J. Agr. Sci. Tech. (2020) Vol. 22(4): 1067-1082

Genetic Structure and Mixed Linear Model-Based Association Analysis for Morphological Traits in a Collection of Tomato Landraces from Iran and Turkey

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ABSTRACT

To extend the genetic base of Iranian tomato germplasm, 93 landraces were collected from the northwest of Iran and East Anatolian of Turkey, along with three commercial cultivars, and their genetic structure were studied using 39 SSR primers. Thirty-five polymorphic SSR loci generated a total of 118 alleles in the studied germplasm. Number of alleles per locus and effective number of alleles averaged 3.37 and 2.47, respectively. Expected heterozygosity of SSRs varied from 0.227 (TMS24) to 0.773 (LEta016), averaged 0.558. The mean number of alleles per genomic-SSRs (3.61) was more than that of EST-SSRs (2.66). Cluster analysis using Neighbour Joining (NJ) method placed 96 tomato genotypes in eight groups. Little congruence was found between NJ dendrogram and geographical distances. Genetic structure analysis of the germplasm using Bayesian method revealed two sub-populations and separated cherry tomatoes from the other landraces and commercial cultivars. Out of the 21 morphological characters, significant ($P \leq 0.05$) marker-trait associations were found for 18 characters. Each of SSR loci TC11, TC948, and Tom236-237 was associated with three characters. The genetic variability, structure, and markers associated with the studied traits in the current study can be used for planning tomato breeding programs and future studies.

Keywords: Association mapping, Bayesian clustering, *Solanum lycopersicum*, SSR.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), is one of the most economically important and widely cultivated plant in *Solanaceae* family (Kulus, 2018a). Because of high homozygosity, ease of controlled hybridization, small genome (900 Mbp), lack of gene duplication, and availability of a large number of mutants and genetic resources, tomato has been a good model system for plant genetic studies (The tomato genome consortium, 2012; Kulus, 2018b). Landrace populations are a significant part of genetic variation in crop species and usually characterized by a good stress tolerance and local adaptability (Corrado *et al*., 2014). Population bottlenecks and both natural and artificial selections occurred during domestication, and new cultivars production have reduced genetic variation in cultivated tomato germplasm (Foolad, 2007; Kulus, 2019). Also, lack of conservation of primary genotypes has caused an overall reduction in the genetic basis of tomato germplasms in the world in recent decades, making it difficult to

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[DOR: 20.1001.1.16807073.2020.22.4.4.6]

identify polymorphisms between elite germplasm (Sim *et al*., 2009).

In the last three decades, most of the farmers in northwest of Iran and East Anatolian of Turkey cultivate tomato hybrids introduced from countries such as USA and Italy. Genetic variation of tomato has decreased in both regions during this time period because of the continuous replacement of many landraces by modern tomato cultivars. In recent years, the cultivation of tomato landraces has been significantly increased in Iran and programs have been started for genetic improvement of these genotypes, but the lack of information about their genetic diversity and structure has limited their utilization in breeding programs (Henareh *et al*., 2015). Globally, several molecular markers have been developed for precise assessment of genetic diversity in plant species, of which Simple Sequence Repeats (SSRs) are the most widely used, because of their polymorphism, reproducibility, and codominant nature (Abdollahi Mandoulakani *et al*., 2015; Amoozadeh *et al*., 2015; Emanuelli *et al*., 2013). The efficiency and usefulness of SSR markers for study of genetic variation in tomato has been demonstrated (He *et al*., 2003; Garcia-Martinez *et al*., 2006; Mazzucato *et al*., 2010; Todorovska *et al*., 2014).

Polygenic inheritance of the quality-related traits in plants makes their genetic description a very challenging task. The availability of genetic stocks and public databases, the appearance of Next Generation Sequencing (NGS)-based genotyping and the increased exploiting natural genetic variability make association mapping an ideal and reliable strategy to identify genes involved in quantitative variation of complex polygenic traits (Ruggieri *et al*., 2014; Tranchida-Lombardo *et al*., 2018). All morpho-physical and fruit quality-related association studies published in tomato to date have stated the usefulness and reliability of this method for dissecting quantitative traits (Mazzucato *et al*., 2008; Ranc *et al*., 2012; Shirasawa *et al*. 2013; Xu *et al*., 2013; Tranchida-Lombardo *et al*., 2018).

To extend the genetic base of Iranian tomato germplasm, 93 landraces were collected from northwest of Iran and East Anatolian of Turkey and an investigation was designed to describe the genetic variability of these tomato landraces using SSR markers for providing fundamental information to utilize these genetic resources in tomato breeding programs. Association between fruit quality and morphological traits and SSR markers were also investigated in this collection.

MATERIALS AND METHODS

Plant Materials and Phenotypic Data

Plant material (Table S1) consisted of 93 tomato landraces (79 from northwest of Iran and 14 from East Anatolian of Turkey) and commercial cultivars Rio Grande, Peto Early CH, and H-2274. The code of each genotype was defined according to the name of the collected geographical origin. The field trial was carried out at Kahriz Station of Agriculture and Natural Resources Research Centre of West Azerbaijan (Urmia, Iran) during 2012 and 2013. To assess the phenotypic diversity, 21 morphological traits (Table 1) were computed based on Union for the Protection Of new Varieties of plants (UPOV) descriptor. Morphological data were averaged for the two years and minimum, maximum, mean, genotypic variance and heritability of the traits were calculated.

DNA Extraction and SSR Analysis

Young leaves of each genotype were used to extract genomic DNA using CTAB method (Saghai-Maroof *et al*., 1984). DNA quality and concentration were determined by spectrophotometer (NanoDrop 1000) and 0.8% agarose gel electrophoresis.

