



Genetic Diversity and Phylogenetic Relationships of Turkish Local Popcorn (*Zea mays everta*) Populations analyzed by Simple Sequence Repeats (SSRs) Markers

Gulay ZULKADIR^{a*} , Leyla IDIKUT^b 

^aDepartment of Organic Agriculture Management, Applied Technology and Management School of Silifke, Mersin University, Silifke, Mersin, TURKEY

^bDepartment of Crop Field, Faculty of Agriculture, Kahramanmaraş Sutcu Imam University, Kahramanmaraş, TURKEY

ARTICLE INFO

Research Article

Corresponding Author: Gulay ZULKADIR, E-mail: gulayzulkadir@gmail.com

Received: 30 September 2019 / Revised: 06 December 2019 / Accepted: 26 December 2019 / Online: 31 May 2021

ABSTRACT

Maize (*Zea mays everta*) is preferred as a good dietary in Turkey and it is important to know its genetic diversity to improve the yield. Genetic markers are very important in determining genetic diversity in popcorn populations. The aim of this study was to evaluate the genetic diversity of landraces popcorn populations by simple sequence repeats (SSR) markers. A hundred seventy five accessions of popcorn from thirty five populations grown in Turkey were analyzed using twenty SSR markers.

As a result of molecular analysis, 65 of 66 alleles obtained were showed polymorphisms and the polymorphism rate was 98.5%. The average number of alleles for each SSR loci was 3.3, and this the number

of alleles varied from 1 to 5. The average the polymorphism information content (PIC) value was calculated to be 0.57 for SSR locus ranging from 0.00 to 0.89. The number and percentage of polymorphic loci of the genotypes were determined to vary between 29/47% and 43.94/71.21 % and the mean values were calculated as 39.114 and 59.265 % respectively. The value of genetic change in the phylogenetic tree obtained from landraces popcorn populations was determined as 0.05, and the genetic difference among genotypes varied from 14.7 to 97.1%. Among the markers used in the study, it was observed that code 'phi064' was the most effective marker for determining genetic diversity in popcorn and the highest allele frequency also on this marker was obtained.

Keywords: Genetic diversity, Landrace, Molecular characterization, Popcorn, SSR

© Ankara University, Faculty of Agriculture

1. Introduction

Maize is the major cereal growing all over the world and it is a considerable cereal ranked as the third in world cereal production after wheat and rice (Adjanohoun et al. 2011). One of the commercially produced plants in the corn variety is popcorn. Popcorn (*Zea mays everta*) can be easily distinguished by plant and seed characteristics among other maize varieties.

Popcorn is preferred in terms of its rich nutritional content, vitamins and minerals. Also, corn is a good dietary product with stomach acidic absorption properties, low calorie and whole grain. The multi-purpose use of the corn has led to intensive research on the plant. Through the breeding studies in popcorn, different breeding methods are applied to ensure suitability for the purpose of efficiency such as adaptation, aquaculture and quality criteria. In order to obtain the necessary variation in breeding activities, registered varieties, local varieties and wild relatives should be screened and the appropriate genes should be transferred to the cultivars by improved techniques. The success in plant breeding is first and foremost provided by an efficient, accurate and rapid selection (Frankel 1972).

Maize spread from Central America to the other regions of the world. In course of time, it adapted to the extreme climatic conditions and thus, now it is characterized by a high degree of genetic variability. Morphological characterization is highly affected by the environment conditions therefore it indicates variability (Aci et al. 2013). On the other hand, molecular characterization is not affected by the environmental conditions and it provides valuable genetic information (Gauthier et al. 2002). In order to reveal the crucial genetic information, molecular markers such as microsatellites (SSR) are used (Yao et al. 2008; Liu et al. 2009; Eschholz et al. 2010) and have been very helpful for determining the diversity.

The aim of this study was to analyze the genetic diversity among different popcorn populations via SSRs markers on the samples collected from the different regions in Turkey.

2. Material and Methods

2.1. Plant material

In this study, one hundred and seventy five accessions of popcorn were used from total 35 population that grown in various cities of Turkey including 34 landraces populations and one standard variety (Table 1). This collection was provided by Department of Plant Gene Resources in Ege Agricultural Research and Antalya West Mediterranean Agricultural Research Institute (Nermin Cin 98 as Standard variety).

Table 1- Genotype No, Registration No, Province, Region, Altitude and Material Color Information of the material used in the research

