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ASSESSMENT OF GENETIC DIVERSITY IN TOMATO LANDRACES USING ISSR MARKERS

Mashhid HENAREH[1*], Atilla DURSUN[2], Babak ABDOLLAHI MANDOULAKANI[3], Kamil HALİLOĞLU[4]

[1]West Azerbaijan Agricultural and Natural Resources Research Center, Urmia, Iran.
[2]Department of Horticulture, Faculty of Agriculture, Ataturk University,Erzurum, Turkey.
[3]Department of Plant Breeding and Biotechnology, Faculty of Agriculture,Urmia University, Urmia, Iran.
[4]Department of Field Crops, Faculty of Agriculture, Ataturk University, Erzurum, Turkey.

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Tomato is one of the most economically important vegetable crops in many parts of the world. Turkey and Iran are the main producers of tomatoes in the world. The objective of this study was to assess the genetic variation of 93 tomato landraces from East Anatolian region of Turkey and North-West of Iran, along with three commercial cultivars using 14 ISSR primers. The percentage of polymorphic loci (PPL) for all primers was 100%. The mean of expected heterozygosity (He) for the primers varied from 0.153 (UBC808) to 0.30 (UBC848). The dendrogram placed the landraces and commercial cultivars into nine groups. The genotypes originating from the same region, often located in the same group or two adjacent groups. The highest likelihood of the data was obtained when population were located into 2 sub-populations (K = 2). These sub-populations had Fst value of 0.16 and 0.21.

Key words: ISSR, genetic variation, Shannon's information index, *Solanum lycopersicum*.

INTRODUCTION

Tomato originated from South America, which is widely grown in both temperate and tropical regions of the world and constitutes a major agricultural industry. Tomato is an excellent model system for plant genetic studies (BENOR *et al*., 2008). This has been due to many reasons, including high self fertility and homozygosity, ease of controlled pollination and hybridization, diploid species with a rather small genome (950 Mbp), lack of gene duplication, the ability to develop haploids, and availability of a wide array of mutants and genetic stocks (including wild species) (FOOLAD, 2007).

According to FAOSTAT (2012), tomato production in Turkey and Iran is 11,350,000 and 6,000,000 tons respectively, which are ranked as the fourth and sixth in the world. East

Corresponding author: M. Henareh**,** West Azerbaijan Agricultural and Natural Resources Research Center, Urmia, Iran. E-mail: mashhid_henareh@yahoo.com Phone:+989144407629. Fax:+98 44 2622220

Anatolian region of Turkey and North-West of Iran are the main producers of tomato in two countries. The cultivated tomato in Turkey and Iran can be separated in two groups, tomato landraces and improved cultivars. Tomato landraces have distinctive organoleptic traits (flavour and aroma) and nutritional value. These genotypes have received intensive attention especially in fresh market tomato. Many landraces were continuously replaced by modern tomato cultivars in these regions in recent years; therefore this germplasm has experienced an overall reduction of its genetic basis. Hence, it is necessary to characterize, preserve and extend this germplasm.

