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GENETIC DIVERSITY ANALYSIS OF ZEA MAYS L. ACCESSIONS BASED ON MICROSATELLITE MARKERS

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ABSTRACT

This study aimed to evaluate the genetic diversity of 21 maize (*Zea mays* L.) accessions cultivated in Kazakhstan using 21 SSR markers. The selected markers revealed substantial polymorphism, with polymorphic information content (PIC) values ranging from 0.557 to 0.962, indicating high marker informativeness. Genetic diversity indices such as the number of alleles (*Na*), effective number of alleles (*Ne*), Shannon's information index (*I*), and Nei's gene diversity index (*uh*) varied significantly among accessions, with the Kazakh accession ZM001 showing the highest diversity. Analysis of Molecular Variance (AMOVA) revealed that 66% of the total genetic variation was attributable to differences among accessions, confirming strong population differentiation (Fst = 0.611) among maize accessions. Cluster analysis, Principal Coordinate Analysis (PCoA), and STRUCTURE analysis consistently grouped accessions according to their geographic origin, distinguishing local, Chinese, and European accessions. These results highlight the effectiveness of SSR markers in revealing genetic structure and demonstrate the existence of untapped allelic variation in the maize germplasm of Kazakhstan.

Key words: Zea mays, SSR markers, genetic diversity, analysis of molecular variance

INTRODUCTION

Maize (Zea mays L.), widely recognized as the «queen of cereals» is among the top three cereal crops globally, along-side wheat and rice [1]. It serves as a vital source of food, livestock feed, and industrial raw material [2; 3]. Owing to its exceptional adaptability and genetic plasticity, maize is cultivated across a wide range of agro-climatic zones. In Kazakhstan, maize is gaining increasing significance, especially in the southern and southeastern regions, where it is grown for both grain and silage purposes [4]. However, the local agro-climatic conditions – such as late spring frosts, summer droughts, and extreme temperature fluctuations – pose serious challenges to stable maize production. This highlights the importance of ongoing breeding efforts aimed at enhancing stress resilience and ensuring yield stability under these conditions.

Modern maize breeding is increasingly supported by molecular techniques that complement conventional selection methods. Among various types of molecular markers, simple sequence repeats (SSRs), also known as microsatellites, have proven particularly effective in analyzing genetic diversity, assessing population structure, and conducting marker-assisted selection (MAS) [5; 6]. SSRs offer several advantages: they are highly polymorphic, co-dominant, reproducible, and uniformly distributed throughout the genome, making them ideal for investigating genetic variation in maize [7; 8].

In Kazakhstan, local and introduced maize cultivars are regularly evaluated in performance trials. Nevertheless, molecular-level characterization remains limited. Most available studies have relied on agro-morphological traits [9; 10], which are often influenced by environmental factors and may not accurately reflect the true genetic potential of accessions. SSR marker-based characterization offers a more reliable alternative, being unaffected by environmental conditions and capable of revealing allelic richness and genetic relationships among genotypes [11].

On a global scale, only a small portion of the available maize genetic diversity is currently exploited in breeding programs [12], raising concerns over the genetic narrowing of commercial hybrids. In contrast, landraces and open-pollinated cultivars preserved in geographically isolated and ecologically diverse regions, such as southern Kazakhstan, represent a valuable repository of untapped alleles, particularly for traits associated with abiotic stress tolerance [13]. Characterizing these genetic resources is essential for preserving

Table 1. The list of maize accessions used for the genotyping

ID	Accessions	Country of origin	Organization provided the seeds
ZM001	Altai-319	Kazakhstan	Krasnovodapad AES
ZM002	Gidro F2	Switzerland (Europe)	Krasnovodapad AES
ZM003	Tulpar -539	Kazakhstan	Krasnovodapad AES
ZM004	F2 S0069	Türkiye (Europe)	Krasnovodapad AES
ZM005	ZPSK-704	Kazakhstan	Krasnovodapad AES
ZM006	Altyn 739	Kazakhstan	Krasnovodapad AES
ZM007	KazZP-777	Kazakhstan	Krasnovodapad AES

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ZM008	Mariam-419	Kazakhstan	Krasnovodapad AES
ZM009	SV Bambus F2	Switzerland (Europe)	Krasnovodapad AES
ZM010	Sunkar-779	Kazakhstan	Krasnovodapad AES
ZM011	Fenikks Spartak	China	PF «Sabyr»
ZM012	Fenikks Atlant	China	PF «Sabyr»
ZM013	Fenikks Celentano	China	PF «Sabyr»
ZM014	Fenikks Takelau	China	PF «Sabyr»
ZM015	Fenikks Bora-Bora	China	PF «Sabyr»
ZM016	Fenikks Gobi	China	PF «Sabyr»
ZM017	LG31642	France (Europe)	PF «Sabyr»
ZM018	Fenikks Avrora	China	PF «Sabyr»
ZM019	LG31555	France (Europe)	PF «Sabyr»
ZM020	Fenikks Francheska	China	PF «Sabyr»
ZM021	Fenikks Bombei	China	PF «Sabyr»

biodiversity and supporting long-term, climate-resilient breeding strategies.