<i>Genotype No</i>	<i>Registration No</i>	<i>Province</i>	<i>Region</i>	<i>Altitude</i>	<i>Material Color</i>	<i>Genotype No</i>	<i>Registration No</i>	<i>Province</i>	<i>Region</i>	<i>Altitude</i>	<i>Material Color</i>
1	TR79913*	Canakkale	Biga	40	Yellow	19	TR78053*	Kutahya	Simav	950	Yellow
2	TR79947*	Balikesir	Gonen	120	Red	20	TR78181*	Usak	Sivas	970	Yellow
3	TR79947*	Balikesir	Gonen	120	Yellow	21	TR76375*	Diyarbakir	Cungus	939	Yellow
4	TR79947*	Balikesir	Gonen	120	Pied	22	TR73761*	Eskisehir	Gunyuzu	916	Yellow
5	TR79987*	Balikesir	Bigadic	437	Dark Red	23	TR73698*	Eskisehir	Beylikova	789	Yellow
6	TR79987*	Balikesir	Bigadic	437	Orange	24	TR74311*	Kayseri	Hacilar	1479	Yellow
7	TR73836*	Eskisehir	Gunyuzu	991	Yellow	25	TR85132*	Tokat	Erbaa	---	Yellow
8	TR73836*	Eskisehir	Gunyuzu	991	Orange	26	TR37977*	Tokat	Merkez	560	Light Yellow
9	TR79988*	Balikesir	Bigadic	437	White	27	Ordu - Dogulu	Ordu	Dogulu	---	Red
10	TR79988*	Balikesir	Bigadic	437	Yellow	28	Konya Pop	Konya	---	---	Red
11	TR73746*	Eskisehir	Gunyuzu	916	Orange	29	Nermin Cin**	---	---	---	Yellow
12	TR73746*	Eskisehir	Gunyuzu	916	Light Orange	30	Tokat Erbaa	Tokat	Erbaa	---	Yellow
13	TR39601*	Artvin	Ardanuc	1300	Red	31	Samsun Bafra	Samsun	Bafra	---	Orange
14	TR79932*	Canakkale	Can	103	White	32	Ordu-Akpınar	Ordu	Akpınar	---	Light Yellow
15	TR78115*	Afyon	İncehisar	1140	Yellow	33	Ordu-Kovanlı	Ordu	Kovanlı	---	Yellow
16	TR76741*	Tekirdag	Sarkoy	120	Dark Red	34	TR54215*	Mugla	Fethiye	1130	Yellow
17	TR38027*	Amasya	Sukuova	400	White/ Yellow	35	TR54215*	Mugla	Fethiye	1130	White
18	TR74236*	Kastamonu	Taskopru	896	Orange						

*, Genotype codes obtained from the Plant Gene Resources Department of Ege Agricultural Research Institute; **, Standard variety. Others populations was collected from various parts of Turkey

2.2. DNA extraction

DNA isolation and SSR analysis were carried out in Kahramanmaraş Sutcu Imam University, Faculty of Agriculture, Department of Field Crops Laboratory and University - Industry - Public Cooperation Development, Application and Research Center (USKIM).

Maize seeds were grown in the greenhouse. Five plants (at 4-5 leave stage) were taken randomly from each population and stored at -80 °C. Single-plant samples were ground to powder in liquid nitrogen using a mortar and pestle. A total genomic DNA was extracted following a modified procedure by Doyle & Doyle (1987).

2.3. SSR analysis

Twenty SSR primer pairs of maize were selected from two of each chromosome, however it was used in previous studies and reported to be effective (Table 2).

Table 2- Name of the SSR markers, Chromosome locations, DNA Sequences and Base Numbers used in the study

Primer Name	Chromosome	DNA Sequence (5'→3')	Primer Name	Chromosome	DNA Sequence (5'→3')
Umc1186	F	TCAAGAACATAATAGGAGGCCAC	Phi015	F	GCAACGTACCGTACCTTCCGA
	6.02			8.00	
	R	AGCCAGCTTGATCTTTAGCATTTG		R	ACGCTGCATTCAATTACCGGAAG
Umc1622	F	CGCTACAAATCCTACTGGTGCTTT	Phi021	F	TTCCATTCTCGTGTCTTGAGTGGTCCA
	2.00			4.00	
	R	CCTCGGATTTTCCAAAACATTTCT		R	CTTGATCACCTTCTCTGCTGTCGCCA
Umc1550	F	CGGGTAATTGGGTACATAACCTC	Phi022	F	TGCGCACCAGCGACTGACC
	4.03			9.00	
	R	GTGCCTCCAACGCCTAGTTTTT		R	GCGGGCGACGCTTCCAAAC
Phi095	F	CCGATCGGCTTTATCACTGTTTAGC	Phi027	F	CACAGCACGTTGCGGATTTCTCT
	1.03			9.03	
	R	ATGCACCATTCTAGCACTATAGCAACACT		R	GCGTACGTACGACGAAGACAC
Umc2101	F	CCCGGCTAGAGCTATAAAGCAAGT	Phi034	F	TAGCGACAGGATGGCCTCTTCT
	3.00			7.00	
	R	CTAGCTAGTTTGGTGCGTGGTGAT		R	GGGGAGCACGCCTTCGTCT
Umc1255	F	GGACTACATCACGCCGGAGAT	Phi064	F	CCGAATTGAAATAGCTGCGAGAACCT
	4.11			1.00	
	R	TTTGGGAGAACAATCGGTTCTGTA		R	ACAATGAACGGTGGTTATCAACACGC
Phi017	F	CGTTGGCGACCAGGGTGC GTTGGAT	Phi084	F	AGAAGGAATCCGATCCATCCAAGC
	9.02			10.0	
	R	TGCAACAGCCATTCGATCATCAAAC		R	CACCCGTA CTTGAGGAAAACCC
Phi057	F	CTCATCAGTGCCGTCGTC CAT	Phi127	F	ATATGCATTGCCTGGA ACTGGAAGGA
	7.01			2.00	
	R	CAGTCGCAAGAAACCGTTGCC		R	AATTCAAACACGCCTCCCGAGTGT
Umc1164	F	AAATAAACGCTCCAAAGAAAGCAA	Umc2050	F	CTCCTGCTGTGATTCTAGGACGA
	4.01			3.00	
	R	GCACGTGTGTGTGTGTTGTTTTTA		R	CTGGATCTCGGCATGGTCTT
Umc1173	F	ATCCGCCAAAAAGGGGAAAA	Umc2292	F	AGCAGAAGAGGACAAACCAGATTC
	4.09			5.00	
	R	TAGAAGTAGCACACGCGCCG		R	ACTTCCGGCATGTCTTGTGTTT