The description and evaluation of germplasm is important for conservation and use of plant genetic resources for current and future agronomic and genetic improvement of the crop (THUL *et al.*, 2011). Heterogeneous landrace populations are important sources of genetic variation and are utilized in plant breeding programs (TERZOPOULOS and BEBELI, 2008). In spite of the potential as a source of variability, the lack of information about agronomic traits and genetic constitution of landraces has limited their use in breeding programs. To identify and estimate the genetic diversity of plants, various methods can be used including morphological, biochemical and molecular markers. Morphological traits have limitations since they are influenced by environmental factors and the developmental stage of the plant (DASHCHI *et al*., 2012). Molecular markers have proved valuable in crop breeding, especially in study of genetic diversity. Different molecular marker systems have been used to determine of genetic variation in tomato such as simple sequence repeats (SSRs) (MAZZUCATO *et al*., 2010), random amplified polymorphic DNA (RAPD) (EZEKIEL *et al*., 2011), amplified fragment length polymorphism (AFLP) (EDRIS *et al*., 2014), sequence-related amplified polymorphism (SRAP) (COMLEKCIOGLU *et al*., 2010) and single nucleotide polymorphism (SNP) (CORRADO *et al*., 2013). Inter simple sequence repeat (ISSR) markers is a simple and quick method that combines most of the advantages of SSRs and AFLP to the universality of RAPD. This marker is a PCR-based technique, which involves amplification of DNA segment between adjacent and inversely oriented microsatellites (SINGH *et al*., 2014). The technique uses microsatellites, usually 16–25 bp long, as primers. These primers can be di-, tri-, tetra- or penta-nucleotides. The usefulness of the ISSR markers for assessing genetic variability in the genus *Solanum* has been demonstrated (EDRIS *et al*., 2014; MANSOUR *et al*., 2009; SHALAEI *et al*., 2014; AGUILERA *et al*., 2011). Utilizing the ISSR markers to distinguish closely related morphotypes within tomato landraces would enable us to better use the full genetic potential of landraces in marker-assisted tomato improvement programs (TERZOPOULOS and BEBELI, 2008). Based on the above considerations, the objective of the current investigation was to assess the genetic variation of tomato landraces collected from of the above mentioned regions by ISSR markers for further planning of germplasm conservation and breeding strategies.

MATERIALS AND METHODS

Plant materials

Ninety-three tomato landraces were collected from north-west of Iran and east Anatolian region of Turkey in 2011. Morphological markers such as size, form and colour of fruit and plant size were used to identify and collect different genotypes. At the time of collection, fruits were harvested from each genotype and then seeds were gathered. Each genotype was coded based on the name of collected site (Table 1, Figure 1). Seeds of these genotypes and three commercial cultivars (Peto Early CH, Rio Grande and H-2274) were planted in small pots in agriculture faculty greenhouse of Ataturk University of Turkey in 2014.