The present study aims to evaluate the genetic diversity among a selected panel of *Zea mays* accessions grown in Kazakhstan using SSR markers. The outcomes of this study are expected to contribute to the development of maize core collection and to provide a scientific foundation for implementing molecular breeding strategies in Kazakhstan.

MATERIALS AND METHODS

The study included 21 accessions (cultivars and hybrids) of common maize (*Zea mays*). By origin, 9 accessions were from China, 7 from Kazakhstan, 2 from Switzerland and

France, and 1 accession from Turkey (Table 1). The hybrids were developed using a two-line (F₁) hybrid system. Seed material was provided by the Krasnovodapad Agricultural Experimental Station (AES) and the peasant farm (PF) «Sabyr».

DNA extraction and SSR genotyping

Genomic DNA was extracted from individual 5-day maize seedlings, with five replicates per accession, using the Dellaporta method with minor modifications [14]. The quality and quantity of the DNA were assessed using a NanoDrop One spectrophotometer (Thermo Fisher Scientific Inc., USA) and 1% agarose gel electrophoresis.

For genotyping 105 maize individuals, 21 SSR markers were selected (Table 2) based on their high levels of polymor-

Table 2. Characteristics of simple sequence repeat (SSR) primers used in microsatellite analysis of Zea mays

	SSRs	Sequence (5'-3')	Chr. No.	Motif	Annealing T (°C)	
1	bnlg1520	F: TCCTCTTGCTCTCCATGTCC	2	(AG)22	58	
		R: ACAGCTGCGTAGCTTCTTCC		,		
2	phi002	F: CATGCAATCAATAACGATGGCGAGT	1	AACG	58	
	pinooz	R: TTAGCGTAACCCTTCTCCAGTCAGC	1	Tirico	30	
3	phi021	F: TTCCATTCTCGTGTTCTTGGAGTGGTCCA	4	AG	58	
3	piliozi	R: CTTGATCACCTTTCCTGCTGTCGCCA	7	AG	<i>3</i> 0	
4	mh:027	F: GCGTACGTACGACGAAGACAC	9	(CCCCT) _m	58	
4	4 phi027	R: CACAGCACGTTGCGGATTTCTCT	9	(GCGCT)n	36	
5	5 1:040	F: ATGTGGCCATCATTCAATGCTGTAGAC	9	CATA	60	
3	phi042	R: ACACATGCAGGTGCAGCCAGA	9	CAIA	υυ	
	-1-:047	F: GGAGATGCTCGCACTGTTCTC	2	ATC	62	
0	6 phi047	R: CTCCACCCTCTTTGACATGGTATG	3	AIC	62	
7	1:050	F: AGGTGCTGGACACAGACTTCAAC	5	CCC	50	
/	7 phi058	R: ACTGAGATCCAGGCTCCTCTTC	3	CCG	58	
0	8 phi061	F: GACGTAAGCCTAGCTCTGCCAT	0	TTOT OTAT	50	
8		R: AAACAAGAACGGCGGTGCTGATTC	9	TTCT-GTAT	58	
0	1:070	F: GCTGAGCGATCAGTTCATCCAG		ACCTC	50	
9	phi070	R: CCATGGCAGGGTCTCTCAAG	6	AGCTG	58	

10	phi076	F: TTCTTCCGCGGCTTCAATTTGACC	4	AGCGGG	58	
	1	R: GCATCAGGACCCGCAGAGTC	-			
11	1 phi079	F: TGGTGCTCGTTGCCAAATCTACGA	4	AGATG	58	
11	pino//	R: GCAGTGGTGGTTTCGAACAGACAA	7	AGAIG	56	
12	phi116	F: TCCCTGCCGGGACTCCTG	7	ACTG/ACG	58	
12	piiiTto	R: GCATACGGCCATGGATGGGA	,	ACTG/ACG	36	
13	phi127	F: ATATGCATTGCCTGGAACTGGAAGGA	2	AGAC	58	
13	piii127	R: AATTCAAACACGCCTCCCGAGTGT	2	AGAC	56	
14	umc1060	F: ACAGGATTTGAGCTTCTGGACATT	5	(CGG)5	58	
1 7	unicrooo	R: GGCCTCTCCTTCATCCTATTCAA		(600)3		
15	15 umc1265	F: GCCTAGTCGCCTACCCAAT	2	(TCAC)4	58	
	unicizos	R: TGTGTTCTTGATTGGGTGAGACAT		(теле)	30	
16	umc1327	F: AGGGTTTTGCTCTTGGAATCTCTC	8	(GCC)4	58	
10	unic 1327	R: GAGGAAGGAGGAGGTCGTATCGT	0	(GCC)4		
17	umc1403	F: GTACAACGGAGGCATTCTCAAGTT	1	(GCA)4	58	
1 /	uniciaos	R: TGTACATGGTGGTCTTGTTGAGGT	1	(GCA)4	36	
18	umc1446	F: GCGCTGCTGCTTCTTAAATTATCT	1	(TAA)7	58	
10	uniciato	R: GATGAGACCACCTACAAGTTCGCT	1	(IAA)/	36	
19	umc1545	F: GAAAACTGCATCAACAACAAGCTG	7	(AAGA)4	58	
19	19 umc1343	R: ATTGGTTGGTTCTTGCTTCCATTA	/	(AAGA)4	56	
20	20 umc1941	F: ACGACGAGACTCTGTTCTGGTTCT	5	(CTG)10	58	
		R: AGGAGGATTACGTCAATCTGTTCG	,	(C10)10	50	
21	umc2189	F: CGTAAGTACAGTACACCAATGGGC	1	(CAG)4	58	
	21 umc2189	R: ACACCGACTACAAGCCTCTCAACT	1	(CAU)4	50	