The total volume of PCR mixture was 20 µL containing 2 µL ddH₂O, 3.5 µL 10X PCR buffer (Mg⁺² added), 1.2 µL dNTP (5 mM), 4 µL F primer (20 µM), 4 µL R primer (20 µM), 5 µL Genomic DNA (100 ng), 0.3 µL DNA Taq polymerase (5 U µL⁻¹, Fermentas).

The PCR reaction was performed in a thermal cycler (Eppendorf Mastercycler Gradient) using an initial 94 °C denaturing step for 5 min followed by 34 cycles of [denaturation at 94 °C for 1 min, annealing for 1 min at the primer's annealing temperature, extension at 72 °C for 1 min] and a final extension at 72 °C for 5 min.

2.4. Data analysis

The presence (1) or absence (0) of PCR amplicons were coded. Then the data base was registered in an MS Excel spreadsheet in order to generate the analysis matrix. Genetic diversity parameters such as Polymorphism Information Content (PIC) as previously described by Laborda et al. (2005); polymorphism rate (P), number of alleles (Na), expected heterozygosity (He) and Shannon's phenetic index (H) were estimated according to the method used by Nei 1972. Cluster analysis by Un-weighted Pair Group Method using Arithmetic Averages (UPGMA) were estimated according to the method used by Rohlf (1992) and genetic variation patterns among the maize genotypes were identified using PopGen32 (Population Genetic Analysis System, Version 32V) and MEGA (Molecular Evolutionary Genetics Analysis) 6 databases software, respectively.

3. Results and Discussion

3.1. SSR polymorphism

The SSR markers selected to analyze the genetic diversity of the maize accessions displayed different characteristic profiles. Thus, different numbers of polymorphic bands, percentage of polymorphism, Polymorphism Information Content (PIC), and expected heterozygosity have been generated using the SSR markers.

SSR markers used to molecular characterization, allele size, allele number and PIC values included 175 popcorn accessions occurring from 35 landraces popcorn population are reported in Table 3.

Table 3- Allele size, number and PIC value information of SSR markers used in molecular characterization of landraces popcorn populations

<i>Primer</i>	<i>Allel Size</i>	<i>Number of Alleles</i>	<i>PIC</i>	<i>Primer</i>	<i>Allel Size</i>	<i>Number of Alleles</i>	<i>PIC</i>
phi015	80-120	4	0.88	phi127	110-129	3	0.40
phi017	100-110	4	0.76	phi064	73-110	5	0.89
phi021	90-120	4	0.87	phi057	160-170	3	0.57
phi034	110-150	4	0.70	umc1550	140-280	3	0.43
umc2292	130-170	5	0.89	phi095	140-180	3	0.56
umc2101	150-180	3	0.50	phi022	150-180	4	0.62
umc2050	120-160	4	0.82	phi027	150-180	4	0.87
umc1622	40-90	2	0.10	umc1164	140-160	2	0.09
umc1186	220-240	2	0.12	umc1173	150-170	3	0.64
				umc1255	130-280	3	0.65

Nineteen of 20 SSR markers were noted to be polymorphic while one marker (phi084) was found to be monomorphic. Total numbers of alleles were detected as 66 and 65 of them were polymorphic. The polymorphism rate was calculated to be as high as 98.5%.

Vivodik et al. (2017) characterizing 40 maize populations with 10 SSR markers determined that they had 65 alleles in total, and that the number of these alleles changed between 4-8 (mean 6.5 alleles) and the PIC value varied from 0.734 to 0.848 (mean PIC 0.810). Riberio et al. (2017) analyzed 48 single hybrid maize varieties commercially used in Brazil with 20 SSR markers and determined the average allele number as 9.8 and the average PIC value as 0.84. Atanda & Olaove (2017) analyzed 24 inbred maize lines with 20 SSR markers and identified total of 101 alleles and found that the average allele number was 5.5 and the average PIC was 0.46.

Allele numbers observed for each locus ranged from 1 (phi084) to 5 (phi064 and umc2292) with an average of 3.3 alleles per locus. The PIC value ranged from 0.00 (phi084) to 0.89 (umc2292 and phi064) with average value of 0.57. Number and percentage of polymorphic loci of populations were observed to range from 29 and 43.94% (population 21) to 47 and 71.21% (population 13) with an average of 39.11 and 59.27%, respectively (Table 4).

