

## Research article

<https://doi.org/10.31489/2026FEB3/66-82>

UDC 633.15

Received: 3.04.2026 | Accepted: 16.04.2026 | Published online: 30.09.2026

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## Microsatellite-based genetic diversity evaluation of *Zea mays* L. accessions from different geographical origins

The purpose of this study was to investigate the genetic diversity and population structure of 51 maize (*Zea mays* L.) accessions originating from Kazakhstan, China, and Europe using simple sequence repeat (SSR) markers. A total of 21 highly polymorphic SSR markers were used to genotype 255 individuals, enabling a comprehensive assessment of allelic variation and genetic relationships. The number of alleles per locus ranged from 1.0 to 2.7, with a mean of 1.517, while expected heterozygosity ( $uHe$ ) averaged 0.192, indicating moderate genetic diversity among the accessions. Genetic diversity analysis by geographic origin revealed that European accessions exhibited the highest allelic richness ( $N_a = 5.9$ ) and genetic diversity, followed by Chinese and Kazakhstani accessions. Despite these differences, all groups exhibited 100% polymorphism, confirming the high informativeness of the selected SSR markers. Polymorphic information content (PIC) values ranged from 0.446 to 0.884, with an average of 0.712, indicating that markers such as *phi047*, *bnlg1520*, and *umc2189* were highly informative. Analysis of molecular variance (AMOVA) demonstrated that 70% of the total genetic variation was attributable to differences among accessions, indicating strong genetic differentiation ( $F_{st} = 0.702$ ) and limited gene flow ( $N_m = 0.212$ ). Cluster analysis, principal coordinate analysis, and STRUCTURE analysis consistently revealed clear genetic separation of the Chinese accessions, whereas European and Kazakhstani accessions showed closer genetic relationships and partial admixture. Overall, the findings highlight substantial genetic diversity and distinct population structure among the maize accessions, providing valuable insights for germplasm conservation and the selection of genetically diverse parental lines for breeding programs.

**Keywords:** *Zea mays*, Kazakhstan, molecular marker, SSR, genetic diversity, polymorphism, genetic structure, genotyping.

### Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide and ranks among the top three staple grains alongside wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) [1]. Owing to its high productivity, wide adaptability, and diverse end uses, maize plays a central role in global food security, livestock production, and industrial applications [2]. It is cultivated across a broad range of agroecological zones, from temperate to tropical regions, reflecting its remarkable genetic plasticity and capacity to adapt to diverse environmental conditions [3].

This extensive adaptability and phenotypic diversity are closely linked to the complex taxonomic structure of maize within the species *Zea mays*. Classification proposed by Hugh Iltis and John Doebley [4] provided a detailed subdivision of the *Zea mays* complex; however, it is now considered partly outdated due to advances in molecular systematic and revised taxonomic approaches. In contrast, modern authoritative databases such as Plants of the World Online (POWO) [5] adopt a simplified and currently accepted view, recognizing only three subspecies within *Zea mays*: *Zea mays* subsp. *mays*, *Zea mays* subsp. *parviglumis* Iltis & Doebley, and *Zea mays* subsp. *huehuetenangensis* (Iltis & Doebley) Doebley.

Within this group, subsp. *mays* is the domesticated form grown extensively across the globe, while subsp. *parviglumis* and subsp. *huehuetenangensis* are wild relatives, or teosintes. These wild subspecies are critical for tracing the evolutionary roots of maize and serve as vital resources for expanding its genetic diversity. Specifically, *Zea mays* subsp. *parviglumis* (Balsas teosinte) is identified as the immediate progenitor

of modern maize, with the distinct morphological variations between them arising from human-driven selection throughout the domestication process [6–8].

Within the cultivated subspecies *Zea mays* subsp. *mays*, an exceptionally high level of genetic and phenotypic diversity is observed, reflecting thousands of years of domestication, selection, and adaptation to diverse agroecological conditions. This diversity is expressed in numerous varieties (often referred to as kernel or endosperm types), which differ in grain structure, biochemical composition, and end-use applications [9].

Among the most widely recognized varieties, dent maize (*var. indentata*) is characterized by a combination of hard and soft endosperm, resulting in a depression at the crown of the kernel; it is predominantly used for animal feed and industrial processing, including starch and bioethanol production. Flint maize (*var. indurata*) possesses a hard outer endosperm layer, making it more resistant to mechanical damage and suitable for cultivation in cooler climates, as well as for food products such as grits and traditional dishes. Sweet maize (*var. saccharata*) is distinguished by mutations affecting sugar metabolism, resulting in high sugar content in immature kernels, and is widely consumed as a vegetable. Popcorn (*var. everta*) has a unique kernel structure with a hard pericarp and dense endosperm, allowing it to expand explosively when heated. Flour maize (*var. amylacea*) contains predominantly soft starch, facilitating milling into flour and its use in traditional foods, particularly in indigenous cultures. Waxy maize (*var. ceratina*) is rich in amylopectin and is valued in the food industry and for specialized starch applications [10–13].