phism and reproducibility [1; 15; 16; 17; 18; 19].

PCR parameters, including the annealing temperature (Ta), were individually optimized for each marker to achieve high amplification efficiency and specificity (Table 2). Each reaction was carried out in a total volume of $20~\mu L$, comprising 20 ng of genomic DNA, 1 U of Taq DNA polymerase, 0.2 mM of each dNTP, 10 pM of each primer, 1.5 mM MgCl₂, and 1× Taq buffer. The thermal cycling program began with an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at the marker-specific Ta °C (Table 2) for 30 seconds, and extension at 72 °C for 1 minute, concluding with a final extension at 72 °C for 5 minutes.

PCR products were separated by capillary electrophoresis using the QIAxcel Connect System (QIAGEN, Germany) with the QIAxcel DNA High-Resolution Kit, QX Alignment Marker (15 bp/3 kb), and QX Size Marker (50 bp/1 kb). Sample analysis was performed using the standard OH500 protocol with a 20-second injection time. SSR alleles were identified by comparing fragment sizes to the reference DNA ladder.

Genetic Diversity and Population Structure Analysis

To assess the genetic diversity among the 21 Zea mays accessions, several diversity indices were calculated using GenAlEx version 6.5 [20]. These included the number of alleles per locus (Na), number of effective alleles (Ne), Shannon's Information Index (I), Nei's gene diversity index (uh), percentage of polymorphic loci (%P), and the polymorphic in-

formation content (PIC). Additionally, genetic differentiation (F_{st}) and gene flow (N_m) were estimated. Based on PIC values, SSR markers were categorized as highly informative (PIC > 0.5), moderately informative (0.25 < PIC \leq 0.5), or slightly informative (PIC < 0.25), following the classification by Botstein et al. [21].

The genetic relationships among accessions were further investigated using several multivariate and model-based approaches. A neighbor-joining (NJ) dendrogram was constructed based on genetic distance matrices in PAST software version 3.19 [22]. Principal Coordinate Analysis (PCoA) was performed in GenAlEx to visualize the genetic clustering of individuals in reduced-dimensional space. Analysis of Molecular Variance (AMOVA) was conducted to partition genetic variation within and among maize accessions.

To infer population structure, Bayesian clustering analysis was carried out using STRUCTURE version 2.3.4 [23], employing the admixture model with correlated allele frequencies. The number of genetic clusters (K) was estimated by running simulations for K values ranging from 1 to 10. The optimal K value was determined using the Δ K method proposed by Evanno et al. [24], as implemented in the CLUMPAK platform [25].

RESULTS

Genetic Diversity Across Accessions

A total of 21 accessions of *Zea mays* (ZM) were analyzed using 21 SSR markers. The *Na* ranged from 1.000 (in accessions ZM11, ZM12, ZM17, ZM19, and ZM20) to 2.619 (ZM001), with an average of 1.710, *Ne* varied from 1.000 to 2.239, averaging 1.521 across all accessions (Table 3). The Shannon Information Index, reflecting genetic diversity, showed the highest value in ZM001 (0.825) and the lowest in several accessions (0.000), with an overall mean of 0.372. Nei's genetic diversity index followed a similar trend, peaking in ZM001 (0.629) and averaging 0.291. Polymorphism

levels ranged from 0% in accessions ZM11, ZM12, ZM17, ZM19, and ZM20 to 100% in ZM009, respectively (Table 3), suggesting that 16 out of 21 studied SSR markers were polymorphic. These findings indicate significant differences in genetic diversity among the accessions studied.

SSR loci showed varying levels of polymorphism. The *Na* per locus ranged from 1.4 to 2.0, averaging 1.71. The highest PIC was observed in the umc1327 marker (0.962), suggesting it to be the most informative marker for detecting genetic variation (Table 4).

