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Research Article

Exploring Genetic Diversity and Population Structure of Turkish Black Sea Region Maize (*Zea mays* L.) Germplasm using SSR Markers

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Abstract

This study aimed to investigate the genetic diversity and population structure of 32 local maize genotypes collected from the Black Sea Region of Turkey using SSR markers. 14 most polymorphic primers yielded a total of 42 bands. An average of 3 alleles per SSR primer was detected, and the number of alleles varied from 1 (phi022) to 6 (umc1571). The unweighted pair-group method with arithmetic means (UPGMA) clustering divided maize accessions into three main populations. According to Nei's genetic distances, DZ-M-145 (Corum) and DZ-M-20 (Trabzon) genotypes were the closest (0.03) genetically related populations, while DZ-M-68 (Artvin) and DZ-M-55 (Rize) were the most genetically distant (0.63) populations. The study identified molecular genetic diversity not mentioned for maize plants from the Black Sea.

Keywords: SSR, maize, genetic, local maize populations, molecular characterization

Türkiye Karadeniz Bölgesi Mısır (*Zea mays* L.) Germplazmının Genetik Çeşitliliğinin ve Popülasyon Yapısının SSR Markörleri Kullanılarak Araştırılması

Öz

Bu çalışma, Türkiye'nin Karadeniz Bölgesi'nden toplanan 32 yerel mısır genotipinin SSR belirteçleri kullanılarak genetik çeşitliliğini ve popülasyon yapısını araştırmayı amaçlamıştır. Moleküler karakterizasyon çalışmasında toplamda 42 bant veren en polimorfik 14 primer kullanılmış, SSR primeri başına ortalama 3 alel saptanmış ve alel sayısı 1 (phi022) ile 6 (umc1571) arasında değişim göstermiştir. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) yöntemi, mısır aksesyonlarını üç ana popülasyona bölmüştür. Nei'nin genetik mesafelerine göre, DZ-M-145 (Çorum) ve DZ-M-20 (Trabzon) genotipleri genetik olarak en yakın (0.03) popülasyonlar iken, DZ-M-68 (Artvin) ve DZ-M-55 (Rize) genetik olarak en uzak (0.63) popülasyonlar olarak tespit edilmiştir. Çalışma, Karadeniz'deki mısır bitkileri için daha önce bahsedilmeyen moleküler genetik çeşitliliği tanımıştır.

Anahtar Kelimeler: SSR, mısır, genetik, yerel mısır popülasyonları, moleküler karakterizasyon

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1. Introduction

Maize (*Zea mays* L.) is the most important crop in the cereals after wheat and rice. According to genetic data, the maize plant was domesticated from its wild relative, Balsas teosinte (*Zea mays subspecies parviglumis*), in Mexico [1]. Annual production of maize exceeds 1 billion tons worldwide [2] and increases day by day. Maize is used as food and feed. It is estimated to be the most produced grain crop in the world by 2025. Its current demand is expected to be double by 2050 [3-4].

Landraces are very important as there is a vast amount of genetic material utilized in crop improvement and development methods [5]. Landraces of maize plants, which are frequently used in breeding programs, are essential genetic resources [6]. To understand the genetic structure of maize, researching genetic diversity is very important. Future breeding programs need parental genotypes with desired characteristics [7]. Therefore, the crop gene pool should be evaluated according to the degree of genetic diversity [8] and the diverse genotypes must be utilized in different breeding programs.

Molecular markers, which offer an effective way of combining genotypic and phenotypic variation, have recently been used in the agricultural sector to determine genetic diversity [9]. Molecular markers play crucial roles in the in-depth detection of genetic diversity modeling in maize and in determining gene flow from the center of origin to migration pathways [10-11-12-13-14-8]. SSR technique or microsatellite polymorphism, which is one of the PCR-based techniques, can be defined as PCR amplification of tandemly repeated sequences [15-16]. In this co-dominant and multi-allelic technique, the number of repeating units determines the polymorphism for fragment lengths, while heterozygous can be distinguished for different fragments in diploid genomes [17] In the SSR technique, which contains locus-specific side primers that produce a high level of polymorphic banding, genetic variation is quite good due to the differences in the number of consecutively repeated SSR units in a locus. SSR or microsatellites are preferred for genetic analysis because they are very strong, polymorphic, and common in plant genomes [16].

Detecting genetic diversity is a crucial step in plant breeding programs that helps in the development of more productive and desired varieties. This study aimed to investigate the molecular diversity of 32 local maize genotypes collected from the Black Sea region of turkey with SSR markers.