Table 1. Codes and geographical origins of the tomato landraces

No.	Landraces code	Origin	No.	Landraces code	Origin	No.	Landraces code	Origin
1	IR.U1	Iran-Urmia	32	IR.MI7	Iran-Miandoab	63	TU.IG11	Turkey-Iğdır
2	IR.U2	Iran-Urmia	33	IR.B	Iran-Bokan	64	TU.IG12	Turkey-Iğdır
3	IR.U3	Iran-Urmia	34	IR.MA1	Iran-Mahabad	65	TU.IG13	Turkey-Iğdır
4	IR.U4	Iran-Urmia	35	IR.MA2	Iran-Mahabad	66	TU.IG14	Turkey-Iğdır
5	IR.U5	Iran-Urmia	36	IR.MA4	Iran-Mahabad	67	IR.U20	Iran-Urmia
6	IR.U6	Iran-Urmia	37	IR.N2	Iran-Naghadeh	68	IR.U21	Iran-Urmia
7	IR.U7	Iran-Urmia	38	IR.Q1	Iran-Qaraziaediin	69	IR.U22	Iran-Urmia
8	IR.U8	Iran-Urmia	39	IR.Q2	Iran-Qaraziaediin	70	IR.U23	Iran-Urmia
9	IR.U10	Iran-Urmia	40	IR.Q3	Iran-Qaraziaediin	71	IR.O6	Iran-Oshnavieh
10	IR.U11	Iran-Urmia	41	IR.Q4	Iran-Qaraziaediin	72	IR.P5	Iran-Piranshahr
11	IR.U12	Iran-Urmia	42	IR.Q5	Iran-Qaraziaediin	73	IR.P6	Iran-Piranshahr
12	IR.U13	Iran-Urmia	43	IR.Q6	Iran-Qaraziaediin	74	IR.P7	Iran-Piranshahr
13	IR.U14	Iran-Urmia	44	IR.Q7	Iran-Qaraziaediin	75	IR.P8	Iran-Piranshahr
14	IR.U15	Iran-Urmia	45	IR.Q8	Iran-Qaraziaediin	76	IR.P9	Iran-Piranshahr
15	IR.U16	Iran-Urmia	46	IR.KH1	Iran-Khoy	77	IR.P10	Iran-Piranshahr
16	IR.O1	Iran-Oshnavieh	47	IR.KH2	Iran-Khoy	78	IR.SR2	Iran-Sardasht
17	IR.O2	Iran-Oshnavieh	48	IR.SA1	Iran-Salmas	79	IR.SR3	Iran-Sardasht
18	IR.O3	Iran-Oshnavieh	49	IR.SA2	Iran-Salmas	80	IR.SR4	Iran-Sardasht
19	IR.O4	Iran-Oshnavieh	50	IR.U18	Iran-Urmia	81	IR.SR5	Iran-Sardasht
20	IR.O5	Iran-Oshnavieh	51	IR.SR1	Iran-Sardasht	82	IR.SR6	Iran-Sardasht
21	IR.P1	Iran-Piranshahr	52	IR.U19	Iran-Urmia	83	IR.SR7	Iran-Sardasht
22	IR.P2	Iran-Piranshahr	53	TU.IG1	Turkey-Iğdır	84	IR.SR8	Iran-Sardasht
23	IR.P3	Iran-Piranshahr	54	TU.IG2	Turkey-Iğdır	85	IR.MA5	Iran-Mahabad
24	IR.P4	Iran-Piranshahr	55	TU.IG3	Turkey-Iğdır	86	IR.MA6	Iran-Mahabad
25	IR.N1	Iran-Naghadeh	56	TU.IG4	Turkey-Iğdır	87	IR.MA7	Iran-Mahabad
26	IR.MI1	Iran-Miandoab	57	TU.IG5	Turkey-Iğdır	88	IR.MA8	Iran-Mahabad
27	IR.MI2	Iran-Miandoab	58	TU.IG6	Turkey-Iğdır	89	IR.MA9	Iran-Mahabad
28	IR.MI3	Iran-Miandoab	59	TU.IG7	Turkey-Iğdır	90	IR.MA10	Iran-Mahabad
29	IR.MI4	Iran-Miandoab	60	TU.IG8	Turkey-Iğdır	91	IR.U24	Iran-Urmia
30	IR.MI5	Iran-Miandoab	61	TU.IG9	Turkey-Iğdır	92	IR.U25	Iran-Urmia
31	IR.MI6	Iran-Miandoab	62	TU.IG10	Turkey-Iğdır	93	IR.U26	Iran-Urmia

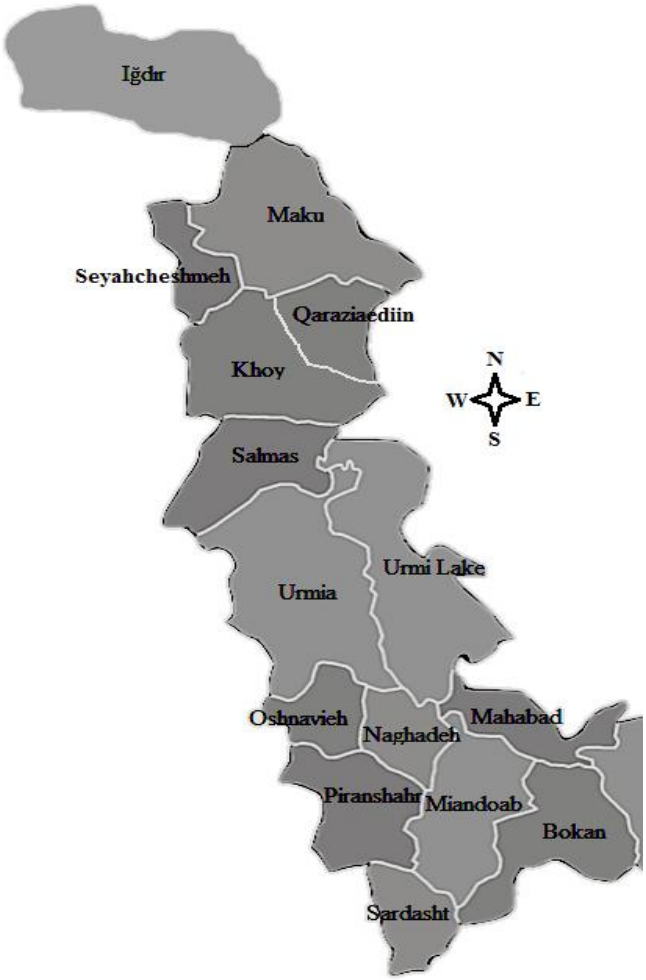

Figure 1. Geographical locations of the tomato landraces used in the current study