Table 3. Genetic diversity of maize accessions used in the study

ZM001 5 2.619 2.239 0.825 0.629 ZM002 5 1.905 1.661 0.492 0.395 ZM003 5 2.190 1.856 0.628 0.495 ZM004 5 1.714 1.482 0.367 0.286 ZM005 5 2.333 2.080 0.722 0.571 ZM006 5 2.286 1.915 0.648 0.495 ZM007 5 2.524 2.106 0.741 0.552 ZM008 5 2.143 1.825 0.597 0.467 ZM009 5 2.429 2.083 0.771 0.614 ZM010 5 2.238 1.962 0.705 0.581 ZM011 5 1.000 1.000 0 0 ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 <th>sions</th> <th>N</th> <th>Na</th> <th>Ne</th> <th>I</th> <th>uh</th> <th>%P</th>	sions	N	Na	Ne	I	uh	% P
ZM003 5 2.190 1.856 0.628 0.495 ZM004 5 1.714 1.482 0.367 0.286 ZM005 5 2.333 2.080 0.722 0.571 ZM006 5 2.286 1.915 0.648 0.495 ZM007 5 2.524 2.106 0.741 0.552 ZM008 5 2.143 1.825 0.597 0.467 ZM009 5 2.429 2.083 0.771 0.614 ZM010 5 2.238 1.962 0.705 0.581 ZM011 5 1.000 1.000 0 0 ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 <td>01</td> <td>5</td> <td>2.619</td> <td>2.239</td> <td>0.825</td> <td>0.629</td> <td>95.24%</td>	01	5	2.619	2.239	0.825	0.629	95.24%
ZM004 5 1.714 1.482 0.367 0.286 ZM005 5 2.333 2.080 0.722 0.571 ZM006 5 2.286 1.915 0.648 0.495 ZM007 5 2.524 2.106 0.741 0.552 ZM008 5 2.143 1.825 0.597 0.467 ZM009 5 2.429 2.083 0.771 0.614 ZM010 5 2.238 1.962 0.705 0.581 ZM011 5 1.000 1.000 0 0 ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 <td>02</td> <td>5</td> <td>1.905</td> <td>1.661</td> <td>0.492</td> <td>0.395</td> <td>66.67%</td>	02	5	1.905	1.661	0.492	0.395	66.67%
ZM005 5 2.333 2.080 0.722 0.571 ZM006 5 2.286 1.915 0.648 0.495 ZM007 5 2.524 2.106 0.741 0.552 ZM008 5 2.143 1.825 0.597 0.467 ZM009 5 2.429 2.083 0.771 0.614 ZM010 5 2.238 1.962 0.705 0.581 ZM011 5 1.000 1.000 0 0 ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238	03	5	2.190	1.856	0.628	0.495	76.19%
ZM006 5 2.286 1.915 0.648 0.495 ZM007 5 2.524 2.106 0.741 0.552 ZM008 5 2.143 1.825 0.597 0.467 ZM009 5 2.429 2.083 0.771 0.614 ZM010 5 2.238 1.962 0.705 0.581 ZM011 5 1.000 1.000 0 0 ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM020 5 1.000	04	5	1.714	1.482	0.367	0.286	47.62%
ZM007 5 2.524 2.106 0.741 0.552 ZM008 5 2.143 1.825 0.597 0.467 ZM009 5 2.429 2.083 0.771 0.614 ZM010 5 2.238 1.962 0.705 0.581 ZM011 5 1.000 1.000 0 0 ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1	05	5	2.333	2.080	0.722	0.571	85.71%
ZM008 5 2.143 1.825 0.597 0.467 ZM009 5 2.429 2.083 0.771 0.614 ZM010 5 2.238 1.962 0.705 0.581 ZM011 5 1.000 1.000 0 0 ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	06	5	2.286	1.915	0.648	0.495	76.19%
ZM009 5 2.429 2.083 0.771 0.614 ZM010 5 2.238 1.962 0.705 0.581 ZM011 5 1.000 1.000 0 0 ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	07	5	2.524	2.106	0.741	0.552	85.71%
ZM010 5 2.238 1.962 0.705 0.581 ZM011 5 1.000 1.000 0 0 ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	08	5	2.143	1.825	0.597	0.467	76.19%
ZM011 5 1.000 1.000 0 0 ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	09	5	2.429	2.083	0.771	0.614	100.00%
ZM012 5 1.000 1.000 0 0 ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	10	5	2.238	1.962	0.705	0.581	95.24%
ZM013 5 2.524 2.133 0.758 0.571 ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	11	5	1.000	1.000	0	0	0.00%
ZM014 5 1.048 1.044 0.032 0.029 ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	12	5	1.000	1.000	0	0	0.00%
ZM015 5 1.095 1.045 0.048 0.038 ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	13	5	2.524	2.133	0.758	0.571	90.48%
ZM016 5 1.190 1.111 0.104 0.086 ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	14	5	1.048	1.044	0.032	0.029	4.76%
ZM017 5 1.000 1.000 0 0 ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	15	5	1.095	1.045	0.048	0.038	9.52%
ZM018 5 1.238 1.134 0.127 0.105 ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	16	5	1.190	1.111	0.104	0.086	19.05%
ZM019 5 1.000 1.000 0 0 ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	17	5	1.000	1.000	0	0	0.00%
ZM020 5 1.000 1.000 0 0 ZM021 5 1.429 1.266 0.239 0.200	18	5	1.238	1.134	0.127	0.105	23.81%
ZM021 5 1.429 1.266 0.239 0.200	19	5	1.000	1.000	0	0	0.00%
	20	5	1.000	1.000	0	0	0.00%
Mean 5 1.710 1.521 0.372 0.201	21	5	1.429	1.266	0.239	0.200	42.86%
1./10 1.321 0.3/2 0.271	ın	5	1.710	1.521	0.372	0.291	47.39%
SE 0.00 0.04 0.03 0.020 0.015	E	0.00	0.04	0.03	0.020	0.015	8.57%