2. Material and Methods

2.1. Plant materials and DNA Extraction

In this study, 32 local maize genotypes collected from 26 Turkish provinces in the Black Sea region were used as plant material (Supp Table 1, Fig. 1). From the young leaves of the plants representing each genotype, 0.8 grams of sample was taken and crushed in liquid nitrogen with the help of mortar and pestle. The crushed samples in powdered form were kept at –80 °C until

the DNA isolation started. Genomic DNA was extracted from the stored samples according to the CTAB protocol [18] with slight modifications [19].

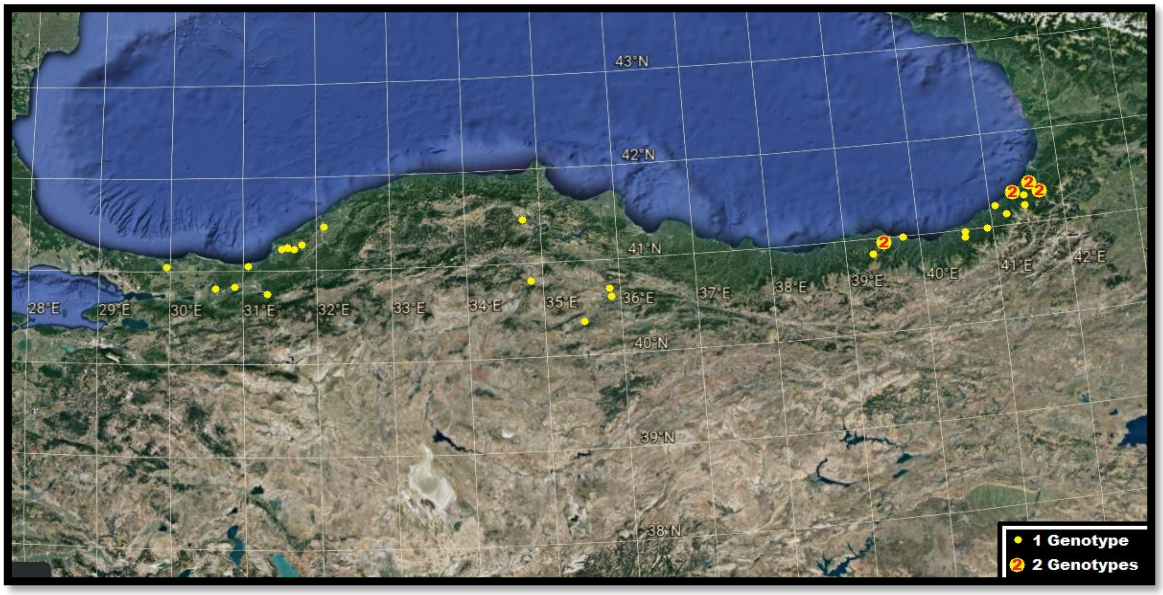

Fig. 1. Locations of 32 maize genotypes used in the study.

2.2. SSR Analysis

A total of 22 SSR markers were tested with 8 randomly isolated DNA samples. Band images of 14 SSR markers were selected as they showed a polymorphic feature. The names, sequences, and annealing temperatures of these markers are shown in Table 1. The electrophoresis procedure was performed by capillary electrophoresis method using ABI 3130xl automatic genetic analyzer device. Amplification of SSR was performed with 12.5µl reaction mixture containing 10-20 ng DNA, 2.0 mM $MgCl_2$, 20 mM (NH4)2 SO4, 75 mM Tris-HCl, %0.01 Tween 20, 200M dNTP, 20 nM 5' M13 universal primer (average base: 40), 200 nM reverse primer (average base: 20), M13 universal primer marked with 200 nM PET, NED, VIC or FAM and 0.7 units Taq DNA polymerase.

Reactions consisted of 5 min pre-denaturation at 94 ℃, 45 seconds of 28 denaturation cycles at 94 ℃, 45 seconds of annealing temperature at 50-54 ℃, 1.5 min of extension step at 72 ℃, and denaturation stage (45 seconds at 94°C, 45 seconds at 52°C, 1.5 min at 72°C) in 8 cycles, and 5 min final extension at 72°C in one cycle.

PCR products were run on 1.8% (w/v) agarose gel using 0.5 X TBE (Tris-Boric Acid-EDTA) buffer for 2 hours. Gel taken from the electrophoresis system was visualized in UV Imager Gel Doc XR + system (Bio-Rad, USA) after immersion in ethidium bromide solution. A 100 bp ladder was used for observing the DNA polymorphism of maize genotypes.