Thirty-nine SSR primer pairs (Table 2) (Areshchenkova and Ganal, 2002; He *et al*., 2003; Bredemeijer *et al*., 2002; Mazzucato *et al*., 2010; Garcia-Martinez *et al*., 2006; Mazzucato *et al*., 2008; Areshchenkova and Ganal, 1999; Areshchenkova, 2000), were used to assess genetic variability in the

[DOR: 20.1001.1.16807073.2020.22.4.4.6]

Table 1. Phenotypic diversity among the tomato genotypes.[a]

Trait	Min	Max	Mean	σ^2_g	h^2 (%)
Cotyledon leaf length (cm)	3.1	5.2	4.1	0.42**	95.45
Cotyledon leaf width (mm)	4.6	7.2	6	0.76**	97.43
Leaf length (cm)	11.3	30.9	23.08	47.43**	99.08
Leaf width (cm)	6.3	22.1	13.63	21.73**	98.64
Days to flowering	72	86	79.86	16.65**	69.46
Flowers/Inflorescence	3.7	7.2	4.82	0.95**	91.34
Fruit set/Cluster (%)	51.5	95	72.64	165.4**	78.20
Fruits/Plant	8	143.7	30.35	1697.69**	98.38
Fruit weight (g)	8.8	232.4	117	7432.66**	99.48
Days to fruit maturity	113.3	143.8	129.9	56.65**	81.54
Fruit diameter (cm)	2.1	9	5.9	4.93**	97.82
Fruit length (cm)	2.5	7.5	5.5	3.49**	97.49
Days to 50% fruit maturity	136.5	172.8	155.7	99.8**	96.64
Pericarp thickness (mm)	2.7	8.8	6.05	3.86**	96.74
Carpels/Fruit	2	12.4	4.91	8.18**	97.73
Seeds/Fruit	40.4	244.5	128.3	5270.41**	97.52
Fruit peduncle length (cm)	1.7	3.6	2.71	0.3**	88.23
Total soluble solids	3.4	6.8	5.03	0.77**	95.08
pH	4.07	4.5	4.28	0.02**	83.33
Acidity	0.34	1.17	0.652	0.07**	93.33
Yield/Plant (kg)	1.4	3.3	2.17	0.35**	87.5

[a] σ^2_g: Genotypic variance, h^2: Heritability. ** Significance at 0.01 level of probability.

studied germplasm. Out of the primer pairs used, 11 were EST-SSRs. PCR amplifications were performed in a volume of 10 µL containing 1X PCR buffer (10 mM Tris–HCl, 50 mM KCl, 1.5 mM MgCl2), 0.5 mM dNTP, 2.5 mM MgCl2, 25 pmol of each primer, 1U Taq DNA polymerase and 50 ng of template DNA. The amplifications were performed in a MultiGene gradient thermal cycler TC9600-G-230V (Labnet International Inc.) with a first denaturation at 94°C for 4 minutes and 35 cycles of 94°C for 1 minute, 50-61°C for 1 minute and 72°C for 2 minutes and a final extension of 72°C for 7 minutes. The PCR products were resolved on 6% (w/v) denaturing polyacrylamide gels (model C-DASG-400-50) at 300 volts for 1.5 to 2.5 hours and visualized under UV light.

Genetic Diversity and Population Structure Analysis

The genotype of the individuals was scored at each locus according to the length of the amplified SSR bands. To characterize the capacity of each primer for polymorphism detection in the studied germplasm, Number of alleles (Na), Number of effective alleles (Ne), Shannon's Information index (I) and mean of expected Heterozygosity (He) were calculated for each locus and the entire studied germplasm using the GenAlEx6.5 software (Peakall and Smouse, 2012).

Cluster analysis was performed using MEGA 4 (Tamura *et al*., 2007) by Neighbour Joining (NJ) method. To investigate the population structure, Bayesian model-based approach was used in the software STRUCTURE 2.3.4 (Pritchard *et al*., 2000) with the no-admixture model and correlated allele frequencies among populations. The number of subpopulations (k) with 10 independent runs were set from 1 to 20 and burn in period and MCMC iterations, both to 100,000. The mean of Fixation index (F_{ST}) values for the clusters obtained from STRUCTURE, were also estimated. STRUCTURE HARVESTER was used to determine the optimal number of k (Evanno *et al*., 2005; Earl and vonHoldt, 2012).

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Table 2. Characteristics of the 35 SSR loci used in the current study.[a]

No.	SSR name	Ta	LG	Na*	Na	Ne	I	He	Allele size (bp)
1	EST268259 (E)	55	1	2	2	1.994	0.692	0.499	122-135
2	EST245053 (E)	58	1	2	2	1.958	0.682	0.489	221-226
3	TMS63 (G)	58	1	3	3	1.457	0.579	0.314	140-158
4	TMS24 (G)	54	2	-	3	1.293	0.450	0.227	362-385
5	TMS2 (G)	54	3	-	4	2.604	1.055	0.616	365-405
6	TMS8 (G)	55	3	-	2	1.929	0.675	0.482	460-496
7	TC11 (E)	58	4	3	2	1.843	0.650	0.457	95-105
8	EST259379 (E)	55	4	3	2	1.367	0.439	0.268	138-150
9	TMS22 (G)	56	4	4	3	1.709	0.737	0.415	155-168
10	TMS39 (G)	58	5	5	3	2.992	1.097	0.666	118-136
11	TMS37 (G)	55.5	5	6	4	3.141	1.196	0.682	186-201
12	EST253712 (E)	56	6	4	3	1.879	0.802	0.468	141-156
13	TC1843 (E)	58	7	3	4	2.375	1.037	0.579	528-593
14	TC948 (E)	58	8	2	3	1.812	0.793	0.448	143-184
15	EST248494 (E)	59	8	2	2	1.913	0.670	0.477	203-207
16	TMS29 (G)	55	8	3	3	2.174	0.844	0.540	340-372
17	Tom236-237 (G)	55	9	3	3	2.378	0.977	0.579	210-255
18	TMS43(G)	54.5	9	2	2	1.732	0.614	0.423	332-346
19	TMS4 (G)	50	10	3	3	2.392	0.978	0.582	225-235
20	TC461 (E)	56	11	3	4	2.216	1.006	0.549	191-204
21	TMS42 (G)	54	11	5	3	2.179	0.917	0.541	282-298
22	TMS52 (G)	53	12	9	5	3.578	1.430	0.721	158-171
23	TMS9 (G)	53	12	5	5	3.347	1.388	0.701	330-358
24	TMS33 (G)	57.5	12	4	4	3.028	1.203	0.670	257-276
25	TMS48 (G)	54	12	3	3	2.289	0.904	0.563	178-200
26	TMS23 (G)	54	12	3	3	2.739	1.053	0.635	382-418
27	TMS7 (G)	51	12	4	4	2.052	0.788	0.513	161-174
28	LEta024 (G)	55	-	4	4	2.983	1.213	0.665	170-188
29	LEtat002 (G)	59	-	3	3	2.684	1.034	0.627	198-207
30	LEta003 (G)	61	-	4	4	3.762	1.353	0.734	142-164
31	LEta019 (G)	58	-	5	5	3.765	1.458	0.734	318-360
32	LEta020 (G)	58	-	4	4	2.459	1.045	0.593	198-208
33	LEta012 (G)	60	-	5	4	2.203	0.999	0.546	364-406
34	LEat002 (G)	59.5	-	4	4	3.826	1.364	0.739	236-255
35	LEta016 (G)	60	-	6	6	4.405	1.579	0.773	208-230
Mean					3.37	2.47	0.963	0.558	
Total					118				