Table 4. Assessment of the genetic diversity of SSR markers

No	Locus	Na	Ne	I	uh	F_{st}	N _m	PIC
1	bnlg1520	2.0	1.8	0.482	0.357	0.626	0.149	0.797
2	phi002	1.9	1.7	0.448	0.348	0.592	0.172	0.684
3	phi021	1.7	1.5	0.375	0.305	0.694	0.110	0.815
4	phi027	1.8	1.6	0.431	0.352	0.600	0.166	0.708
5	phi042	1.5	1.4	0.293	0.238	0.735	0.090	0.790
6	phi047	1.8	1.6	0.412	0.329	0.698	0.108	0.878
7	phi058	1.7	1.4	0.330	0.252	0.667	0.125	0.606
8	phi061	1.6	1.4	0.306	0.248	0.738	0.089	0.758
9	phi070	1.6	1.5	0.321	0.248	0.713	0.100	0.691
10	phi076	1.9	1.6	0.443	0.343	0.657	0.131	0.802
11	phi079	1.6	1.5	0.367	0.310	0.665	0.126	0.768

12	phi116	1.9	1.5	0.431	0.333	0.660	0.129	0.785
13	phi127	1.8	1.6	0.406	0.305	0.651	0.134	0.706
14	umc1060	1.9	1.7	0.432	0.329	0.629	0.147	0.716
15	umc1265	2.0	1.7	0.490	0.367	0.635	0.144	0.864
16	umc1327	1.5	1.3	0.258	0.210	0.668	0.124	0.962
17	umc1403	1.7	1.5	0.361	0.276	0.702	0.106	0.828
18	umc1446	1.6	1.4	0.281	0.214	0.749	0.084	0.723
19	umc1545	1.7	1.5	0.364	0.290	0.695	0.110	0.766
20	umc1941	1.4	1.3	0.218	0.181	0.739	0.088	0.557
21	umc2189	1.7	1.5	0.354	0.281	0.736	0.090	0.862
M	ean	1.71	1.52	0.372	0.291	0.679	0.120	0.765
S	SE	0.04	0.03	0.020	0.015	0.010	0.006	0.093

Notes: Na – number of alleles per locus; Ne – effective number of alleles; I - Shannon's Information Index; uh – Nei's genetic diversity index; F_{st} – genetic differentiation; N_{tt} – gene flow; PIC – polymorphic information content; SE – Standard error

Nei's genetic diversity per locus averaged 0.291, and the Shannon index had a mean of 0.372, indicating moderate genetic variation across the loci. The average F_{st} value of 0.679 indicates substantial genetic differentiation among accessions, and the average N_m value (representing gene flow) was 0.120, indicating restricted gene flow between them (Table 4).

Analysis of Molecular Variance (AMOVA)

To evaluate the distribution of genetic variation among and within maize accessions, an AMOVA was performed (Figure 1). The results showed that 66% of the total genetic variation was attributed to differences among accessions, while 34% was due to variation within accessions (Table 3). This indicates a high level of genetic differentiation among the studied accessions.

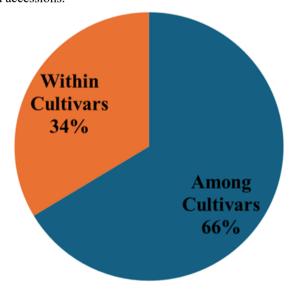


Figure 1. Analysis of Molecular Variance of maize accessions

The estimated F_{st} was 0.611, suggesting a substantial genetic divergence between the groups. The calculated N_m was 0.159, indicating limited genetic exchange among accessions. This may be due to geographic isolation, differences in breeding programs, or restricted distribution of seed material.

To assess the genetic relationships among countries of origin and 21 maize (*Zea mays*) accessions, a dendrogram was constructed using the neighbor-joining method based on SSR

marker data (Figure 2).

The phylogenetic tree clearly separated the samples into three distinct clusters. Chinese accessions formed a separate clade, strongly supported by a bootstrap value of 100. European and Kazakhstani samples grouped together in a well-supported clade (bootstrap = 100), indicating a closer genetic relationship between them compared to the Chinese group (Figure 2A).

The dendrogram divided the accessions into three main clusters (Figure 2B). The first cluster predominantly comprises accessions of Chinese origin (ZM012, ZM014, ZM015, ZM016, ZM020, etc.), as well as one from Turkey (ZM004), which may indicate the proximity of their genetic profiles, likely due to a shared breeding history or similar agro-ecological conditions.