Table 1. SSR primer names, sequences, and annealing temperatures used in this study

Primer name	Sequence	Annealing temp (^{0}C)
Umc1675	ATCGCGACGAGTTAATTCAAACAT	52
Phi022	GTTCTTCCTCTTCCCCATCAGTCT	52
Umc2084	ACGAGCGAGTGGAGAATAGG	52
bnlg1839	AGCAGACGGAGGAAACAAGA	52
umc1743	TGGACTTCGAAAATTCTCTTCAGC	52
umc1993	CTTTTCTGCTACTCCTGCCTGC	52
phi 032	CTCCAGCAAGTGATGCTGGAC	52
umc1571	GCACTTCATAACCTCTCTGCAGGT	52
umc1450	ACAGCTCTTCTTGGCATCGT	52
umc1893	TCCAGTGCCACCCCTAGATAGTAA	54
umc1432	GGCCATGATACAGCAAGAAATGAT	54
umc1279	CAATCCAATCCGTTGCAGGTC	50
umc1129	GAGAGTATGCTACTCGCCGC	50
umc2016	AGAGACGACATGTCTATCCTTGCC	50

2.3. Data Analysis

SSRs were scored as either present or absent according to the presence of bands as 1 and absence as 0, respectively. A matrix was created by determining genetic distances, according to [20]. PIC (Polymorphism Information Content) was determined, according to [21]. At the same time, the effective number of alleles, gene diversity, Shannon's information index, and unbiased heterozygosity were calculated in PopGene ver 1.32 [2]. Analysis of molecular variance (AMOVA) and UPGMA (Cluster analysis) was performed using R software [20]. STRUCTURE software was used for the Bayesian clustering model. The cluster numbers (K) were detected by the protocol of [23].

3. Results and Disscussion

3.1. SSR Genetic Diversity

Bands were detected and selected in 14 of 22 SSR primers obtained from the website MaizeGDB.org and 42 alleles were obtained from these bands. All primers used were 100% polymorphic. An average of 3 alleles per SSR primer was determined (Table 2). Most alleles were detected at the umc1571 locus (6), and the least alleles were seen at the phi022 (1) locus. The PIC value varied between 0.43-0.95, and the mean PIC value was 0.65 (Table 2).

Table 2. Primer names, allele numbers, PIC values and Polymorphism rates obtained from this study.

Primer Names	DNA sequences (5' - 3')	Allele Numbers	PIC Values	Polymorphism (%)
umc1675	**F**-ATCGCGACGAGTTAATTCAAACAT **R**-TACGATGTCTTCAGTGTGACACCA	2	**0.43**	100
phi022	**F**-GTTCTTCCTCTTCCCCATCAGTCT **R**-ATAGCTGCGCGTAAAGCAACC	1	0.53	100
umc1526	**F**- ACGAGCGAGTGGAGAATAGG **R**-AGCCCAGTACGTGGGGTC	3	0.61	100
bnlg1839	**F**-AGCAGACGGAGGAAACAAGA **R**-TCTCCCTCTCCCTCTTGACA	2	0.51	100
umc1743	**F**-TGGACTTCGAAAATTCTCTTCAGC **R**-GAGAGGAGGAGCTTCACGAGC	4	0.67	100
umc1993	**F**-CTTTTCTGCTACTCCTGCCTGC **R**-CTAGCTGATGGAGGCTGTAGCG	2	0.59	100
phi032	**F**-CTCCAGCAAGTGATGCTGGAC **R**-GACACCCGGATCAATGATGGAAC	2	0.44	100
umc1571	**F**-GCACTTCATAACCTCTCTGCAGGT **R**-CACCGAGGAGCACGACAGTATTAT	6	0.81	100
umc1450	**F**-ACAGCTCTTCTTGGCATCGT **R**-GACTTTGCTGGTCAGCTGGT	4	**0.95**	100
umc1893	**F**-TCCAGTGCCACCCCTAGATAGTAA **R**-ACCCCAGAGTATCTCATCACCCTT	3	0.62	100
umc1432	**F**-GGCCATGATACAGCAAGAAATGAT **R**-TACTAGATGATGACTGACCCAGCG	5	0.79	100
umc1279	**F**-CAATCCAATCCGTTGCAGGTC **R**-GATGAGCTTGACGACGCCTG	2	0.69	100
umc1129	**F**-GAGAGTATGCTACTCGCCGC **R**-GACGAGTTTGGAGTGCCATT	3	0.74	100
umc2016	**F**-AGAGACGACATGTCTATCCTTGCC **R**-ATTGCATTGCATTCAGCTGTTGT	3	0.65	100
Mean		**3**	**0.65**	**100**