DNA extraction and PCR procedure

Genomic DNA was extracted from young leaves of three weeks seedlings of five plants from each genotype using CTAB method (SAGHAI-MAROOF *et al*., 1984) with a few modifications intended (two consecutive extractions with Chloroform-isoamyl alcohol). DNA concentration and quality were assessed by spectrophotometer (NanoDrop 1000) and 0.8% agarose gel electrophoresis. PCR amplifications were performed in a volume of 10 µl containing 1X PCR buffer, 2.5 mM $MgCl_2$, 0.5 mM dNTP, 10 pmol primer, 0.5 U Taq DNA polymerase and 30 ng of template DNA. The amplifications were carried out in a MultiGene gradient thermal cycler TC9600-G-230V (Labnet International Inc.) programmed for an initial denaturation of 4 min at

94ºC, followed by 35 cycles consisting of 40 s at 94ºC, 40 s at 53 to 56 ºC depending on the primer, 2 min at 72ºC, and a extension of 6 min at 72ºC. The amplification products were separated by polyacrylamide Mega-Gel dual vertical electrophoresis (model C-DASG-400-50) at 300 volts for 2.5 to 3 h (WANG *et al*., 2003; UTOMO *et al*., 2009). The gel solution consisted of 6% (w/v) acrylamid/bisacrylamid (19:1) in 0.5 TBE buffer, 0.07% (w/v) ammonium persulfate, and 0.08% (w/v) TEMED. The gel was visualized under UV light and photographed. This study was carried out in Laboratory of Genetic and Biotechnology, Department of Field Crops, Agriculture Faculty, Ataturk University, Turkey in 2014.

Data analysis

For statistical analysis, ISSR markers were binary-coded using 1 indicating the presence, and 0 indicating the absence of a band for in-line bands in each genotype. Number of loci, number of polymorphic loci, percentage of polymorphic loci (PPL), mean of expected heterozygosity (He), Shannon's information index (I) and number of effective alleles (Ne) were calculated in order to characterize the capacity of each primer to detect polymorphism and to evaluate the discriminating ability of each primer in this collection. These parameters were estimated using GenAlEx 6.41 (PEAKALL and SMOUSE, 2006). Cluster analysis was performed using the method of Minimum Evolution and Number of differences coefficients using the Mega 4 program. To identify population structure of genotypes, Structure 2.3.1 software (PRITCHARD *et al*., 2000) was run with a "burn-in" setting of 10000 followed by 50000 MCMC iterations using the admixture model at different values of K (K from 1 to 20). Optimal K value was determined according to the method of K delta. Pair-wise F-statistics (Fst) values were estimated among sub-populations.