This broad spectrum of forms within *Zea mays* subsp. *mays* highlights its remarkable genetic plasticity and adaptability. The diversity of kernel types is not only of economic importance but also reflects underlying allelic variation at key loci controlling starch composition, texture, and metabolic pathways. Such extensive intrasubspecific variation makes cultivated maize one of the most genetically diverse crop species and underscores its significance as a model organism for genetic, genomic, and breeding studies [14–16].

Given this high level of genetic diversity, reliable tools are required to accurately assess variation both within and among maize populations. Molecular markers have become essential in this context, providing a robust alternative to traditional agro-morphological evaluation. Among them, simple sequence repeat (SSR) markers are particularly widely used due to their high polymorphism, co-dominant inheritance, reproducibility, and genome-wide distribution [17, 18]. SSR markers enable precise detection of allelic variation, making them highly suitable for studies of genetic diversity, population structure, and relationships among plants [19].

Numerous studies have successfully applied SSR markers to characterize maize germplasm worldwide, revealing substantial levels of genetic variation and facilitating the development of core collections and breeding strategies [20–25]. These approaches have proven especially valuable for identifying unique alleles and underutilized genetic resources, which are critical for broadening the genetic base of modern breeding programs.

In maize, the use of SSR markers is particularly relevant due to the presence of numerous hybrid varieties, each with distinct parental contributions, as well as diverse local landraces. These hybrids, while improving yield and stress tolerance, can also reduce genetic diversity if a limited set of parental lines is repeatedly used. SSR-based analyses, therefore, provide an essential tool for evaluating and preserving genetic variation in both landraces and hybrids, supporting informed breeding and conservation efforts [26, 27].

Although SSR-based studies of maize have been widely conducted worldwide, research on maize genetic diversity using SSR markers in Kazakhstan remains scarce. Previous studies in the region have mostly focused on agro-morphological traits [28–31] or small panels of genotypes, such as the 21 accessions analyzed in our earlier work [32], which provided preliminary information but did not fully capture the genetic diversity of local germplasm.

This study extends prior research by examining 51 maize accessions, including local landraces from Kazakhstan and global hybrid varieties. This larger, more diverse panel enables a more comprehensive assessment of genetic diversity and population structure using SSR markers. Such analyses not only provide insights into the extent of variation within cultivated maize in Kazakhstan but also inform breeding programs by identifying genetically distinct lines and potential sources of beneficial alleles for future maize hybrid development.

### Experimental

**Plant material.** The study included 51 accessions (hybrids) of common maize (*Zea mays* subsp. *mays* L.). By origin, accessions are divided into three groups: from Kazakhstan (17), China (10), and Europe (24). The European accessions were represented by Germany (9), Switzerland (7), Türkiye (3),

France (2), Russia (2), and Greece (1). The hybrids were developed using a population, single-, two- (F<sub>1</sub>), and three-line hybrid systems. Seed material was provided by the Krasnovodopad Agricultural Experimental Station (AES) and the peasant farm (PF) “Sabyr” (Fig. 1; Tab. 1).

