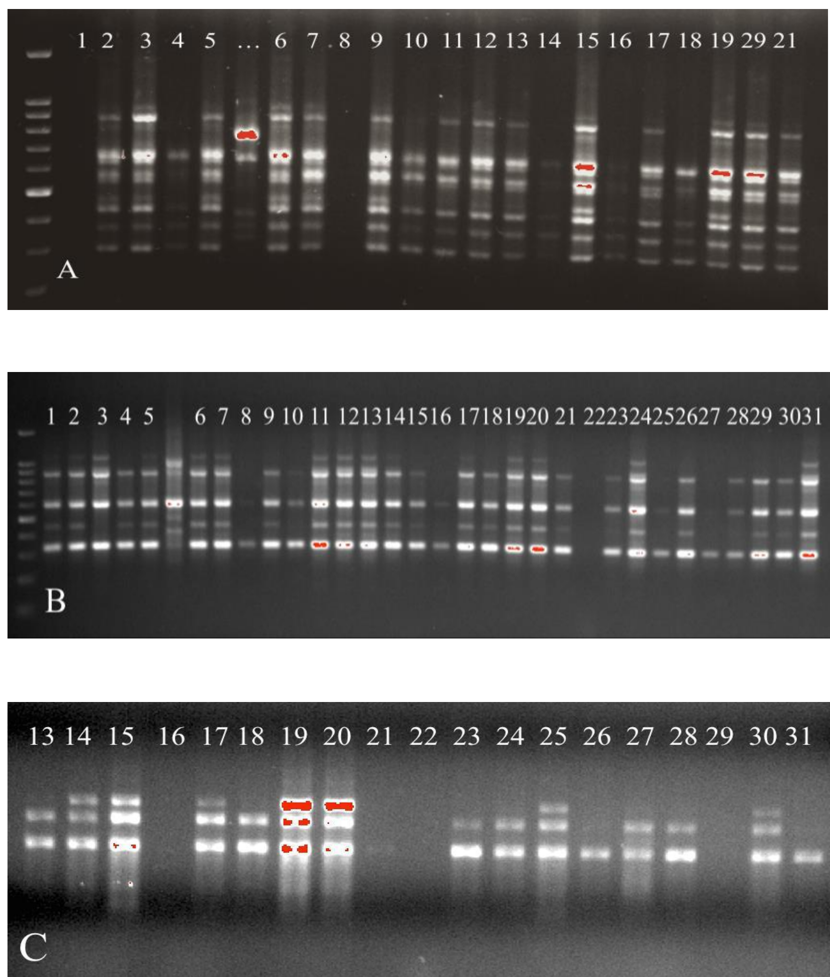

Figure 2. ISSR electhophoretic pattern of some tomato accessions obtained by primer UBC825 (A), UBC857 (B) and HB8 (C)

In total, 50 scorable bands were obtained from studied tomato accessions, where 18 out of them were monomorphic, occurring in all representatives, and 32 were polymorphic, representing 63.3% of all the amplified loci (Table 3).

Primer UBC825 and HB8 were the most (10) and the less (3) allele produced primers. The higher polymorphism detected primers were UBC825 and UBC814. Primer UBC860 and UBC825 generated the greatest diversity index with a value of 0.89 and 0.85 respectively. The smallest diversity identified by primer UBC808, with an index of 0.34. The average genetic diversity index was 0.61 (Table 3).

Table 3. Polymorphism and genetic diversity index exhibited by ISSR primers in tomato

Primers	Sequence (5'-3')	Number of alleles	Number of polymorphic alleles	Polymorphism percentage (%)	Genetic diversity index
UBC 808	(AGAGAC)2AGA GC	4	2	50	0.34
ISSR 814	(CT)8TG	7	6	86	0.58
UBC 825	(ACACAC)2ACA CT	10	7	70	0.85
UBC 834	(AGAGAG)2AGA GYT	6	3	50	0.50
ISSR 857	(AC)8YT	6	4	67	0.71
UBC 860	(TGTGTG)2TGT GRA	9	5	56	0.89
ISSR HB8	(GA)6GG	3	2	67	0.50
ISSR HB9	(GT)6GG	5	3	60	0.52
Average		6.25	4.0	63.25	0.61

Experimental studies revealed different level of polymorphism for ISSR primers and tomato genotypes. For instance, SHAHLAEI and colleagues (2014) have used 10 ISSR primers for genetic diversity analysis of 10 tomato accessions and observed 86 bands with 20 (23.25%) being polymorphic where, an average resolving power value of markers was 1.55. Mansour and colleagues (MANSOUR *et al.,* 2010) reported high polymorphism in ISSR analysis (100%). In other research, the high level of polymorphism of 62% and an average number of polymorphic bands of 3.5 per primer were observed (EDRIS *et al.,* 2014). Contrary, low level of polymorphism (34%) in ISSR analysis of Brazilian tomato cultivars was reported (AGUILERA *et al.,* 2011). ISSR markers were used for genetic assessment of Greek tomato accessions and proved to be useful in describing genetic diversity among Greek landraces (TERZOPOULOS and BEBELI, 2008).