RESULTS AND DISCUSSION

Out of 20 ISSR primers tested, 14 primers produced clear amplified products and discernible banding pattern (Table 2). Fourteen primers amplified 185 visible and scorable loci which all were polymorphic (100%). The number of total loci per primer ranged from 6 (UBC867 and UBC820) to 26 (UBC855), with an average of 13.2. In a genetic diversity assessment of 41 tomato accessions using 12 ISSR primers, 120 amplified bands were produced, 59 (57.8%) of which were polymorphic (TERZOPOULOS and BEBELI, 2008). High percentage of polymorphism detected in our study might be due to the high number of landraces used, use of polyacrylamide instead agarose gel for separation of ISSR bands and vast geographical distribution of the origins of the landraces. Noteworthy, high genetic diversity in this germplasm has been also observed for morphological characteristics (HENAREH, 2015). For example, fruit weight ranged from 8.8 to 232.4 gr; nine different fruit shapes and four distinct fruit color were observed in this tomato germplasm. Also, high polymorphism in polyacrylamide gel for ISSR markers has been already reported in grapevine (MORENO *et al.*, 1998). The maximum and minimum values of heterozygosity, Shannon's information index and number of effective alleles were observed for primers UBC848 and UBC808, respectively (Table 2). Primers UBC848 and A7, with 0.3 and 0.29 values of highest heterozygosity, have (CA) and (AG) repetitive motifs. In general, primers with (AC), (AG), (GA), (CT) and (TC) repeats show higher polymorphism than primers with other di-, tri- or tetra-nucleotide repeats (REDDY *et al*., 2002). In study of foxtail millet (genotype Yugu1) genome, more dinucleotide fragments containing AT, TA, AG, GA, CT and TC units were isolated, compared with other kinds of dinucleotide repeats (ZHANG *et al*., 2014).

Cluster analysis using number of differences coefficients and method of the Minimum Evolution placed the genotypes into nine clusters (Figure 2). The most of Urmia genotypes (73%), three commercial cultivars and a few number of the other genotypes located in cluster I. The dendrogram revealed close relationship among three commercial cultivars. These commercial cultivars are similar in view of some morphological traits such as fruit size, fruit firmness, fruit ripening and vegetative growth. The most of genotypes this group was alike in fruit firmness and plant size and these genotypes were suitable for processed food industries. Cluster II consisted of 78.65% of Iğdır genotypes, 50% of Sardasht genotypes and 15.5% of Urmia landraces. Landraces grouped in this group, had soft fruit and most of these genotypes were fresh market tomato. The remaining Iğdır genotypes, IR.U19 and IR.P5, located in cluster III. The landraces of this group had intermediate fruit firmness, intermediate fruit size and flat shape at blossom end of fruit. The genotypes of fourth cluster showed morphological similarity in view of depression at peduncle end of fruit, fruit pH and vegetative growth. In cluster V, six genotypes from different regions were placed. Group VI with 11 genotypes consisted landraces with large fruits. The genotypes placed in cluster VII were similar for morphological characters such as fruit size, fruit shape, fruit firmness, fruit ripening, pH and total soluble solids (TSS). Out of 8 landraces situated in group VIII, 4 genotypes belonged to Miandoab region. Traits fruit firmness, shape at blossom end of fruit and fruit ripening of these genotypes were alike. 83.3% landraces of Qaraziaediin, Khoy and Salmas regions with intermediate fruit firmness and early ripening were located in group IX.

Table 2. Primer sequences, annealing temperatures, total loci, polymorphic loci, PPL, He, I and Ne of the ISSR primers used in the current study

Primer	Sequnce (5′→ 3′)	Anealing temperature	Total loci	Polymorphic loci	PPL	He	I	Ne
A7	$(AG)_{10}T$	54	9	9	100	0.29	0.452	1.446
UBC811	$(GA)_8C$	54	19	19	100	0.193	0.331	1.257
UBC867	$(GGC)_8$	54	6	6	100	0.233	0.374	1.378
UBC820	$(GT)_8C$	55	6	6	100	0.265	0.407	1.452
A12	$(GA)_6CC$	53	19	19	100	0.223	0.362	1.342
UBC848	$(CA)_8R*G$	54	8	8	100	0.3	0.468	1.478
UBC855	$(AC)_8Y*T$	56	26	26	100	0.174	0.297	1.241
UBC818	$(CA)_8G$	54	7	7	100	0.236	0.376	1.381
UBC849	$(GT)_8CG$	54	13	13	100	0.202	0.334	1.296
UBC808	$(AG)_8C$	54	18	18	100	0.153	0.268	1.202
UBC840	$(GA)_8Y*T$	54	9	9	100	0.222	0.36	1.323
UBC815	$(CT)_8T$	54	10	10	100	0.180	0.304	1.251
UBC 880	$(GGAGA)_3$	53	25	25	100	0.184	0.321	1.239
430	$(TGG)_7A$	56	11	11	100	0.247	0.405	1.345
Total			185	185				
Mean			13.2	13.2	100	0.224	0.365	1.336