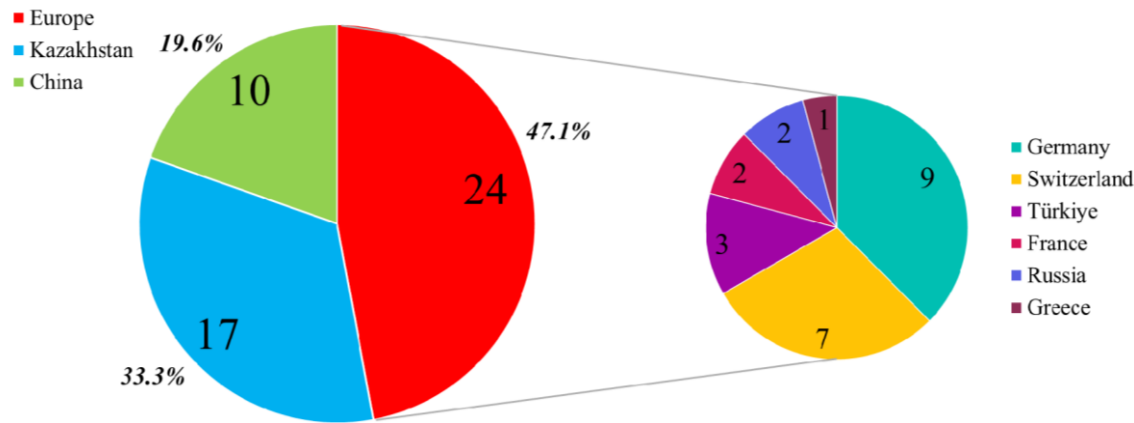


Figure 1. Distribution of maize accessions used in this study by country of origin

Table 1

Characteristics of maize accessions used for SSR genotyping

| №  | ID     | Accessions         | Country of origin    | Developing system       |
|----|--------|--------------------|----------------------|-------------------------|
| 1  | ZM-001 | Altai-319          | Kazakhstan           | Three-line hybrid       |
| 2  | ZM-002 | Gidro F2           | Switzerland (Europe) | Two-line (F1) hybrid    |
| 3  | ZM-003 | Tulpar-539         | Kazakhstan           | Two-line (F1) hybrid    |
| 4  | ZM-004 | F2 S0069           | Türkiye (Europe)     | Two-line (F1) hybrid    |
| 5  | ZM-005 | ZPSK-704           | Kazakhstan           | Two-line (F1) hybrid    |
| 6  | ZM-006 | Altyn 739          | Kazakhstan           | Two-line (F1) hybrid    |
| 7  | ZM-007 | KazZP-777          | Kazakhstan           | Three-line hybrid       |
| 8  | ZM-008 | Mariam-419         | Kazakhstan           | Two-line (F1) hybrid    |
| 9  | ZM-009 | SV Bambus F2       | Switzerland (Europe) | Two-line (F1) hybrid    |
| 10 | ZM-010 | Sunkar-779         | Kazakhstan           | Three-line hybrid       |
| 11 | ZM-011 | Fenikks Spartak    | China                | Two-line (F1) hybrid    |
| 12 | ZM-012 | Fenikks Atlant     | China                | Two-line (F1) hybrid    |
| 13 | ZM-013 | Fenikks Chelentano | China                | Two-line (F1) hybrid    |
| 14 | ZM-014 | Fenikks Takelau    | China                | Two-line (F1) hybrid    |
| 15 | ZM-015 | Fenikks Bora-bora  | China                | Two-line (F1) hybrid    |
| 16 | ZM-016 | Fenikks Gobi       | China                | Two-line (F1) hybrid    |
| 17 | ZM-017 | LG31642            | France (Europe)      | Two-line (F1) hybrid    |
| 18 | ZM-018 | Fenikks Avrora     | China                | Two-line (F1) hybrid    |
| 19 | ZM-019 | LG31555            | France (Europe)      | Two-line (F1) hybrid    |
| 20 | ZM-020 | Fenikks Francheska | China                | Two-line (F1) hybrid    |
| 21 | ZM-021 | Fenikks Bombei     | China                | Two-line (F1) hybrid    |
| 22 | ZM-022 | Symbat-1           | Kazakhstan           | Two-line (F1) hybrid    |
| 23 | ZM-024 | BG23-1637          | Kazakhstan           | Two-line (F1) hybrid    |
| 24 | ZM-025 | Briso              | Germany (Europe)     | Two-line (F1) hybrid    |
| 25 | ZM-026 | Skap 620           | Russia (Europe)      | Two-line (F1) hybrid    |
| 26 | ZM-027 | Hamilton           | Greece (Europe)      | Single-line (F1) hybrid |
| 27 | ZM-029 | KVS Intelegenes    | Germany (Europe)     | Two-line (F1) hybrid    |
| 28 | ZM-030 | KVS Kaleido        | Germany (Europe)     | Two-line (F1) hybrid    |
| 29 | ZM-038 | Dala aru 446P      | Kazakhstan           | Population hybrid       |
| 30 | ZM-039 | KazLK444           | Kazakhstan           | Two-line (F1) hybrid    |
| 31 | ZM-040 | KazLK575           | Kazakhstan           | Two-line (F1) hybrid    |
| 32 | ZM-041 | KazLK780           | Kazakhstan           | Two-line (F1) hybrid    |
| 33 | ZM-042 | KazNIIZiR 90       | Kazakhstan           | Two-line (F1) hybrid    |