The second cluster shows a mixed structure and comprises accessions from China (ZM011, ZM013), as well as from Kazakhstan (ZM003, ZM008, ZM010), and also representatives from Switzerland (ZM009) and France (ZM017, ZM019) (Figure 2B). This may suggest the presence of introgression or shared ancestral lines, reflecting the use of similar germplasm in breeding programs.

The third cluster is characterized by the highest node support (bootstrap values of 99 and above) and mainly includes accessions of Kazakhstani origin (ZM001, ZM005, ZM006, ZM007), along with one accessions from Switzerland (ZM002). Such clustering indicates a high degree of genetic similarity within this group.

The bootstrap support levels of most branches range from moderate to high, which confirms the reliability of the clustering structure. The obtained results are consistent with the geographical origin of the samples and indicate the presence of both regionally specific genotypes and significant gene flow between countries.

Principal Coordinate Analysis (PCoA)

To further explore the genetic relationships among the three populations and 21 maize (*Zea mays*) accessions, principal coordinate analysis was performed based on SSR marker data (Figure 3).

The PCoA revealed clear genetic differentiation among the populations. The first coordinate (Coord.1), explaining

Table 5. Summary of Analysis of Molecular Variance

Source	df	SS	MS	Est. Var.	%	F _{st}	N _m
Among Accessions	20	4013.600	200.680	36.444	66%		
Within Accessions	84	1550.800	18.462	18.462	34%		
Total	104	5564.400		54.906	100%	0.611	0.159

Notes: df – degrees of freedom; SS – sum of squares; MS – mean squared; Est.var. – estimates of variance; % – percentage of variation; F_{st} – fixation index; N_{m} – gene flow value;

^{*} p < 0.001; $N_m = (1 - F_{et})/4F_{et}$

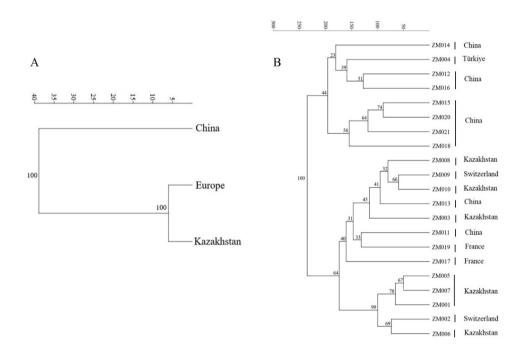


Figure 2. Dendrogram illustrating genetic relationships among (A) countries of origin of accessions and (B) 21 maize (*Zea mays*) accessions based on SSR marker data using the neighbor-joining method

89.98% of the total variation, separated the Chinese samples from those of Kazakhstan and Europe. The second coordinate (Coord.2), accounting for 10.02% of the variation, further distinguished the European samples from the Kazakhstani group. These results indicate distinct genetic clustering among the regional groups, with Chinese accessions showing the greatest divergence (Figure 3A).

A similar analysis was performed among accessions. The first two principal coordinates accounted for 30.85% and 15.68% of the total genetic variation, respectively, together explaining 46.53% of the observed variation (Figure 3B).

The PCoA plot revealed a clear separation of the accessions into several distinct groups, which is generally consistent with the clustering observed in the dendrogram (Figure 2B). Accessions of Chinese origin (e.g., ZM012, ZM014, ZM015, ZM016, ZM020) clustered together predominantly in the lower left quadrant, suggesting a high degree of genetic similarity likely resulting from shared breeding backgrounds and similar selection pressures.

The accessions of Kazakhstan origin (ZM001, ZM003, ZM005, ZM006, ZM007, ZM010) were mainly distributed in the lower right quadrant, indicating a close genetic relationship among these genotypes (Figure 3B). Notably, ZM006 appeared somewhat separated from other accessions of this

group, potentially reflecting unique genetic contributions or localized adaptation.

Accessions from European countries, such as Switzerland (ZM002, ZM009) and France (ZM017, ZM019), were positioned in the upper right and central regions of the plot, showing moderate genetic differentiation relative to the Chinese and Kazakhstan groups (Figure 3B).

Additionally, accessions ZM018 and ZM021 occupied isolated positions on the plot, suggesting distinct genetic profiles and potential unique ancestry or introgression events.

Overall, the PCoA results corroborate the findings from the dendrogram analysis, confirming the presence of regional genetic structuring among the analyzed maize accessions and highlighting the influence of both geographic origin and breeding history on their genetic diversity.

Population Structure Analysis

The population structure of the 21 maize accessions was analyzed using a model-based Bayesian clustering approach (STRUCTURE analysis) (Figure 4). The results indicated the presence of three distinct genetic clusters (K=3), which is consistent with the dendrogram and PCoA results.