PIC. Polymorphism information content

The lowest Shannon's information index, genetic diversity, and unbiased heterozygosity were detected in Izmit province. The lowest effective alleles number (Ne) was detected in Corum province. The highest gene diversity parameters were seen in the Trabzon province (Table 3). The value of polymorphic alleles number was found higher than [8] and [24]. The PIC value determines the effectiveness of the discriminant power between polymorphic loci [25] and genotypes [26] mean PIC value of 0.65 was calculated during this study and was found higher than [27-7-28-29-8] and [24]. The mean number of effective alleles was 1.25 during is the investigation and in agreement with [17]. The mean gene diversity (He) value was 0.18 and was found lower than [31] and [28]. Obtaining higher values for various diversity indices during the current investigation proposed the presence of higher genetic diversity in the studied maize germplasm.

Table 3. Genetic diversity indices computed among 32 maize genotypes using 14 SSR primers.

Provinces	Ne	I	He	uHe
Amasya	1.314	0.250	0.173	0.207
Artvin	1.369	0.338	0.221	0.235
Corum	1.01	0.15	0.12	0.13
Duzce	1.12	0.17	0.14	0.12
Izmit	1.09	0.13	0.11	0.09
Karabuk	1.15	0.18	0.19	0.14
Rize	1.371	0.335	0.221	0.245
Sakarya	1.264	0.244	0.161	0.194
Trabzon	1.442	0.380	0.257	0.293
Zonguldak	1.321	0.285	0.190	0.211
Mean	1.245	0.246	0.178	0.187

NE: Effective alleles number, **He:** Gene diversity, **I:** Shannon's information index, **uHe:** Unbiased heterozygosity

The mean genetic distance between all genotypes was 0.28. The lowest genetic distance (0.03) was present between DZM-68 (Artvin) and DZM-55 (Rize). The highest genetic distance (0.63) was observed between DZM-145 (Corum) and DZM-20 (Trabzon) (Supp Fig. 1). Identification of genetically diverse genotypes is critical in breeding programs and can be observed with distant genetic distances between genotypes. With this determination, the possibility of heterosis or heterobeltiosis increases in crosses. Therefore, it can be said that DZM-20 and DZM-145 genotypes are the most distant genotypes and can be recommended as parents for maize breeding activities.

Analysis of molecular variance (AMOVA) reflected major variation within population (96%) as compared to among populations (4%) (Table 4). The presence of higher variation within the population as compared to among the population may be due to gene flow, genetic drift, foreign pollination, ecotype variation, adaptation [32], human activities, and environmental transformation [33].

Table 4. Analysis of molecular variance to determine the genetic diversity of 32 Maize genotypes.

Source	Df	SS	MS	Est. Var.	%
Among Pops	9	10.727	2.145	0.069	4%
Within Pops	22	40.298	1.832	1.832	96%
Total	31	51.025		1.901	100%

Est. Var: Estimated variance, **MS:** Mean square, **SS:** Sum of squares, **Df:** Degrees of freedom

The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method separated the 32 maize genotypes into three main groups as A, B, and C (Supp Fig. 2). Maize genotypes observing a higher level of genetic similarity were clustered very close to each other and vice versa. Group A comprised of 15 genotypes and was divided into two subgroups as A1 and A2. Group B clustered 14 genotypes and was divided into two subgroups as B1 and B2. Group C revealed 3 genotypes and was divided into two subgroups as C1 and C2.

The principal component analysis divided 32 maize genotypes into three populations (Fig. 2). The results obtained were compatible with UPGMA.