Table 4 - Number and percentage of polymorphic loci of maize populations by Nei 1973 method

Population No	Polymorphic Locus Number	Polymorphic Locus Percentage (% P)	Population No	Polymorphic Locus Number	Polymorphic Locus Percentage (% P)	Population No	Polymorphic Locus Number	Polymorphic Locus Percentage (% P)
1	40	60.61	13	47	71.21	25	39	59.09
2	39	59.09	14	41	62.12	26	35	53.03
3	38	57.58	15	38	57.58	27	41	62.12
4	38	57.58	16	43	65.15	28	41	62.12
5	39	59.09	17	38	57.58	29	41	62.12
6	39	59.09	18	42	63.64	30	40	60.61
7	38	57.58	19	44	66.67	31	36	54.55
8	41	62.12	20	33	50.00	32	39	59.09
9	37	56.06	21	29	43.94	33	39	59.09
10	41	62.12	22	40	60.61	34	43	65.15
11	37	56.06	23	36	54.55	35	44	66.67
12	40	60.61	24	33	50.00	Average	39.114	59.265

Molin et al. (2013) analyzed 48 local popcorn populations in Rio Grande do Sul and Parana in Brazil with 47 SSR markers and identified the polymorphic index as 78.3%. Sharma et al. (2010) analyzed 48 local maize varieties in India with 42 SSR primers and as a result recorded 60% polymorphism rate.

3.2. Genetic Differentiation

The parameters n_a , n_e , h , I and gen frequency revealed the genetic structure of the accessions (Table 5). Among polymorphic loci, the mean observed number of alleles (n_a) varied from 1 (phi084) to 2 (all other alleles) and mean n_a was 1.99; the mean effective number of alleles (n_e) varied between 1.00 (phi084) and 1.99 (phi015-3) with an average of 1.65 for all accessions. The total gen diversity (h) made according to Nei (1973) method was ranging from 0.0 to 0.5 with the average value of 0.36. The Shannon's information index (I) varied between 0.00 and 0.69 with an average of 0.53 for all accessions. Finally, the gene frequency (f) values; f_0 value ranged 0.00 to 0.98 with an average of 0.58 and the f_1 value ranged 0.0171 to 1.0000 with an average of 0.42.

Polymorphism limit of alleles is accepted as 95%, thus alleles are considered to be monomorphic if the frequency is 95% or more, however it is polymorphic when the allele frequency is below 95%.

Accordingly, the allele frequencies in phi017-4 ($f_0=0.9714$), phi034-1 ($f_0=0.9829$), umc2292-1 ($f_0=0.9714$), umc2292-2 ($f_0=0.9714$) and umc2050-4 ($f_0=0.9829$) were 95%, therefore, they were regarded as monomorphic. Polymorphic alleles are evaluated in their own, the allele frequency for 0 allele ranged from $f_0=0.0000$ (phi084) to $f_0=0.9314$ (phi064-5) and the allele frequency for 1 allele ranged from $f_1=0.0686$ (phi064-5) to $f_1=1.0000$ (phi084).

The investigated parameters, total genetic diversity (H_t), genetic diversity within the population (H_s), inter-population genetic differentiation (G_{st}) and gene flow (N_m) revealed the genetic structure of the accessions (Table 6).

Table 5- The mean observed number of alleles, mean effective number of alleles, genetic diversity according to Nei (1973) and Shannon's information index values of genetic variation of all loci