Continuation of Table 1

| №  | ID     | Accessions      | Country of origin    | Developing system    |
|----|--------|-----------------|----------------------|----------------------|
| 34 | ZM-044 | KVS Alkanto     | Germany (Europe)     | Two-line (F1) hybrid |
| 35 | ZM-045 | KVS Atako       | Germany (Europe)     | Three-line hybrid    |
| 36 | ZM-048 | KVS Elektro     | Germany (Europe)     | Two-line (F1) hybrid |
| 37 | ZM-049 | Kerubino OK-69  | Germany (Europe)     | Two-line (F1) hybrid |
| 38 | ZM-050 | Kefrankos       | Germany (Europe)     | Two-line (F1) hybrid |
| 39 | ZM-051 | Kleopatras      | Germany (Europe)     | Two-line (F1) hybrid |
| 40 | ZM-056 | M11G99          | Türkiye (Europe)     | Two-line (F1) hybrid |
| 41 | ZM-057 | M-16C45         | Türkiye (Europe)     | Two-line (F1) hybrid |
| 42 | ZM-062 | SI Bambus       | Switzerland (Europe) | Two-line (F1) hybrid |
| 43 | ZM-063 | SI Gladius      | Switzerland (Europe) | Two-line (F1) hybrid |
| 44 | ZM-065 | SI Cursor       | Switzerland (Europe) | Two-line (F1) hybrid |
| 45 | ZM-066 | SI Maileston    | Switzerland (Europe) | Two-line (F1) hybrid |
| 46 | ZM-067 | SI Fontero      | Switzerland (Europe) | Two-line (F1) hybrid |
| 47 | ZM-069 | Skap 301CB      | Russia (Europe)      | Two-line (F1) hybrid |
| 48 | ZM-070 | Taulsizdik 20CB | Kazakhstan           | Two-line (F1) hybrid |
| 49 | ZM-072 | Tomiris         | Kazakhstan           | Three-line hybrid    |
| 50 | ZM-073 | Hyuaksi 948     | China                | Two-line (F1) hybrid |
| 51 | ZM-074 | Turan480        | Kazakhstan           | Three-line hybrid    |

**DNA extraction.** Genomic DNA was extracted from 5-day-old maize seedlings (five replicates per accession) using a modified Dellaporta protocol [33]. The quality and quantity of the extracted DNA were assessed using a NanoDrop One spectrophotometer (Thermo Fisher Scientific Inc., USA) and 1 % agarose gel electrophoresis. For PCR analysis, genomic DNA was normalized to 100 ng/μL.

**SSR genotyping.** A set of 21 SSR markers (Tab. 2) was selected for genotyping 255 maize individuals based on their high levels of polymorphism, reproducibility, and informativeness, with five replicates per accession. These markers have been validated in previous studies, ensuring reliable and comparable results [20–25].

PCR parameters, including the annealing temperature (Ta), were individually optimized for each marker to achieve high amplification efficiency and specificity (Tab. 2). Each reaction was carried out in a total volume of 20 μL, comprising 100 ng of genomic DNA, 1 U of Taq DNA polymerase, 0.2 mM of each dNTP, 10 pM of each primer, 1.5 mM MgCl<sub>2</sub>, 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.

Table 2

## Characteristics of SSR markers used for analysis

| № | Primer          | Primer Sequence (5'-3')                                           | Motif                | Chr. No. | Ta (°C) | Associated genes and traits [34]                            |
|---|-----------------|-------------------------------------------------------------------|----------------------|----------|---------|-------------------------------------------------------------|
| 1 | <i>bnlg1520</i> | F: TCCTCTTGCTCTCCATGTCC<br>R: ACAGCTGCGTAGCTTCTTCC                | (AG) <sub>22</sub>   | 2        | 58      | -                                                           |
| 2 | <i>phi002</i>   | F: CATGCAATCAATAACGATGGCGAGT<br>R: TTAGCGTAACCTTCTCCAGTCAGC       | AACG                 | 1        | 58      | <i>agal2</i> , 100 kernel weight, leaf below flag leaf      |
| 3 | <i>phi021</i>   | F: TTCCATTCTCGTGTTCTTGGAGTGGTCCA<br>R: CTTGATCACCTTTCCTGCTGTCGCCA | AG                   | 4        | 58      | <i>adh2</i>                                                 |
| 4 | <i>phi027</i>   | F: GCGTACGTACGACGAAGACAC<br>R: CACAGCACGTTGCGGATTTCTCT            | (GCGCT) <sub>n</sub> | 9        | 58      | <i>wx1</i> , amino acids, leaf below flag leaf, metabolites |