[a] E: EST-SSR, G: Genomic-SSR, Ta: Annealing temperature, LG: Linkage Group, Na*: Number of alleles detected in previous studies, Na: Number of alleles, Ne: Effective Number of alleles, I: Shannon's Information index, He: Mean of expected Heterozygocity.

[DOR: 20.1001.1.16807073.2020.22.4.4.6]

Association Mapping Analysis

Pair-wise r^2 between 35 SSR loci and their *P*-values (using 1000 permutations) were estimated using TASSEL 3 (Bradbury *et al*., 2007). This parameter was calculated for each Linkage Group (LG) and for genomic- and EST-SSRs as well. To identify marker-trait associations, Mixed Linear Model (MLM), which incorporates both Q- and kinship (K)-matrices as covariates in the analysis, was used. K-matrix, the matrix of pair-wise relationship of genotypes, was estimated based on SSR data using the software TASSEL 3. The Q-matrix was obtained at K= 2 using STRUCTURE 2.3.4. A threshold for significant associations was adopted at a False Discovery Rate (FDR) of 0.01 using Bonferroni's correction (Šidàk, 1967).

RESULTS

Morphological Analysis

Analysis of variance revealed significant differences ($P \leq 0.01$) and a large range of variation among genotypes for all the characters studied. For example, percentage of fruit set per cluster ranged from 51.5 to 95, number of fruits per plant from 8 to 143.7, fruit weight from 8.8 to 232.4 g, Total Soluble Solids (TSSs) from 3.4 to 6.8 and yield per plant from 1.4 to 3.3 kg. The heritability varied from 68.5% for days to flowering to 99.48% for fruit weight (Table 1).

Genetic Diversity

Out of the 39 SSR loci used for germplasm genotyping, 35 loci (89.74%) generated 118 alleles (Table 2). Loci TC1107 and EST258529 amplified monomorphic banding pattern and loci TMS35 and TMS60 failed to yield PCR fragments. The number of alleles per locus ranged from 2 (EST268259, EST245053, TMS8, TC11, EST259379, EST248494 and TMS43) to 6 (LEta016), averaged 3.37. Size of the allele fragments varied from 95 (TC11) to 593 bp (TC1843). The minimum and maximum of Ne, I, and He were observed for loci TMS24 and LEta016, respectively. These parameters in the studied landraces averaged 2.47, 0.963, and 0.558, respectively.

Cluster Analysis

Cluster analysis using NJ method placed 96 genotypes in eight groups (Figure 1). Out of the 16 landraces located in the first group, eight were from two adjacent regions of Piranshahr and Sardasht. Three commercial cultivars were placed in the second group in the vicinity of each other. Most of the landraces originating from Urmia were located in cluster IV. Cherry tomato landraces constituted 45.5 and 85.7% of the landraces in groups V and VII, respectively. Landraces collected from Iğdır (Turkey) distributed in different clusters.

Population Structure

Inferring the appropriate number of clusters using STRUCTURE HARVESTER

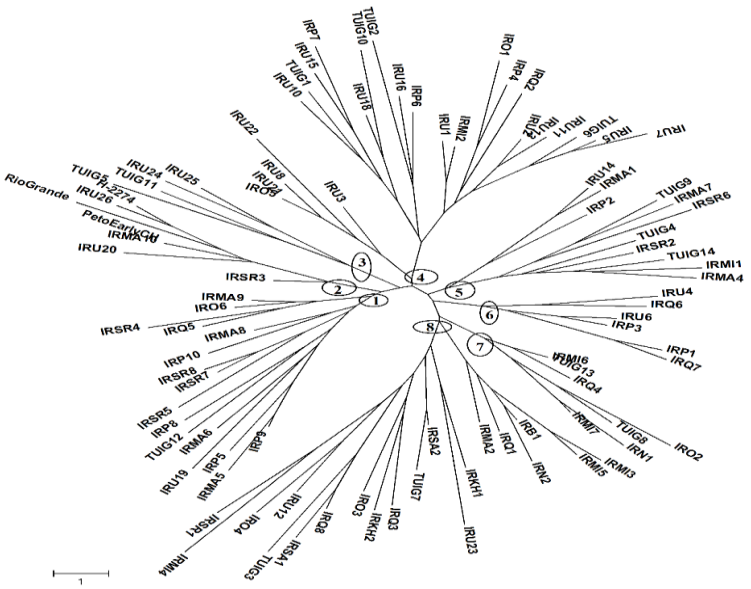

Figure 1. Neighbor joining tree of the 93 tomato landraces and three commercial cultivars using 35 SSR loci.

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showed the highest peak at k= 2 (Figure S1), suggesting two genetically distinct groups in the analyzed tomato germplasm (Figure 2). F_{ST} values of the groups were 0.13 and 0.20, respectively.

Following setting the number of clusters to two, inferred ancestry estimates of genotypes (Q-matrix) was obtained for the subpopulation using STRUCTURE output (Table S2). Model-based clustering put cherry tomatoes in group I and separated them from the remaining landraces. Of the eight tomato landraces from Sardasht, seven were cherry tomatoes (group I). A lot of landraces originating from the divers geographical locations along with commercial cultivars were placed in cluster II.