In our study polymorphism percentage ranged from 50 to 90% and an average number of polymorphic bands of 4.0 per primer were observed, which is high enough.

The similarity coefficient based on ISSR markers used ranged from 0.52 to 0.98 in our research. According to Jaccard`s similarity index, the lowest genetic distance was found between ‘AG1224’ and ‘Gronastiy’ (0.52), ‘Orange’ and ‘AG1224’ (0.54), ‘Evgeniya’ and ‘AG1224’ (0.55), ‘Vkusniy 3’ and ‘AG2695’ (0.57), and ‘Gronastiy’ and ‘Yablinka rozi’ (0.57) accessions. As it is shown by the dendrogram, one of the accessions of each pairs, except the last one, is local unknown population, whereas other belongs to introduced materials. The last pairs include two accessions form Ukraine and Poland.

Among several accessions studied the higher degree of similarity were found. The highest similarity of 0.98 was determined between ‘Zafar’ and ‘Azerbaijan-94’, ‘Khachmaz-1’ and ‘Azerbaijan-94’, ‘Khachmaz-1’ and ‘Severyanka’, and ‘Shakar’ and ‘Absheron-1’. The value of similarity was equal to 0.95 for ‘Azerbaijan-94’ and ‘Severyanka’ genotypes. This can be explained by narrow genetic differences between those accessions.

A dendrogram was generated based on UPGMA analysis, using Jaccard similarity coefficients that can be divided into 6 distinct clusters on a value of 0.74 (Figure 3). The first, fourth and the sixth clusters consisted of only one accession each. The only accession of the cluster 1, ‘Orange’ from Poland, was the most divergent among the other accessions. Accessions, belonged to other two above-mentioned clusters, ‘Absheron 3’ and ‘AG1224’, were the accessions with an unknown status.

The second group includes four genotypes belonging to the Russia, France and

Azerbaijan collections. The only local cultivar joined in this cluster, 'Gurman', is one of the accessions threatened to extinct.

The third cluster consists of 7 accessions whereas one of them ('Gronastiy') is more divergent that those of others. One out of seven accessions belongs to Russia, while other each of three to Azerbaijan and Poland materials respectively. 'Garatag', joined in this cluster, is an old cultivar that not cultivated anymore and is threatened to extinct. Yet another local genotypes represented in this cluster ('Absheron-2' and 'AG2695') were collected from the 'Absheron' region of country.