PPL: percentage of polymorphic loci, He: mean of expected heterozygocity, I : Shannon's information index, Ne : number of effective alleles

Results from the structure analysis of the current germplasm are given in Figure 3. The highest likelihood of the data was obtained when population were grouped into 2 sub-populations (K = 2). Low values of K obtained in this study could be due to the high amount of gene flow between the collected regions of the landraces. It is important to note that the vicinity of the genotypes in cluster analysis were similar to the results obtained by structure analysis. Clusters 1, 2, 3, 4 (58 genotypes) placed in sub-population 1 and groups 5, 6, 7, 8, 9 (38 genotypes) in sub-population 2. These sub-populations (1and 2) had Fst values of 0.16 and 0.21 respectively. Fst is frequently used as a summary of genetic differentiation among groups. It has been suggested that Fst depends on the allele frequencies at a locus, as it exhibits a variety of peculiar properties related to genetic diversity (JAKOBSSON *et al*., 2013). Nevertheless genetic variation in sub-population 2 was more than that of sub-population 1.

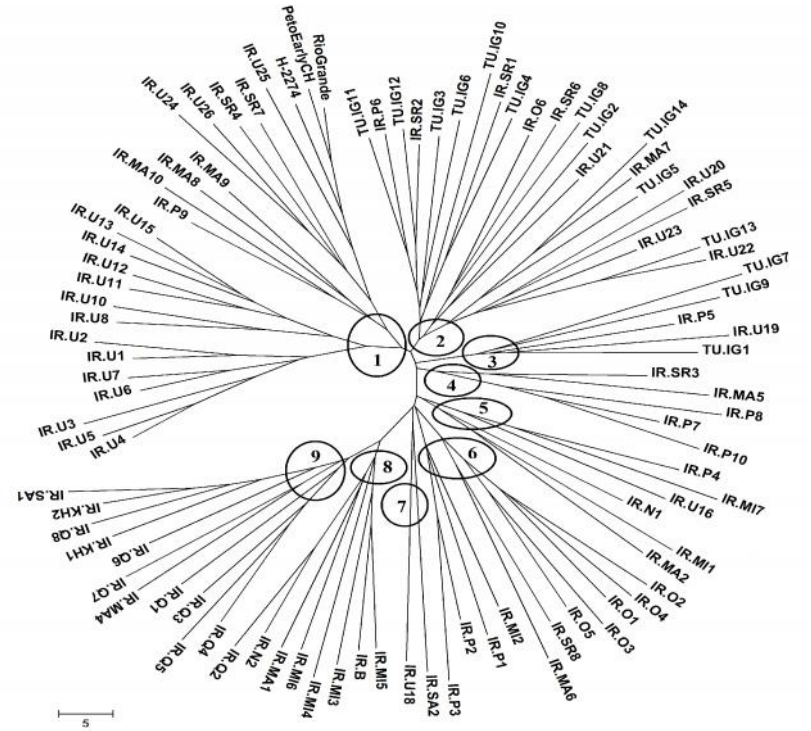

Figure 2. Genetic distances of the tomato landraces and commercial cultivars obtained using 14 ISSR primers constructed with Minimum Evolution clustering using number of differences coefficients