LD Decay and Association Mapping Analysis

The LD extent (r^2) in the studied germplasm (Figure 3) ranged from 0.001 (LG 5) to 0.057 (LG 12), averaging 0.018. LD extent for genomic-SSRs (0.019) was slightly more than that of EST-SSRs (0.011).

Out of the 21 studied traits, associated markers were found for 18 traits (Table 3). Seven markers (29.16%), out of the 24 associated markers, were EST-SSRs. No linked SSR markers were detected for cotyledon leaf width, days to flowering, and fruit weight. Only one associated marker was identified for each trait of leaf length and width, carpel numbers in fruit, seed numbers in fruit, TSS, and yield. The most number of the associated markers (five markers) were found for pericarp thickness, three markers on LG 12 (year 2012) and two markers on LGs 4 and 8 (year 2013). All three markers associated with cotyledon leaf length in both years were common. The identified associated markers for all traits (exept for pericarp thickness) were on different LGs. Two out of the three markers associated with each trait fruit set/cluster and fruit lenght were the same in both years. Marker LEta016 was associated with number of days to 50% fruit maturity in both years and explained 15.3 and 14.7% of the variation of this trait in 2012 and 2013, respectively. Marker LEta020 showed significant association with TSS only in 2012 and illustrated 12.1% of its variation. Markers EST259379, TMS29, LEta020 and EST253712 were associated with pH and markers TMS63 and TMS7 were associated with acidity. Marker TMS23 on LG 12 revealed significant association with yield and explained 9.5% of the yield total variation. Marker TC11 was associated with cotyledon leaf lenght, days to fruit maturity, and days to 50% fruit maturity. Associated markers Tom236-237 were common for fruit set/cluster, days to fruit maturity, and fruit peduncle lenght. Marker TMS7 was also associated with fruits/plant, fruit lenght and

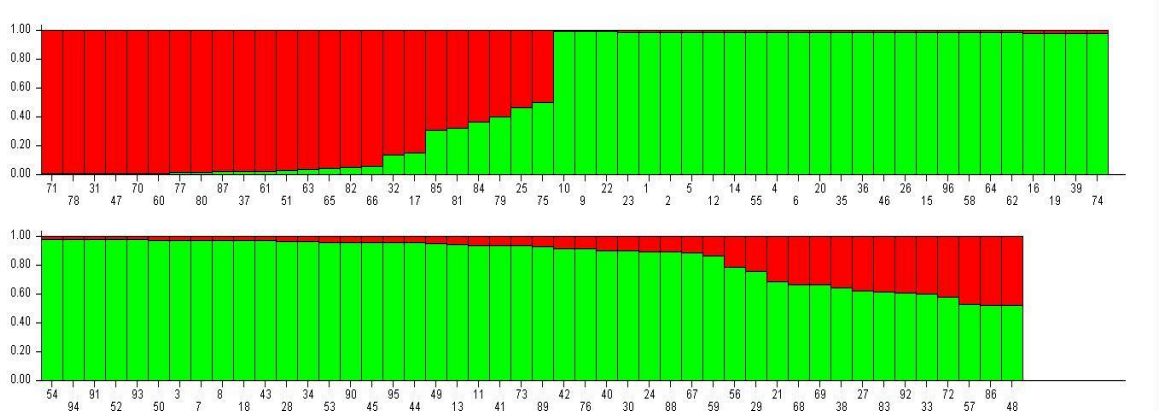

Figure 2. A Bayesian model-based clustering of the analyzed landraces demonstrated the occurrence of two clusters within the tomato germplasm based on 35 SSR loci. Bar colours and lengths represent inferred clusters and Q, respectively, identified by STRUCTURE for K= 2.

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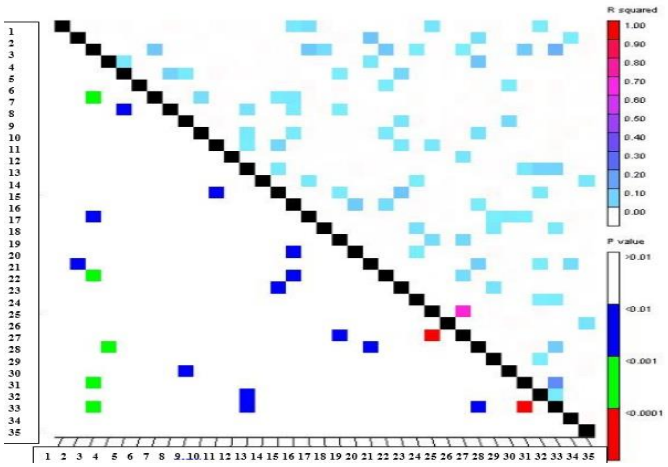

Figure 3. Linkage Disequilibrium (LD) values (r^2) throughout the tomato genome. Markers were ordered on the x and y axes. Marker numbers corresponded to Table 2. Each cell of the heat map represents a single marker pair. The r^2 values for each marker pair are on the top half of the heat map and are represented from 0.0 (white) increasing equal increments of 0.1 to 1.0 (red). The *P*-values of each r^2 estimate are on the bottom half of the heat map and are represented from non-significant (*P*> 0.05; white) to highly significant (*P*< 0.0001; red).

Table 3. List of the markers linked to various traits and their R^2 and associated *P*-values. [a]