The first cluster (represented in red) comprised predominantly Chinese accessions, as well as some accessions with

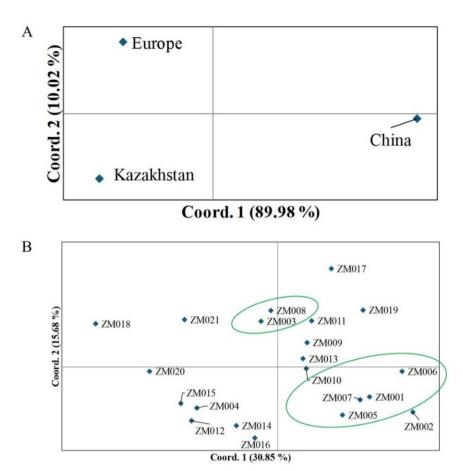


Figure 3. Principal Coordinate Analysis plot showing the genetic relationships among (A) origin countries of accessions and (B) 21 maize (*Zea mays*) accessions based on SSR marker data. Kazakh accessions are indicated with a green circle

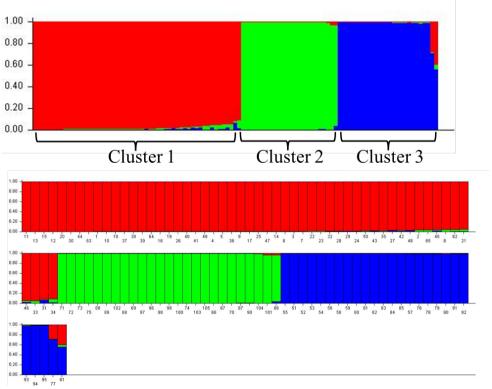


Figure 4. Population structure of 21 maize accessions. Distribution of maize accessions by clusters at K = 3

mixed ancestry components. This suggests a high level of genetic similarity within this group, likely due to shared breeding backgrounds or standard selection practices.

The second cluster (green) primarily consisted of accessions from Kazakhstan, as well as a few from European countries (Figure 4), indicating a degree of genetic admixture and

possible introgression events resulting from shared germplasm pools.

The third cluster (blue) contained accessions from Switzerland and certain Kazakhstani accessions, indicating a separate gene pool and possible distinct selection histories (Figure 4).

Several accessions displayed evidence of admixture, as indicated by the presence of multiple color components in their membership coefficients, suggesting historical gene flow or hybridization among populations.

Overall, the STRUCTURE analysis (Figure 4) corroborates the findings from the dendrogram (Figure 2) and PCoA (Figure 3), supporting the existence of both regionally distinct and admixed genotypes among the studied maize accessions.

DISCUSSION

In the present study, substantial variability was observed across several genetic diversity indices among 21 maize (*Zea mays*) accessions grown in Kazakhstan, as demonstrated by wide ranges in the number of alleles per locus, effective alleles, Shannon's information index, and Nei's genetic diversity index. These findings are consistent with previous reports emphasizing the effectiveness of SSR markers in analyzing maize genetic structure.

For instance, Bocianowski et al. (2021) reported that the use of 30 SSR primers enabled the detection of 112 markers, with the number of alleles per locus ranging from 1 to 17, and an average of 3.7 alleles per locus. Despite a higher mean Na reported in their study, the overall distribution of alleles and the high informativeness of specific loci (e.g., phi061 with PIC = 0.497) underscore the significant reservoir of genetic diversity present in maize accessions.

In our study, the average Na was 1.71, and the PIC values ranged from 0.557 to 0.962, confirming the presence of both moderately and highly informative markers. Notably, the umc1327 marker exhibited the highest PIC value (0.962), highlighting its potential utility for genotype identification and future breeding applications. Similarly, Bocianowski et al. identified markers such as phi021 and phi061 as significantly associated with multiple quantitative traits (e.g., grain yield, grain moisture, ear length), which aligns with our findings regarding the multifunctional value of specific SSR loci.

The study by Kumari et al. (2018) further supports the suitability of SSR markers for diversity assessment. Although their research utilized only 22 markers on eight maize genotypes, they reported mean PIC and genetic diversity values of 0.297 and 0.373, respectively, which are lower than those obtained in our research. This discrepancy can likely be attributed to the smaller sample size and more limited geographical representation in the study of Kumari with co-authors. In contrast, our analysis included both local and introduced accessions, thus providing a broader scope of genetic variation and enabling the identification of unique allelic profiles, particularly among Kazakh and Chinese accessions. The AM-OVA in our work revealed that most genetic variation (66%) was attributable to differences among accessions. This finding is consistent with Bocianowski et al., who explained substantial differentiation by geographic origin and differences in breeding programs. The F_{st} (0.611) and N_m (0.159) observed in our study indicate limited inter-population exchange, a trend also noted by other authors, underscoring the importance of conserving regional genetic resources.

The phylogenetic analysis and PCoA collectively demonstrate clear genetic structuring among the studied accessions from Kazakhstan, China, and Europe. The phylogenetic tree revealed that Chinese accessions formed a distinct clade, while Kazakhstani and European samples were grouped separately, indicating limited gene flow and possible geographic isolation. This pattern was further supported by the PCoA results, where the first coordinate (89.98% of variation) clearly separated Chinese samples, while the second coordinate (10.02%) distinguished between Kazakhstan and Europe. Similar clusters based on geographical and breeding relationships were also reported in studies by Bocianowski et al. and Kumari et al. Further studies incorporating more markers and broader sampling could provide deeper insights into the evolutionary history and population dynamics of the species.