Loci	Number of Accession	n_a^*	n_e^*	h^*	I^*	Gene Frequency		Loci	Number of Accession	n_a^*	n_e^*	h^*	I^*	Gene Frequency	
						Allel 0	Allel 1							Allel 0	Allel 1
						phi015-1	175							20.00	12.127
phi015-2	175	20.00	19.994	0.499	0.693	0.509	0.491	phi064-1	175	20.00	13.243	0.245	0.410	0.857	0.143
phi015-3	175	20.00	19.999	0.500	0.693	0.497	0.503	phi064-2	175	20.00	19.767	0.494	0.687	0.446	0.554
phi015-4	175	20.00	13.968	0.284	0.458	0.829	0.171	phi064-3	175	20.00	18.760	0.467	0.660	0.629	0.371
phi017-1	175	20.00	14.115	0.291	0.467	0.823	0.177	phi064-4	175	20.00	19.716	0.493	0.686	0.440	0.560
phi017-2	175	20.00	19.660	0.491	0.685	0.434	0.566	phi064-5	175	20.00	11.464	0.128	0.250	0.931	0.069
phi017-3	175	20.00	19.054	0.475	0.668	0.389	0.611	phi057-1	175	20.00	18.654	0.464	0.657	0.366	0.634
phi017-4	175	20.00	10.588	0.055	0.130	0.971	0.029	phi057-2	175	20.00	19.890	0.497	0.690	0.463	0.537
phi021-1	175	20.00	19.600	0.489	0.683	0.571	0.429	phi057-3	175	20.00	16.897	0.408	0.598	0.714	0.286
phi021-2	175	20.00	19.890	0.497	0.690	0.463	0.537	umc1550-1	175	20.00	18.861	0.470	0.663	0.623	0.377
phi021-3	175	20.00	18.434	0.457	0.650	0.646	0.354	umc1550-2	175	20.00	15.151	0.340	0.523	0.217	0.783
phi021-4	175	20.00	14.410	0.306	0.484	0.811	0.189	umc1550-3	175	20.00	17.959	0.443	0.635	0.669	0.331
phi034-1	175	20.00	10.349	0.033	0.087	0.983	0.017	phi095-1	175	20.00	15.299	0.346	0.531	0.777	0.223
phi034-2	175	20.00	19.054	0.475	0.668	0.611	0.389	phi095-2	175	20.00	16.037	0.376	0.564	0.251	0.749
phi034-3	175	20.00	16.614	0.398	0.588	0.274	0.726	phi095-3	175	20.00	15.743	0.365	0.551	0.760	0.240
phi034-4	175	20.00	11.595	0.137	0.265	0.926	0.074	phi022-1	175	20.00	15.743	0.365	0.551	0.240	0.760
umc2292-1	175	20.00	10.588	0.055	0.130	0.971	0.029	phi022-2	175	20.00	12.818	0.220	0.378	0.874	0.126
umc2292-2	175	20.00	10.588	0.055	0.130	0.971	0.029	phi022-3	175	20.00	13.100	0.237	0.400	0.863	0.137
umc2292-3	175	20.00	19.890	0.497	0.690	0.463	0.537	phi022-4	175	20.00	19.854	0.496	0.690	0.543	0.457
umc2292-4	175	20.00	13.100	0.236	0.400	0.863	0.137	phi027-1	175	20.00	18.202	0.451	0.643	0.657	0.343
umc2292-5	175	20.00	19.465	0.486	0.679	0.417	0.583	phi027-2	175	20.00	19.854	0.496	0.690	0.457	0.543
umc2101-1	175	20.00	19.231	0.480	0.673	0.400	0.600	phi027-3	175	20.00	19.231	0.480	0.673	0.600	0.400
umc2101-2	175	20.00	18.202	0.450	0.643	0.343	0.657	phi027-4	175	20.00	15.596	0.359	0.544	0.766	0.234
umc2101-3	175	20.00	11.595	0.137	0.265	0.926	0.074	umc1164-1	175	20.00	13.100	0.237	0.400	0.137	0.863
umc2050-1	175	20.00	19.968	0.499	0.692	0.520	0.480	umc1164-2	175	20.00	19.392	0.484	0.677	0.589	0.411
umc2050-2	175	20.00	19.716	0.492	0.686	0.440	0.560	umc1173-1	175	20.00	19.767	0.494	0.687	0.446	0.554
umc2050-3	175	20.00	19.392	0.484	0.677	0.589	0.411	umc1173-2	175	20.00	19.535	0.488	0.681	0.577	0.423
umc2050-4	175	20.00	10.349	0.033	0.087	0.983	0.017	umc1173-3	175	20.00	19.968	0.499	0.692	0.520	0.480
umc1622-1	175	20.00	13.968	0.284	0.458	0.829	0.171	umc1255-1	175	20.00	18.202	0.451	0.643	0.657	0.343
umc1622-2	175	20.00	11.464	0.127	0.250	0.069	0.931	umc1255-2	175	20.00	19.231	0.480	0.673	0.400	0.600
umc1186-1	175	20.00	11.595	0.137	0.265	0.074	0.926	umc1255-3	175	20.00	19.767	0.494	0.687	0.554	0.446
umc1186-2	175	20.00	13.676	0.268	0.440	0.840	0.160	Average	175	19.848	16.435	0.361	0.530	0.589	0.420
phi127-1	175	20.00	17.833	0.439	0.631	0.326	0.674	Standard Error	175	0.340	0.1543	0.193			
phi127-2	175	20.00	14.706	0.320	0.500	0.200	0.800								

* n_a = The mean observed number of alleles; * n_e = The mean effective number of alleles (Kimura & Crow 1964); * h = Genetic diversity according to Nei (1973); * I = Shannon's information index (Lewontin 1972).

While H_t varied between 0.0000 (phi084) and 0.5000 (phi015-3) with an average of 0.3606; H_s ranged from 0.0000 (phi084) to 0.4206 (umc1255) and the average was 0.2391. G_{st} values were identified to vary between 0.1290 (phi095-1) and 0.6706 (phi017-4) with an average of 0.3369. N_m values of genotypes ranged from 0.2456 to 3.3772 with a whole average value of 0.9840; the highest values on phi095-1 allele and the lowest on phi017-4 allele were observed. In general, it was observed that the phi095 coded marker was the most polymorphic marker in determining the diversity of genotypes for gene flow than other markers. On the other hand, phi084 coded marker revealed that it was not an effective marker for the determination of gene flow under this study.

In our study the genetic variation determined was lower than other that Vivodik et al. (2017) found for the 40 maize genotypes. Similarly, Zhang et al. (2016) examined 290 inbred maize lines by 201 SSR markers, and the diversity they determined was 0.70. The study accomplished by Tahir & Maeruf (2016) on 9 corn genotypes with 18 SSR markers were reported that genetic diversity was between 0.20 and 0.82.

Table 6- Total genetic diversity, intra-population genetic diversity, inter-population genetic differentiation and gene flow data in determined SSR loci by Nei 1978 method