Marker	LG	2012		2013		Marker	LG	2012		2013	
		R^2	*P*-value	R^2	*P*-value			R^2	*P*-value	R^2	*P*-value
Cotyledon leaf length						Days to 50% fruit maturity					
TC11	4	0.049	0.038	0.062	0.022	LEta016	-	0.153	0.040	0.147	0.048
TMS37	5	0.112	0.022	0.119	0.021	TC11	4	-	-	0.062	0.028
TMS48	12	0.072	0.044	0.089	0.026	Pericarp thickness					
Leaf length						TMS9	12	0.121	0.027	-	-
TMS43	9	-	-	0.046	0.033	TMS48	12	0.089	0.014	-	-
Leaf width						TMS23	12	0.082	0.023	-	-
TC948	8	0.089	0.017	0.099	0.010	EST259379	4	-	-	0.050	0.019
Flowers/ Inflorescence						TC948	8	-	-	0.067	0.033
EST245053	1	0.084	0.035	-	-	Carpels/Fruit					
TMS39	5	0.088	0.049	-	-	TMS39	5	-	-	0.118	0.009
LEta019	-	0.164	0.019	-	-	Seeds/Fruit					
Fruit set/ Cluster (%)						TMS4	10	0.095	0.025	0.089	0.033
Tom236-237	9	0.080	0.025	0.063	0.046	Fruit peduncle length					
LEtat002	-	0.07	0.038	0.064	0.042	EST268259	1	0.047	0.038	-	-
TMS2	3	-	-	0.1	0.042	TMS8	12	0.041	0.036	-	-
Fruits/Plant						Tom236-237	9	0.063	0.040	-	-
TMS37	5	0.115	0.026	-	-	TSS					
TMS7	12	0.14	0.011	-	-	LEta020	-	0.121	0.038	-	-
Days to fruit maturity						pH					
TC11	4	0.049	0.044	-	-	EST259379	4	0.068	0.022	-	-
Tom236-237	9	0.109	0.013	-	-	TMS29	8	0.149	0.018	-	-
TMS4	10	-	-	0.087	0.026	LEta020	-	0.129	0.024	-	-
Fruit diameter						EST253712	6	0.094	0.025	-	-
TC461	11	0.09	0.046	0.092	0.049	Acidity					
TC948	8	-	-	0.115	0.011	TMS63	1	0.102	0.019	-	-
Fruit length						TMS7	12	-	-	0.096	0.034
TMS8	3	0.039	0.047	-	-	Yield/Plant					
TMS43	9	0.045	0.04	0.039	0.035	TMS23	12	0.095	0.014	-	-
TMS7	12	0.119	0.009	0.068	0.041						

[a] LG: Linkage group.

[DOR: 20.1001.1.16807073.2020.22.4.4.6]

acidity. Markers TMS37, TMS48, TMS43, TMS39, TMS23, EST259379 and LEta020 were found to be associated each with two traits.

DISCUSSION

Generally, genetic diversity in plants detectable by molecular markers depends on the reproduction mode, the domestication history, and the size of the analyzed samples. First studies with molecular markers have clearly indicated low level of genetic diversity in the cultivated tomato germplasm in contrast to other self-pollinating species (Williams and Clair, 1993). High numbers of alleles per polymorphic SSR locus (8.5) were reported for several wild tomato accessions (Alvarez *et al*., 2001) while cultivated tomato germplasm generated values close to 2.5 (He *et al*., 2003; Tam *et al*., 2005). Early studies also indicated that traditional cultivars from South America maintained more genetic diversity than modern tomato cultivars (Williams and Clair, 1993).

The number of alleles per locus in our study averaged 3.37. The Ne, I, and He in the landraces averaged 2.47, 0.963, and 0.558, respectively. In a genetic diversity study of 30 tomato genotypes using 25 SSR loci, Dhaliwal *et al*. (2011) reported a value of 2.86 for average number of alleles per locus. In assessment of genetic diversity in 61 accessions of Italian cultivated tomato using 29 SSRs, He was recorded 0.44 (Mazzucato *et al*., 2008). The high number of alleles per locus and He detected in our study may be due to the wide geographical regions of the collection sites and high numbers of the studied landraces. The less number of alleles per locus, found for EST-SSRs compared to genomic SSRs, might be attributed to the the intensive protection of sequences and low frequency of mutation in coding regions of the genome (Ellis and Burke, 2007; Zeng *et al*., 2010). In diversity assessment of 36 *Gossypium* species using 20 genomic- and 27 EST-SSRs, the average number of alleles per locus was 2.33 and 3.6, respectively (Tabbasam *et al*., 2014).

Grouping obtained with NJ cluster analysis was not in concordance with geographical distances of the landraces and did not give a reasonable category. This might be due to the gene flow among regions, even in two countries (Iran and Turkey). Despite having genotypes with different fruit shape, genetic structure analysis divided the studied germplasm into two genetically distinct groups. F_{ST} (a measure of population differentiation due to genetic structure) value of the groups was 0.13 and 0.20, respectively. F_{ST} values of 0 and 1 show non-differentiation and perfect differentiation between an original population and its sub-populations, respectively. The F_{ST} range from 0 to 0.05 indicates small genetic differentiation, the ranges from 0.05 to 0.15, 0.15 to 0.25, and above 0.25 exhibits moderate, large, and very large genetic differentiation, respectively (Cho *et al*., 2008). Nevertheless, in our study, genetic variation in sub-populations 1 and 2 were moderate and large, respectively.

Population structure analysis separated cherry tomatoes from the remaining genotypes. In study of 48 Spanish tomato genotypes using 19 SSRs and 7 AFLPs (Garcia-Martinez *et al*., 2006) and 35 Brazilian cultivars and landraces using 20 RAPDs (Carelli *et al*., 2006), similar results were also obtained. Cherry tomatoes have small fruits, characterized by small leaves and flowers, a lot of flowers and fruits per plant, a lot of seed per fruit and high vegetative growth. These characters can be found in *S. pimpinellifolium*. The investigations have demonstrated that the genome of *S. lycopersicum* var. cerasiforme is a mixture of *S. lycopersicum* and *S. pimpinellifolium* genomes due to the frequent hybridizations between these species (Nesbitt and Tanksley, 2002; Ranc *et al*., 2008). These reasons may explain why cherry tomatoes constituted a separate cluster.

SSR markers used in our study were applied to identify marker-trait associations. In the recent years, association mapping has been widely used to identify candidate genes affecting complex quantitative traits (Hall *et*

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al., 2010). Unbiased estimation of LD and population structure in the used collection are the prerequisites of the association mapping studies (Fusari *et al*., 2008). LD over genetic distance is high in tomato and decayed at 6-8 cM within 102 tomato varieties, 6-14 cM within 39 processing varieties, and 3-16 cM within 24 fresh market varieties (Robbins *et al*., 2011). The low level of LD (0.018) was observed in the whole collection in the current study, although more SSR markers with enough genome coverage are needed to have a thorough estimation of the r^2.