Overall, our results underscore the importance of SSR markers in assessing genetic diversity and structuring maize collections in Kazakhstan. The highly informative markers identified in this study can be effectively utilized in marker-assisted selection programs to develop new hybrids with enhanced adaptive and agronomic traits.

CONCLUSIONS

The present study demonstrates a moderate to high level of genetic diversity among maize accessions grown in Kazakhstan, as revealed by 21 SSR markers. Significant genetic differentiation was observed among accessions, largely corresponding to their geographic and breeding origin. The highest genetic diversity was recorded in local Kazakh accessions, indicating their potential value for breeding programs. Among the tested SSRs, several markers - including umc1327 (PIC = 0.962), umc1265 (0.864), umc2189 (0.862), phi047 (0.878), phi021 (0.815), phi076 (0.802), and bnlg1520 (0.797) demonstrated high informativeness and are suitable for application in future marker-assisted selection, genetic fingerprinting, and conservation strategies. Overall, the findings emphasize the importance of incorporating molecular tools in the development of genetically diverse and climate-resilient maize accessions in Kazakhstan.

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УДК 633.15

АНАЛИЗ ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ ОБРАЗЦОВ *ZEA MAYS* L. НА ОСНОВЕ МИКРОСАТЕЛЛИТНЫХ МАРКЕРОВ

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АННОТАЦИЯ

В данном исследовании была проведена оценка генетического разнообразия 21 сортов и гибридов кукурузы (Zea mays L.), возделываемых в Казахстане, с использованием 21 SSR-маркера. Использование выбранных маркеров позволило выявить значительный уровень полиморфизма, при этом значения информативности маркеров (PIC) варьировали от 0.557 до 0.962, что свидетельствует о высокой информативности. Показатели генетического разнообразия, такие как общее количество аллелей (Na), эффективное число аллелей (Ne), информационный индекс Шеннона (I) и индекс генетического разнообразия по Nei (I), значительно различались между образцами, при этом казахстанский образец ZM001 показал наибольшее разнообразие. Анализ молекулярной дисперсии (AMOVA) показал, что 66% общей генетической вариации обусловлены различиями между образцами, что подтверждает высокую степень дифференциации популяций (I) среди образцов кукурузы. Кластерный анализ, анализ главных координат (PCoA) и анализ STRUCTURE группировали образцы по их географическому происхождению, различая местные, китайские и европейские образцы. Полученные результаты подчеркивают эффективность использованных в данном ичледовании SSR-маркеров в выявлении генетической структуры и демонстрируют наличие неиспользованного аллельного разнообразия в коллекции кукурузы Казахстана.

Ключевые слова: Zea mays, SSR-маркеры, генетическое разнообразие, анализ молекулярной дисперсии.

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$ZEA\ MAYS\ L.\ YЛГІЛЕРІНІҢ ГЕНЕТИКАЛЫҚ АЛУАНТҰРЛІЛІГІН МИКРОСАТЕЛЛИТТІ МАРКЕРЛЕР НЕГІЗІНДЕ ТАЛДАУ$

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ТҮЙІН

Бұл зерттеуде Қазақстанда өсірілетін жүгерінің ($Zea\ mays\ L.$) 21 сорттары мен гибридтерінің генетикалық алуантүрлілігі 21 SSR маркерінің көмегімен бағаланды. Таңдалған маркерлер елеулі полиморфизмді көрсетті, олардың полиморфты ақпараттық мазмұнының (PIC) мәндері 0.557 мен 0.962 аралығында өзгеріп, маркерлердің жоғары ақпараттылығын көрсетті. Генетикалық алуантүрлілік көрсеткіштері – аллельдер саны (Na), тиімді аллельдер саны (Ne), Шеннон ақпарат индексі (I) және Nei генетикалық алуантүрлілік индексі (uh) – үлгілер арасында айтарлықтай ерекшеленді. Қазақстандық ZM001 үлгісі ең жоғары генетикалық алуантүрлілікті көрсетті. Молекулалық дисперсияны талдау (AMOVA) жалпы генетикалық вариацияның 66%-ы үлгілер арасындағы айырмашылықтармен байланысты екенін көрсетті, бұл жүгері үлгілері арасында айқын популяциялық жіктелуді (F_{st} = 0.611) дәлелдейді. Кластерлік талдау, негізгі координаттар бойынша талдау (PCoA) және STRUCTURE талдауы үлгілерді географиялық шығу тегі бойынша бірізді топтарға бөліп, жергілікті, қытайлық және еуропалық үлгілерді ажыратты. Бұл нәтижелер SSR маркерлерінің генетикалық құрылымды анықтаудағы тиімділігін және Қазақстандағы жүгері генофондында пайдаланылмаған аллельдік алуантүрліліктің бар екенін көрсетеді.

Кілт сөздер: Zea mays, SSR маркерлері, генетикалық алуантұрлілік, молекулалық дисперсиялық талдау.

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