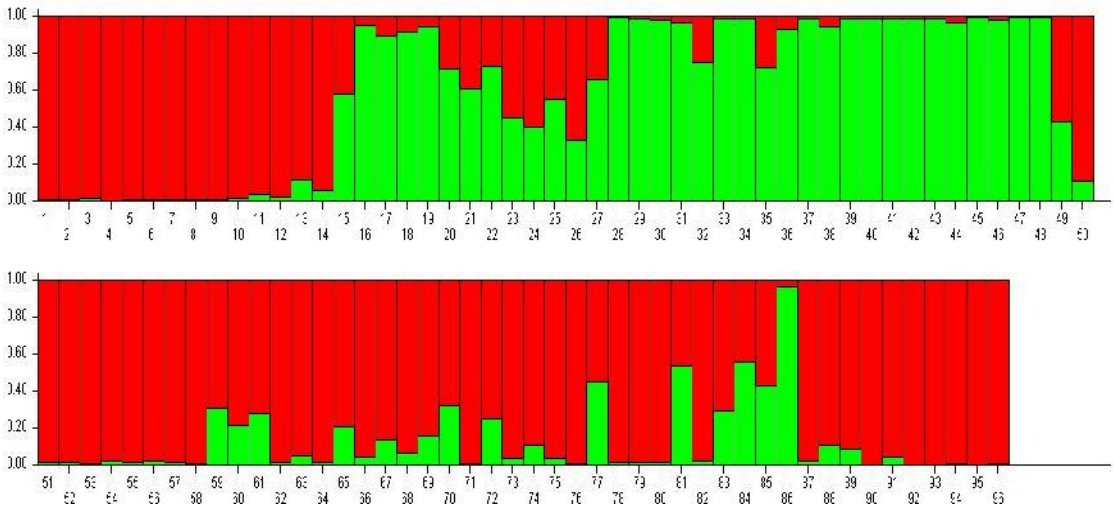

Figure 3. A Bayesian model-based clustering of analysed landraces and cultivars. Bar plots show the membership coefficient estimate (Q) for each landrace and cultivar for the inferred clusters with maximum log-likelihood probability. Bar colours and lengths represent inferred clusters and Q, respectively, identified by STRUCTURE for K = 2. Numbers in the figure show the tomato landraces according Table 1, 94 = Peto Early CH, 95 = Rio Grande and 96 = H-2274.

CONCLUSIONS

The current study demonstrated the existence of high genetic variation in the tomato germplasm studied. This genetic variation can be explored in tomato breeding programs for hybrid cultivars production. Also, grouping of the tomato landraces was in concordance with their geographical distribution areas. The genotypes originating from the same region, often located in the same group or two adjacent groups. Finally, our study established that the ISSR marker could be used as an efficient tool for studying genetic diversity among tomato landraces collected from diverse geographical locations, but different molecular marker systems need to be combined for an accurate and comprehensive assessment of genetic diversity.

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UTVRĐIVANJE GENETIČKOG DIVERZITETA KULTIVARA PARADAIZA PRIMNEOM ISSR MARKERA

Mashhid HENAREH[1*], Atilla DURSUN[2], Babak ABDOLLAHI MANDOULAKANI[3], Kamil HALİLOĞLU[4]

[1]Zapadni Ardžebedžan poljoprivredni i istraživački centar za prirodne resurse, Urmia, Iran.
[2]Odeljenje hortikulture, Poljoprivrenbdi fakultet, Ataturk univerzitet,Erzurum, Turska.
[3]Odeljenje za oplemenjivanje I biotehnologiju,Poljoprivredni fakultet,Urmia Univerzitet, Urmia, Iran.
[4]Odeljenje za ratarstvo, Poljoprivredni fakultet, Ataturk Univerzitet, Erzurum, Turska

Izvod

Cilj istraživanja je spitivanje genetičkog variranja 93 kultivara paradaiza uporedo sa tri komercijalne sorte u regionima istočne Anadolije u Turskoj i Severozapadnom regionu Irana primenom 14 ISSR markera. Procenat polimorfnih lokusa (PPL) je bio 100. Prosečna očekivana heterozigotnost (He) prajmera je varirala od 0.15 (UBC808) do 0.30 (UBC848). Kultivari i ispitivane sorte su se u dendogramu grupisale u deve=t grupa. Genotipovi poreklom iz istog rgeiona su često grupisani u istom klasteru. Najveća verovatnoća podataka je dobijena kada su se populacije locirale u 2 sub - populacije (K = 2) . The highest likelihood of the data was obtained when population were located into 2 sub-populations (K = 2). Fst vrednost ovih populacija je bio 0,16 I 0,21.

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