The results of association mapping studies were influenced by a number of factors including type and size of mapping population, traits examined, number of environments and years used for phenotyping, and type and genome coverage of molecular markers (Ruggieri *et al*., 2014). As previously reported for tomato (Ranc *et al*., 2012), the size of our tomato collection was enough for association mapping studies. The population used in our study represented a huge amount of diversity for most of the traits targeted. To identify associated markers with low level of interactions with environment, phenotyping was performed in two years, although more phenotyping data over several years and environments are needed for identification of reliable associated markers for further breeding programs.

Since previous investigations demonstrated the high efficiency of the MLM method in detecting false associations in tomato populations (Ranc *et al*., 2012), this model was used in our study and idetified 24 associated markers for 18 traits. Markers LEta016 (R^2= 15.3%) and TMS37 (R^2= 14.7%) (associated with days to 50% fruit maturity and cotyledon leaf length, respectively) would be interesting for marker-assisted selection because of the high R^2 values and stability in both years. Markers TMS7 and TMS39 were highly associated (*P*= 0.009) with fruit length and carpels/fruit, respectively. The highly significant associated markers showing a great effect on targeted traits might be appropriate candidates for future marker assisted selection programs, although such markers should be validated in different mapping populations or germplasms. Three markers associated with cotyledon leaf length and two out of the three markers associated with fruit set/cluster and fruit lenght were similar in both years. Marker LEta020 with a R^2 value of 12.1% had significant association with TSS only in 2012. Markers EST259379, TMS29, LEta020 and EST253712 were associated with pH and markers TMS63 and TMS7 were associated with acidity. In contrast to our investigation, Mazzucato *et al*. (2008) reported that EST253712 was associated with fruit weight, locule number and inflorscence type. They also indicated association between TMS63 and friut shape. This probably suggests the pleiotropy effects of these SSR loci. Marker TC11 showed to be significantly associated with cotyledon leaf lenght, days to fruit maturity and days to 50% fruit maturity. Significant association was also detected between marker TC948 and leaf width, fruit diameter and pericarp thickness. Possitive significant correlation has been already reported among these traits (Henareh *et al*., 2016). Associated marker Tom236-237 was common for fruit set/cluster, days to fruit maturity, and fruit peduncle lenght. In another investigation, this marker was significantly associated with green shoulder (Mazzucato *et al*., 2008). Several other markers such as TMS7, TMS37, TMS48, TMS43, TMS39, TMS23, EST259379 and LEta020 were found to be associated each with more than one trait. The pleiotropic effects of the same genes or genetic linkage could be the reasons of such co-localized associations, as previously shown for QTLs (Lecomte *et al*., 2004).

In conclusion, phenotypic evaluation on the 21 studied traits revealed a broad phenotypic variability within the tomato collection investigated. Population structure analysis clearly differentiated cherry tomato landraces from the remaining ones, but grouping of tomato landraces was not in congruence with their geographical information. This study revealed that tomato landraces grown in these regions have maintained enough genetic diversity that would be valuable for utilization in tomato breeding programmes. These

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Table S1. Description of the tomato landraces used in the current study.

Code	Fruit size	Fruit shape	Origin	Longitude (° ′)	Latitude (° ′)	Code	Fruit size	Fruit shape	Origin	Longitude (° ′)	Latitude (° ′)
IRU1	L	Obc	I-U	45 14	37 32	IRMI7	S	F	I-Mi	46 08	36 59
IRU2	L	Obc	I-U	45 13	37 30	IRB	I	Ci	I-B	46 13	36 34
IRU3	L	Obl	I-U	45 13	37 30	IRMA1	I	Obo	I-Ma	45 47	36 56
IRU4	L	Obl	I-U	45 13	37 26	IRMA2	L	Obl	I-Ma	45 48	36 52
IRU5	L	Ci	I-U	45 08	37 30	IRMA4	I	Obo	I-Ma	45 45	36 50
IRU6	I	O	I-U	45 11	37 23	IRMA5	S	Obl	I-Ma	45 41	36 42
IRU7	L	Co	I-U	45 11	37 23	IRMA6	S	Obl	I-Ma	45 40	36 44
IRU8	I	Obl	I-U	45 13	37 29	IRMA7	S	Cy	I-Ma	45 40	36 44
IRU10	I	Obc	I-U	45 09	37 23	IRMA8	L	Obl	I-Ma	45 41	36 42
IRU11	L	Obc	I-U	45 05	37 26	IRMA9	L	Co	I-Ma	45 44	36 48
IRU12	L	F	I-U	45 05	37 26	IRMA10	I	Co	I-Ma	45 44	36 48
IRU13	I	Co	I-U	44 58	37 52	IRQ1	L	Obl	I-Q	45 02	38 54
IRU14	I	O	I-U	45 02	37 51	IRQ2	I	Obo	I-Q	45 02	38 53
IRU15	I	Obl	I-U	45 02	37 51	IRQ3	L	Obl	I-Q	44 57	38 53
IRU16	I	Obo	I-U	45 01	37 50	IRQ4	L	F	I-Q	44 57	38 53
IRU18	L	Ci	I-U	45 02	37 59	IRQ5	I	Obc	I-Q	45 02	38 50
IRU19	I	O	I-U	44 59	37 57	IRQ6	L	Obc	I-Q	45 02	38 50
IRU20	I	Co	I-U	44 58	37 58	IRQ7	I	Co	I-Q	45 02	38 50
IRU21	L	Ci	I-U	45 03	37 43	IRQ8	I	Obc	I-Q	45 08	38 46
IRU22	L	Obc	I-U	45 02	37 37	IRKH1	I	Obl	I-K	45 12	38 42
IRU23	Vs	P	I-U	44 51	37 25	IRKH2	S	Ci	I-K	44 50	38 34
IRU24	L	Obl	I-U	45 10	37 42	IRSA1	S	O	I-Sal	44 45	38 10
IRU25	I	Ci	I-U	45 10	37 42	IRSA2	L	Obl	I-Sal	44 44	38 09
IRU26	I	Obo	I-U	45 03	37 41	IRSR1	Vs	Ci	I-Sar	45 33	36 12
IRO1	I	O	I-O	45 07	37 09	IRSR2	Vs	Ci	I-Sar	45 30	36 16
IRO2	S	Obl	I-O	45 07	37 09	IRSR3	S	Ci	I-Sar	45 30	36 16
IRO3	I	Obl	I-O	45 06	37 02	IRSR4	Vs	O	I-Sar	45 30	36 04
IRO4	I	Obl	I-O	45 06	37 02	IRSR5	S	Obl	I-Sar	45 30	36 04
IRO5	L	Co	I-O	45 08	36 59	IRSR6	S	Ci	I-Sar	45 30	36 04
IRO6	Vs	P	I-O	45 07	37 12	IRSR7	I	F	I-Sar	45 30	36 04
IRP1	L	F	I-P	45 19	36 47	IRSR8	S	Obl	I-Sar	45 28	36 17
IRP2	L	Obc	I-P	45 19	36 47	TUIG1	I	Obl	T-I	43 59	39 57
IRP3	I	Obc	I-P	45 12	36 51	TUIG2	L	Co	T-I	43 58	39 59
IRP4	I	Ci	I-P	45 14	36 49	TUIG3	I	Co	T-I	43 58	39 59
IRP5	I	P	I-P	45 07	36 37	TUIG4	I	Co	T-I	43 59	40 00
IRP6	I	Obc	I-P	45 07	36 37	TUIG5	I	F	T-I	44 04	40 01
IRP7	I	O	I-P	45 05	36 48	TUIG6	L	Co	T-I	44 04	40 01
IRP8	S	Co	I-P	45 11	36 42	TUIG7	I	F	T-I	44 04	40 01
IRP9	L	F	I-P	45 11	36 42	TUIG8	S	Obl	T-I	44 04	40 01
IRP10	S	Co	I-P	45 13	36 39	TUIG9	S	Obl	T-I	44 04	40 01
IRN1	S	O	I-N	45 30	37 00	TUIG10	I	Co	T-I	44 01	39 58
IRN2	S	F	I-N	45 15	36 59	TUIG11	S	Obl	T-I	44 01	39 58
IRMI1	I	Obo	I-Mi	46 01	36 55	TUIG12	L	Co	T-I	44 01	39 58
IRMI2	L	Ci	I-Mi	46 01	36 55	TUIG13	Vs	Ci	T-I	44 01	39 58
IRMI3	I	Co	I-Mi	46 10	36 56	TUIG14	S	Obl	T-I	44 01	39 52
IRMI4	I	O	I-Mi	46 10	36 56	Peto Early CH	I	O	I		
IRMI5	I	Co	I-Mi	46 08	37 00	Rio Grande	I	Obo	I		
IRMI6	S	Obl	I-Mi	46 08	36 59	H-2274	I	Ci	T		