<i>Loci</i>	H_t	H_s	G_{st}	N_m^*	<i>Loci</i>	H_t	H_s	G_{st}	N_m^*	<i>Loci</i>	H_t	H_s	G_{st}	N_m^*
phi015-1	0.175	0.109	0.374	0.835	umc2101-2	0.450	0.338	0.249	1.505	umc1550-2	0.3400	0.2057	0.3949	0.7661
phi015-2	0.499	0.260	0.478	0.544	umc2101-3	0.137	0.077	0.434	0.649	umc1550-3	0.4432	0.2971	0.3295	10.174
phi015-3	0.500	0.306	0.387	0.790	umc2050-1	0.499	0.306	0.386	0.793	phi095-1	0.3464	0.3017	0.1290	33.772
phi015-4	0.284	0.128	0.549	0.410	umc2050-2	0.492	0.315	0.359	0.889	phi095-2	0.3764	0.3246	0.1378	31.297
phi017-1	0.291	0.187	0.357	0.900	umc2050-3	0.484	0.347	0.282	1.269	phi095-3	0.3648	0.3154	0.1353	31.944
phi017-2	0.491	0.324	0.339	0.973	umc2050-4	0.033	0.022	0.321	1.054	phi022-1	0.3648	0.1463	0.5990	0.3347
phi017-3	0.475	0.224	0.528	0.445	umc1622-1	0.284	0.096	0.662	0.255	phi022-2	0.2198	0.1143	0.4801	0.5415
phi017-4	0.055	0.018	0.670	0.245	umc1622-2	0.127	0.054	0.570	0.376	phi022-3	0.2367	0.1646	0.3046	11.413
phi021-1	0.489	0.352	0.281	1.277	umc1186-1	0.137	0.054	0.601	0.331	phi022-4	0.4963	0.3337	0.3276	10.261
phi021-2	0.497	0.342	0.310	1.110	umc1186-2	0.268	0.123	0.540	0.424	phi027-1	0.4506	0.2331	0.4826	0.5360
phi021-3	0.457	0.374	0.180	2.267	phi127-1	0.439	0.274	0.375	0.831	phi027-2	0.4963	0.2834	0.4289	0.6656
phi021-4	0.306	0.246	0.193	2.086	phi127-2	0.320	0.214	0.328	1.021	phi027-3	0.4800	0.3474	0.2762	13.103
phi034-1	0.033	0.013	0.593	0.343	phi127-3	0.477	0.269	0.435	0.648	phi027-4	0.3588	0.2469	0.3120	11.027
phi034-2	0.475	0.306	0.355	0.906	phi064-1	0.244	0.137	0.440	0.636	umc1164-1	0.2367	0.1829	0.2274	16.990
phi034-3	0.398	0.242	0.391	0.777	phi064-2	0.494	0.310	0.370	0.848	umc1164-2	0.4843	0.3474	0.2826	12.691
phi034-4	0.137	0.096	0.302	1.155	phi064-3	0.466	0.297	0.363	0.875	umc1173-1	0.4941	0.3977	0.1951	20.630
umc2292-1	0.055	0.036	0.341	0.965	phi064-4	0.492	0.283	0.424	0.676	umc1173-2	0.4881	0.3200	0.3444	0.9518
umc2292-2	0.055	0.027	0.505	0.488	phi064-5	0.127	0.086	0.320	1.062	umc1173-3	0.4992	0.3200	0.3590	0.8929
umc2292-3	0.497	0.306	0.384	0.802	phi057-1	0.463	0.352	0.241	1.572	umc1255-1	0.4506	0.3520	0.2188	17.848
umc2292-4	0.236	0.146	0.381	0.809	phi057-2	0.497	0.402	0.191	2.118	umc1255-2	0.4800	0.3886	0.1905	21.250
umc2292-5	0.486	0.292	0.398	0.755	phi057-3	0.408	0.338	0.171	2.420	umc1255-3	0.4941	0.4206	0.1488	28.597
umc2101-1	0.480	0.338	0.295	1.193	umc1550-1	0.469	0.352	0.250	1.493	Average	0.3606	0.2391	0.3369	0.9840
										Std. Error	0.0238	0.0137		

H_t , Total genetic diversity; H_s , Genetic diversity within the population; G_{st} , Inter-population genetic differentiation; * N_m = Gene flow. E.g.; $N_m = 0.5 (1 - G_{st}) / G_{st}$; (McDermott & McDonald 1993)

3.3. Genetic relationship and cluster analyses

The amount of genetic change was determined as 0.05 and genetic differences among genotypes ranged between 14.7 and 97.1%. According to our findings, while the lowest genetic distance was observed between 8.3 and 9.3 (Eskisehir-Balıkesir); 18.5 and 22.5 (Kastamonu-Eskisehir); 33.3 and 34.4 (Kovanlı/Ordu-Mugla) coded genotypes with the average of 14.7%, the highest genetic distance was determined between 15.3 and 26.4 (Afyon-Tokat) genotypes with 97.1%.

Generally, when the genetic distance values are examined, variations among genotypes were observed to be very high and even among individuals of the same population, genetic differences were large.

A hundred seventy five genotypes were classified in two large clusters (Figure 1). While the first group had genotypes of population 15 (Incehisar/Afyon), the second group had all other genotypes. Then, second group divided into two sub-groups. The first branch of the second group had genotypes 35.2 and 1.2; the second branch of the second group had other genotypes with two sub-groups and then each group was divided into other sub-groups.

When the local populations were compared with the standard variety, it was relationship with all genotypes except for 15 number genotype. Because parental of standard varieties were obtained from populations collected from Turkey. This state is evidence that the local variety is selected from the country's populations and also the accuracy of the research conducted by us. It can be explained by cause moving to different region of the country with open pollination of popcorn genotypes as reason for being sub-groups under 2 groups and interconnected groups. Generally with telling, the distribution of local populations into groups was predominantly by provinces close to each other. In also, some populations obtained from different regions of Turkey were seen to be within the same group. Comertpay (2008), in analysis of the local corn according to the UPGMA method determined that the distribution of genotypes in groups does not show specific distribution; Warburton et al. (2005) reported that the elite corn lines were not divided into groups according to environmental factors and morphological characteristics. Our study

was supported by these two studies, while populations except for 15 number population were being in the groups and subgroup, population of 15 number placed in a separate group.