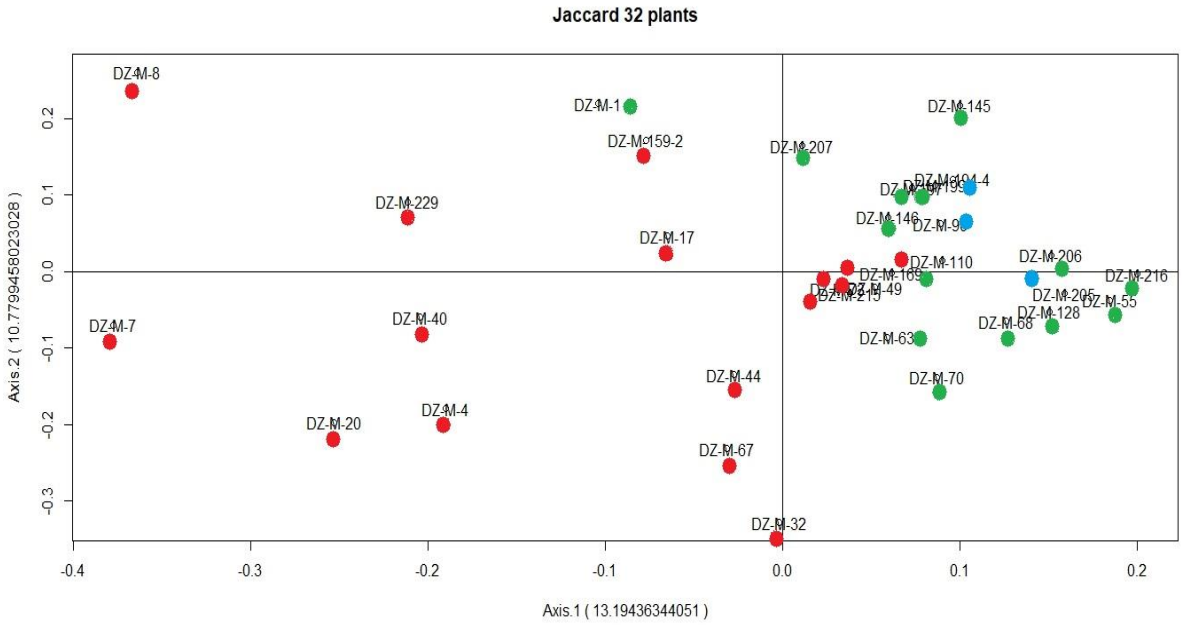

Fig. 2. Principle component analysis of 32 maize genotypes.

The number of groups (K) ranged from 1 to 10, according to STRUCTURE analyses. The most suitable goodness of fit was observed at the value of K = 3 (Fig. 3). The Bayesian clustering model constructed in STRUCTURE software separated the 32 maize genotypes into three clusters observing consistency with the UPGMA and principal component analyses (Supp Fig. 3). As a result, it can be said that the obtained genetic diversity findings contain beneficial information for maize breeders.

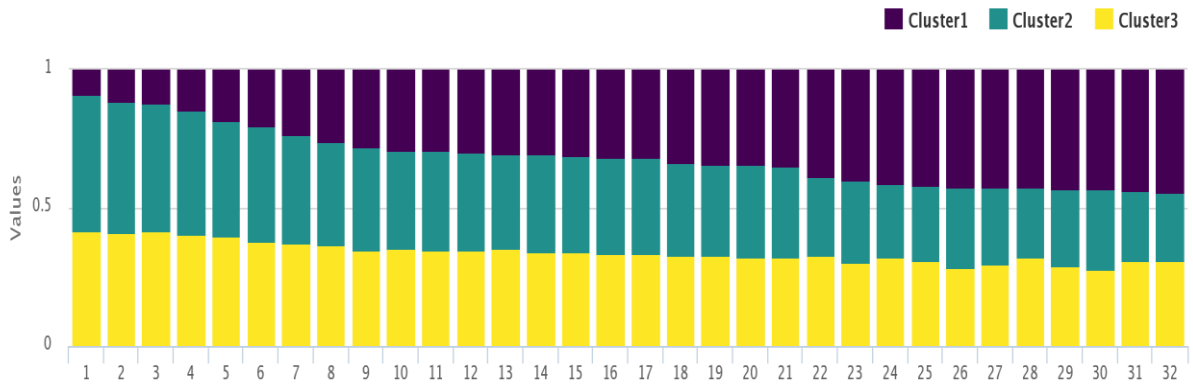

Fig. 3. Structure-based clustering of 32 maize genotypes using 14 SSR markers.

4. Conclusion

A sufficient amount of genetic diversity at the molecular level was reported among the studied 32 maize genotypes. The average of 3 alleles per SSR primer was detected, and the number of alleles varied from 1 (phi022) to 6 (umc1571). Clustering algorithms i.e., Bayesian-based clustering, UPGMA, and PCA separated the 32 maize genotypes into three groups. Analysis of molecular variance (AMOVA) reflected major variation within the population (96%) as compared to among populations (4%). Nei's genetic distances resulted in DZ-M-68 (Artvin) and DZ-M-55 (Rize) as the most genetically distant genotypes and can be implemented in future maize breeding programs.

Ethics in Publishing

There are no ethical issues regarding the publication of this study

Author Contributions

NB and FK contributed equally to this paper, and all authors made substantial contributions. NB and AY conceived and designed the study. All authors participated in the analysis of the results obtained from data and interpretation of the data. NB, MAN, AY, MA, FK, MGT, FSB wrote the manuscript. All authors reviewed and edited the manuscript and approved the final version before submission.

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