Fruit size (L: Large, I: Intermediate, S: Small, Vs: Very small); Fruit shape (Obc: Obcordate, Obl: Oblate, Ci: Circular, O: Ovate, Co: Cordate, F: Flattened, Obo: Obovate, P: Pyriform, Cy: Cylindrical); Origin (I-U: Iran-Urmia,I-O: Iran-Oshnavieh, I-P: Iran-Piranshahr, I-N: Iran-Naghadeh, I-Mi: Iran-Miandoab, I-B: Iran-Bokan, I-Ma: Iran-Mahabad, I-Q: Iran-Qaraziaediin, I-K: Iran-Khoy, Iran-Sal: Iran-Salmas, I-Sar: Iran-Sardasht, T-I: Turkey-Iğdır, I: Iran, T: Turkey).

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Table S2 . The estimated cluster membership coefficients of tomato landraces obtained with STRUCTURE software at K=2.

Genotype code	Genotype number	Sub-population		Genotype code	Genotype number	Sub-population	
		I	II			I	II
IRU1	1	0.009	0.991	IRMI7	32	0.863	0.137
IRU2	2	0.009	0.991	IRB	33	0.393	0.607
IRU3	3	0.24	0.976	IRMA1	34	0.032	0.968
IRU4	4	0.10	0.990	IRMA2	35	0.010	0.990
IRU5	5	0.009	0.991	IRMA4	36	0.010	0.990
IRU6	6	0.10	0.990	IRMA5	85	0.687	0.313
IRU7	7	0.25	0.975	IRMA6	86	0.474	0.526
IRU8	8	0.26	0.974	IRMA7	87	0.978	0.022
IRU10	9	0.007	0.993	IRMA8	88	0.106	0.894
IRU11	10	0.006	0.994	IRMA9	89	0.070	0.930
IRU12	11	0.063	0.937	IRMA10	90	0.038	0.962
IRU13	12	0.009	0.991	IRQ1	38	0.357	0.643
IRU14	13	0.054	0.946	IRQ2	39	0.016	0.984
IRU15	14	0.009	0.991	IRQ3	40	0.093	0.907
IRU16	15	0.012	0.988	IRQ4	41	0.064	0.936
IRU18	50	0.022	0.978	IRQ5	42	0.082	0.918
IRU19	52	0.021	0.979	IRQ6	43	0.027	0.973
IRU20	67	0.113	0.887	IRQ7	44	0.042	0.958
IRU21	68	0.330	0.670	IRQ8	45	0.040	0.960
IRU22	69	0.330	0.670	IRKH1	46	0.010	0.990
IRU23	70	0.987	0.013	IRKH2	47	0.990	0.010
IRU24	91	0.019	0.981	IRSA1	48	0.478	0.522
IRU25	92	0.391	0.609	IRSA2	49	0.044	0.956
IRU26	93	0.021	0.979	IRSR1	51	0.971	0.029
IRO1	16	0.015	0.985	IRSR2	78	0.991	0.009
IRO2	17	0.843	0.157	IRSR3	79	0.594	0.406
IRO3	18	0.026	0.974	IRSR4	80	0.979	0.021
IRO4	19	0.015	0.985	IRSR5	81	0.672	0.328
IRO5	20	0.010	0.990	IRSR6	82	0.943	0.057
IRO6	71	0.991	0.009	IRSR7	83	0.384	0.616
IRP1	21	0.310	0.690	IRSR8	84	0.635	0.365
IRP2	22	0.007	0.993	TUIG1	53	0.037	0.963
IRP3	23	0.008	0.992	TUIG2	54	0.017	0.983
IRP4	24	0.106	0.894	TUIG3	55	0.009	0.991
IRP5	72	0.416	0.584	TUIG4	56	0.208	0.792
IRP6	73	0.064	0.936	TUIG5	57	0.470	0.530
IRP7	74	0.016	0.984	TUIG6	58	0.013	0.987
IRP8	75	0.500	0.500	TUIG7	59	0.134	0.866
IRP9	76	0.082	0.918	TUIG8	60	0.986	0.014
IRP10	77	0.980	0.020	TUIG9	61	0.976	0.024
IRN1	25	0.535	0.465	TUIG10	62	0.014	0.986
IRN2	37	0.977	0.023	TUIG11	63	0.964	0.036
IRMI1	26	0.011	0.989	TUIG12	64	0.013	0.987
IRMI2	27	0.378	0.622	TUIG13	65	0.952	0.048
IRMI3	28	0.029	0.971	TUIG14	66	0.938	0.062
IRMI4	29	0.241	0.759	PetoEarlyCH	94	0.017	0.983
IRMI5	30	0.097	0.903	RioGrande	95	0.040	0.960
IRMI6	31	0.990	0.010	H-2274	96	0.012	0.988