The reasons of the high genetic diversity of genotypes are; the genotypes used are open fertilizer material, be grown in different places, be high adaptability and it can be concluded that hundreds of seeds can be taken from one plant.

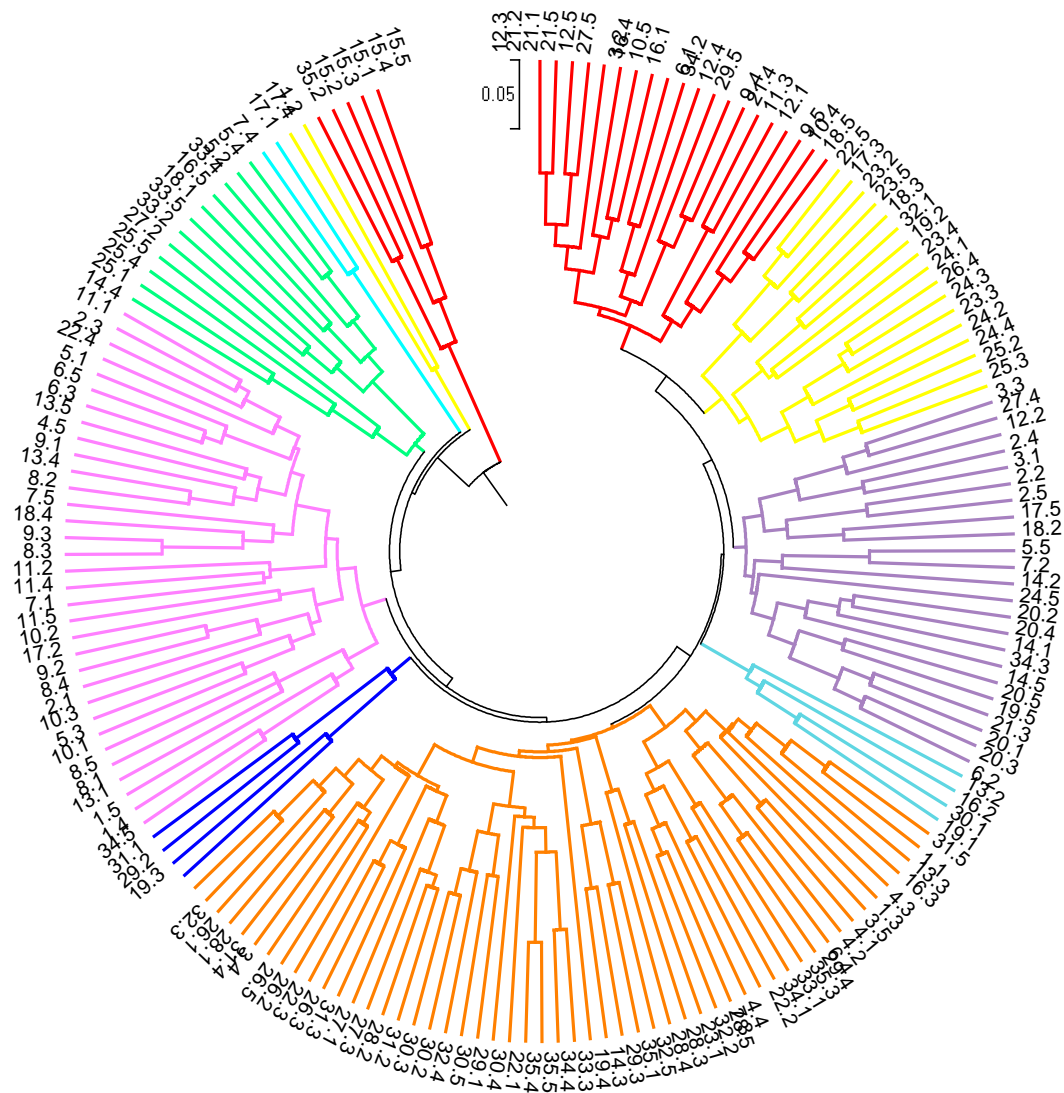


Figure 1- Cluster analysis of 175 accessions of popcorn based on UPGMA difference index through SSR markers polymorphism

4. Conclusions

Local popcorn populations are known to be richer in their genetic diversity than their hybrid counterparts. The use of genotypes in different groups in the breeding studies and having high evolution is beneficial in obtaining healthier and more effective results. At each stage of the study, examining genetic differences or richness within and between populations were observed high differences in each parameter. It was concluded that these differences would be an important resource of providing with a wide crop variety in terms of breeding and researchers.

This study was be first time for reveal of genome map of the genetic diversity on popcorn populations in Turkey. In addition, this study will give direction local hybrid seed production.

Acknowledgements

This study was supported by the Scientific Research Projects Unit of the Kahramanmaraş Sutcu Imam University (Project number: 2014/3-27 D).