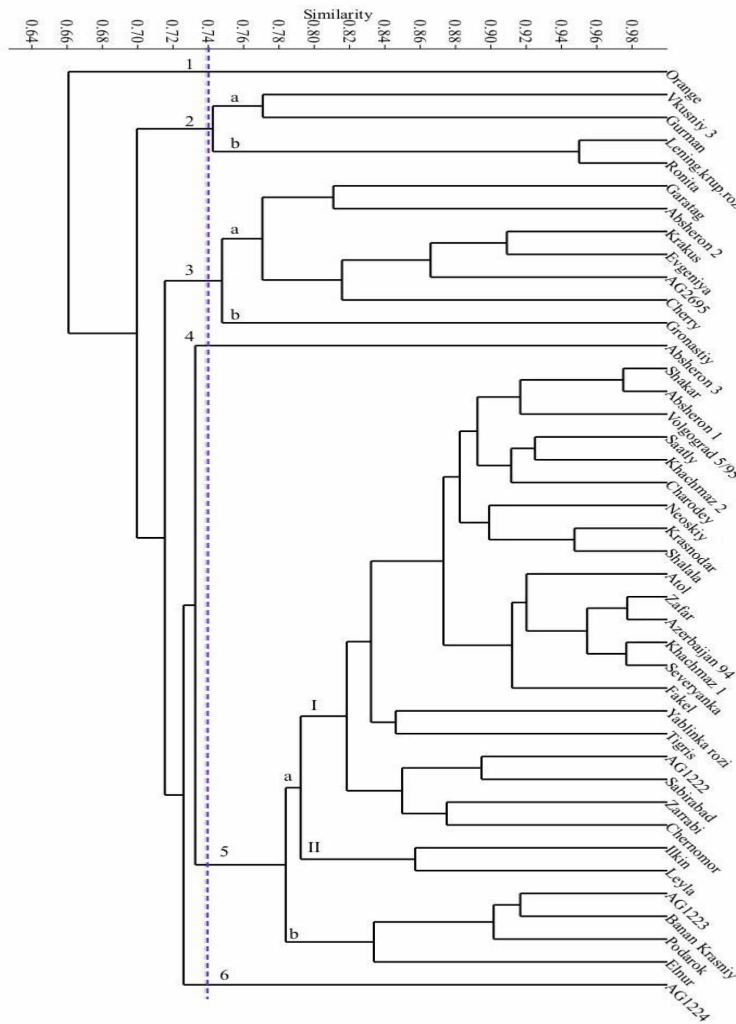

Figure 3. UPGMA dendrogram based on Jaccard coefficient among studied tomato accessions

The fifth cluster was the most abundant and contained all other 27 studied accessions.

This cluster divided in detail into two sub-clusters, where the most representative sub-cluster ‘a’ was further separated into two groups. Sub-cluster ‘b’ contains four assorted accessions of several countries. 11 accessions of main I group of sub-cluster ‘a’ are local materials, 8 belongs to Russian materials, and two, one from Poland and another from Ukraine. II group of sub-cluster ‘a’ consists of two advanced cultivars from same breeder in Azerbaijan.

Local tomato accessions joined in fifth cluster are grown nearly at all the regions of country (Figure 1). ‘Shalala’ is only greenhouse tomato cultivars among studied local accessions. ‘Shakar’, ‘Zafar’ and ‘Elnur’ cultivars are cultivated at the Lankaran and Guba-Khachmaz region. ‘Leyla’ and ‘Zarrabi’ are mainly grown at the Lankaran region. ‘Azerbaijan-94’ is a new released cultivar that grown at the Ganja-Gazakh region, whereas ‘Ilkin’ can be found in all the vegetable producing regions (SHARIFOVA *et al.,* 2013). Concerning the local populations of mentioned cluster, two were from Guba-Khachmaz (‘Khachmaz-1’ and ‘Khachmaz-2’), two from Aran (‘Saatly’ and ‘Sabirabad’) and two from Absheron (‘Absheron-1’ and ‘Absheron-3’) regions respectively, whereas other two (‘AG1222’ and ‘AG1223’) were totally unknown populations.

Thus, all main clusters includes accessions originated from different countries. Hence, there was a tendency of together clustering for accessions from the same or adjacent geographic origin, the accessions collected or originated from different geographic regions were also found placed into the same cluster, or from same geographic area placed into different clusters. This indicates that, the geographical origin of accessions has no influence on the clusters obtained. Such results were also obtained in a number of studies and explained that the accessions from different regions might have similar genetic background and those from the same origin might also have different genetic background (KENENI *et al.,* 2005; GASHAW *et al.,* 2007; CELKA *et al.,* 2010; SHARIFOVA *et al.,* 2013). All those results suggested that selection of parent genotypes based on geographical origin only was not an accurate indicator of genetic diversity.

Consequently, although data obtained in our research were efficient for the discrimination of genetic distance among genotypes, an accurate selection of parental genotypes based on more detailed study of genetic diversity should be considered for successful breeding programmes. Nevertheless, to distinguish the accessions with unknown origin and biological status, further comprehensive assessment using of different types and more polymorphic molecular markers could be necessary.

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ANALIZA GENETIČKOG DIVERZITETA RAZLIČITIH GENOTIPA PARADAJZA POMOĆU ISSR DNK MARKERA

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Izvod

ISSR marker su korišćeni za analizu variranja između 41 genotipa pradajza. Ukupno 50 traka je dobijeno, gde su 32 bile polimorfne, što predstavlja 63.3% ukupno amplifikovnih lokusa. Procenat polimorfizma varira između 50 do 90% a prosečan broj polimorfnih traka je 4.0. Prosečan indeks genetičkog diverziteta je 0.61. Prajmeri UBC860 i UBC825 generišu najveći indeks diverziteta sa vrednostima 0.89 I 0.85. Namanji diverzitet je identifikovan prajmerom UBC808, sa indeksom 0.34. Genetička sličnost između ispitivnaih genotipova varira od 0.52 do 0.98. Klaster analiza na osnosu Jacardovog koeficijenta grupiše genotipove u 6 klastera na vrednosti 0.74. Najniža genetička distance je dobijena između "Gronastiy" i "AG1224" (0.52), "Orange" i "AG1224" (0.54), i "Evgeniya" i "AG1224" (0.55). Najveća sličnost od 0.98 je dobijena između "Zafar" i "Azerbaijan-94", "Khachmaz-1" i "Azerbaijan-94", "Khachmaz-1" i "Severyanka", i "Shakar" i "Absheron-1" genotipova.

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