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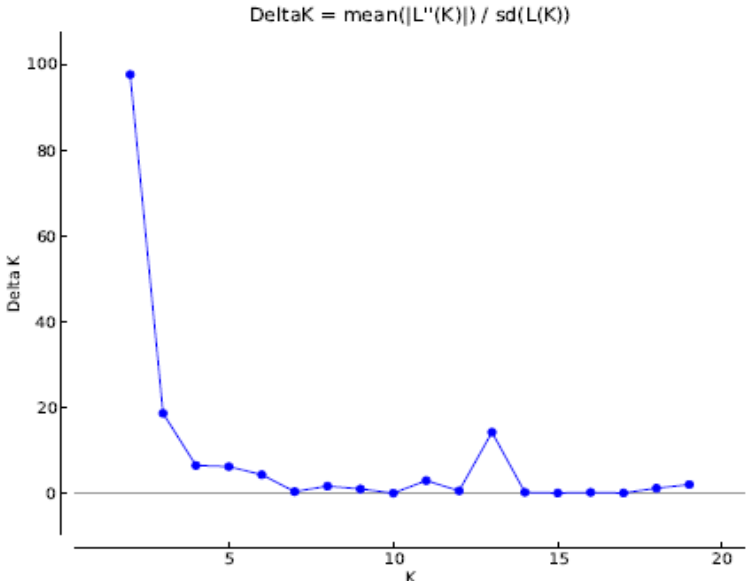

Figure S1. Estimation of the optimum number of sub-populations for tomato genotypes according to the Evanno's method. The graph shows DeltaK for each K value.

landraces are well adapted to the growing environments of the collection sites and stresses, therefore, we suggest to replace some modern cultivars by elite landraces. The association mapping approach used allowed detection of 24 SSRs associated with 18 traits. The use of the markers highly associated with a given trait in both years could be a valuable starting point for marker-aided selection. The findings suggest that use of SSR markers and a highly valid statistical model (MLM) are appropriate for identification of the associations with the traits targeted. In addition, identified SSRs could be exploited as markers aiming the specific-interest traits for assisted selection in tomato breeding programs. A further validation and confirmation of the markers in a different set of accessions or mapping populations would be in any case necessary.

ACKNOWLEDGEMENTS

This work was supported by Laboratory of Genetic and Biotechnology, Department of Field Crops, Agriculture Faculty, Ataturk University, Turkey and West Azerbaijan Agricultural and Natural Resources Research Center, AREEO, Urmia, Iran.

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[DOR: 20.1001.1.16807073.2020.22.4.4.6]

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ساختار ژنتیکی و تجزیه ارتباط صفات مورفولوژیکی بر اساس مدل خطی مخلوط در ارقام محلی گوجه‌فرنگی ایران و ترکیه

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چکیده

به منظور گسترش پایه ژنتیکی ژرم‌پلاسم گوجه‌فرنگی ایران، ۹۳ رقم محلی از شمال‌غرب ایران و منطقه آناتولی شرقی ترکیه جمع‌آوری و ساختار ژنتیکی آنها به همراه سه رقم تجاری با ۳۹ جفت آغازگر SSR مطالعه شد. ۳۵ مکان چندشکل SSR در مجموع ۱۱۸ آلل تولید کردند. متوسط تعداد آلل در هر مکان و تعداد آلل موثر به ترتیب ۳/۳۷ و ۲/۴۷ آلل بود. هتروزیگوسیتی مورد انتظار در آغازگرها از ۰/۲۲۷ (TMS24) تا ۰/۷۷۳ (LEta016) متغیر و میانگین آن ۰/۵۵۸ بود. میانگین تعداد آلل SSRهای ژنومی (۳/۶۱) بیشتر از EST-SSRها (۲/۶۶) بود. تجزیهٔ خوشه‌ای با روش Neighbour joining، ۹۶

[DOR: 20.1001.1.16807073.2020.22.4.4.6]

ژنوتیپ گوجه‌فرنگی را در هشت گروه قرار داد. همگرایی کمی بین گروهبندی حاصل تجزیه خوشه‌ای و فواصل جغرافیایی ارقام وجود داشت. تجزیه ساختار ژنتیکی با استفاده از روش Bayesian، ژرم‌پلاسم مورد مطالعه را به دو گروه تقسیم کرد و ارقام گوجه‌فرنگی‌های ریز (چری) را از سایر ارقام محلی و تجاری متمایز کرد. متوسط شاخص تثبیت (F_{ST}) برای دو گروه ۰/۱۳ و ۰/۲ بود. از ۲۱ صفت مورفولوژیک مورد مطالعه، ۱۸ صفت با نشانگرهای SSR ارتباط معنی‌داری ($P\leq0.05$) نشان دادند. نشانگرهای TC11، TC948 و Tom236-237 هر کدام با سه صفت ارتباط معنی‌داری نشان دادند. نتایج حاصل از مطالعه تنوع و ساختار ژنتیکی و نشانگرهای پیوسته شناسایی شده در این تحقیق، در طراحی برنامه‌های اصلاحی گوجه‌فرنگی و در مطالعات آینده مورد استفاده قرار گیرد.

[DOR: 20.1001.1.16807073.2020.22.4.4.6]