References

- Aci M M, Revilla P, Morsli A, Djemel A, Belalia N, Kadri Y, Khelifi-Saloui M, Ordás B & Khelifi L (2013). Genetic diversity in Algerian maize (*Zea mays* L.) landraces using SSR markers. *Maydica* 58: 304-310
- Adjanohoun A, Allagbe M, Noumavo P A, Gotoechan-Hodonou H, Sikirou R, Dossa K K, GleleKakaï R, Kotchoni S O & Baba-Moussa L (2011). Effects of plant growth promoting rhizobacteria on field grown maize. *Journal of Animal & Plant Sciences* 11(3): 1457-1465
- Atanda A S & Olaoye G (2017). Multiplex-Ready PCR assay of SSR marker diversity among quality protein maize inbred parental lines. *South African Journal of Plant and Soil* 34(2): 149-154 <https://doi.org/10.1080/02571862.2016.1211325>
- Comertpay G (2008). Characterization of open pollinated Turkish maize populations using morphological traits and SSRs molecular markers. Cukurova University of Cukurova Institute of Natural and Applied Science Field Crops Department, PhD Thesis, Adana (Published)
- Doyle J J & Doyle J L (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* v.19 pp.11-15
- Eschholz T W, Stamp P, Peter R, Leipner J & Hund A (2010). Genetic structure and history of swiss maize (*Zea mays* L. ssp. *mays*) landraces. *Genetic Resources and Crop Evolution* 57(1): 71-84 <https://doi.org/10.1007/s10722-009-9452-0>
- Frankel O H (1972). The significance, utilization and conservation of crop genetic resources. FAO, Rome
- Gauthier P, Gouesnard B, Dallard J, Redaelli R, Rebourg C, Charcosset A, Boyat A (2002). RFLP diversity and relationships among traditional european maize populations. *Theoretical and Applied Genetics* 105(1): 91-99 <https://doi.org/10.1007/s00122-002-0903-7>
- Kimura M & Crow J F (1964). The Number of alleles that can be maintained in a finite population. *Genetics* 49(4): 725
- Laborda P R, Oliveira K M, Garcia A A F, Paterniani M E A G Z, De Souza A P (2005). Tropical maize germplasm: What can we say about its genetic diversity in the light of molecular markers? *Theoretical and Applied Genetics* 111(7): 1288-1299 <https://doi.org/10.1007/s00122-005-0055-7>
- Lewontin R C (1972). The Apportionment of human diversity. *Evolutionary Biology* 6: 381-398
- Liu W S, Dong M, Song Z P, Wei W (2009). Genetic diversity pattern of *Stipapurpurea* populations in the hinterland of Qinghai-Tibet Plateau. *Annals of Applied Biology* 154: 57-65 <https://doi.org/10.1111/j.1744-7348.2008.00274.x>
- McDermott J M & McDonald B A (1993). Gene flow in plant pathosystems. *Annual Review of Phytopathology* 31(1): 353-373
- Molin D, Coelho C J, Máximo D S, Ferreira F S, Gardingo J R, Matiello R R (2013). Genetic diversity in the germplasm of tropical maize landraces determined using molecular markers. *Genetics and Molecular Research* 12(1): 99-114
- Nei M (1972). Genetic distance between populations. *The American Naturalist* 106(949): 283
- Nei M (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences* 70(12): 3321
- Nei M (1978). The theory of genetic distance and evolution of human races. *Japanese Journal of Human Genetics* 23(4): 341
- Ribeiro C A G, Pinto M D O, Maciel T E F, Pastina M M, Barros E G D, Guimarães C T (2017). Universal tail sequence-SSR applied to molecular characterization of tropical maize hybrids. *Scientia Agricola* 74(2): 163-168 <https://doi.org/10.1590/1678-992x-2016-0079>
- Rohlf F J (1992). Program numerical taxonomy and multivariate analysis system. Version 1.70 New York
- Sharma L, Prasanna B M, Ramesh B (2010). Analysis of phenotypic and microsatellite-based diversity of maize landraces in India, especially from the North East Himalayan region. *Genetica* 138(6): 619-631 <https://doi.org/10.1007/s10709-010-9436-1>
- Tahir N A & Maeruf M S (2016). Assessment of salinity tolerance and SSR profile differentiation in nine maize genotypes (*Zea mays* L.). *Maydica* 61 M18: 1-8
- Vivodík M, Gálová Z, Balážová Ž, Petrovičová L (2017). Genetic variation of European maize genotypes (*Zea mays* L.) detected using SSR markers. *Potravinárstvo Slovak Journal of Food Sciences* 11(1): 126-131
- Warburton M L, Ribaut J M, Franco J, Crossa J, Dubreuil P, Betrán FJ (2005). Genetic characterization of 218 elite CIMMYT inbred maize lines using RFLP markers. *Euphytica* 142:97-106 <https://doi.org/10.1007/s10681-005-0817-y>
- Yao Q L, Yang K C, Pan G T, Rong T Z (2008). Genetic diversity of maize (*Zea mays* L.) landraces from southwest China based on SSR data. *J. Genet. Genomics* 34: 851-860 [https://doi.org/10.1016/S1673-8527\(07\)60096-4](https://doi.org/10.1016/S1673-8527(07)60096-4)
- Zhang J, Guo J, Liu Y, Zhang D, Zhao Y, Zhu L, Huang Y, Zhang Z, Chen J (2016). Genome-wide association study identifies genetic factors for grain filling rate and grain drying rate in maize. *Euphytica* 212(2): 201-21 <https://doi.org/10.1007/s10681-016-1756-5>



© 2021 by the authors. Licensee Ankara University, Faculty of Agriculture, Ankara, Turkey. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).