Continuation of Table 2

| №  | Primer         | Primer Sequence (5'-3')        | Motif     | Chr. No. | Ta (°C) | Associated genes and traits [34]                                       |
|----|----------------|--------------------------------|-----------|----------|---------|------------------------------------------------------------------------|
| 5  | <i>phi042</i>  | F: ATGTGGCCATCATTCAATGCTGTAGAC | CATA      | 9        | 60      | <i>sus1</i> , photoperiod growing-degree days to silk, node number     |
|    |                | R: ACACATGCAGGTGCAGCCAGA       |           |          |         |                                                                        |
| 6  | <i>phi047</i>  | F: GGAGATGCTCGCACTGTTCTC       | ATC       | 3        | 62      | <i>mybr17</i>                                                          |
|    |                | R: CTCCACCCTCTTTGACATGGTATG    |           |          |         |                                                                        |
| 7  | <i>phi058</i>  | F: AGGTGCTGGACACAGACTTCAAC     | CCG       | 5        | 58      | <i>col12</i> , tassel branch number, ear height, plant height          |
|    |                | R: ACTGAGATCCAGGCTCCTCTTC      |           |          |         |                                                                        |
| 8  | <i>phi061</i>  | F: GACGTAAGCCTAGCTCTGCCAT      | TTCT-GTAT | 9        | 58      | <i>wx1</i> , amino acids, leaf below flag leaf, metabolites            |
|    |                | R: AAACAAGAACGGCGGTGCTGATTC    |           |          |         |                                                                        |
| 9  | <i>phi070</i>  | F: GCTGAGCGATCAGTTCATCCAG      | AGCTG     | 6        | 58      | <i>mlg3</i>                                                            |
|    |                | R: CCATGGCAGGGTCTCTCAAG        |           |          |         |                                                                        |
| 10 | <i>phi076</i>  | F: TTCTTCCGCGGCTTCAATTTGACC    | AGCGGG    | 4        | 58      | -                                                                      |
|    |                | R: GCATCAGGACCCGCAGAGTC        |           |          |         |                                                                        |
| 11 | <i>phi079</i>  | F: TGGTGCTCGTTGCCAAATCTACGA    | AGATG     | 4        | 58      | <i>gpc1</i>                                                            |
|    |                | R: GCAGTGGTGGTTTCGAACAGACAA    |           |          |         |                                                                        |
| 12 | <i>phi116</i>  | F: TCCCTGCCGGGACTCCTG          | ACTG/ACG  | 7        | 58      | <i>phi116</i>                                                          |
|    |                | R: GCATACGGCCATGGATGGGA        |           |          |         |                                                                        |
| 13 | <i>phi127</i>  | F: ATATGCATTGCCTGGAAGTGAAGGA   | AGAC      | 2        | 58      | -                                                                      |
|    |                | R: AATTCAAACACGCCTCCCGAGTGT    |           |          |         |                                                                        |
| 14 | <i>umc1060</i> | F: ACAGGATTTGAGCTTCTGGACATT    | (CGG)5    | 5        | 58      | <i>umc1060</i>                                                         |
|    |                | R: GGCCTCTCCTTCATCCTATTCAA     |           |          |         |                                                                        |
| 15 | <i>umc1265</i> | F: GCCTAGTCGCCTACCCTACCAAT     | (TCAC)4   | 2        | 58      | <i>ereb197</i> , tassel spike length; leaf angle, tassel branch number |
|    |                | R: TGTGTTCTTGATTGGGTGAGACAT    |           |          |         |                                                                        |
| 16 | <i>umc1327</i> | F: AGGGTTTTGCTCTTGGAATCTCTC    | (GCC)4    | 8        | 58      | <i>umc1327</i> , anthesis date                                         |
|    |                | R: GAGGAAGGAGGAGGTCGTATCGT     |           |          |         |                                                                        |
| 17 | <i>umc1403</i> | F: GTACAACGGAGGCATTCTCAAGTT    | (GCA)4    | 1        | 58      | <i>xyl5</i> , 100 kernel weight                                        |
|    |                | R: TGTACATGGTGGTCTTGTTGAGGT    |           |          |         |                                                                        |
| 18 | <i>umc1446</i> | F: GCGCTGCTGCTTCTTAAATTATCT    | (TAA)7    | 1        | 58      | <i>kcs15</i>                                                           |
|    |                | R: GATGAGACCACCTACAAGTTCGCT    |           |          |         |                                                                        |
| 19 | <i>umc1545</i> | F: GAAAAGTGCATCAACAACAAGCTG    | (AAGA)4   | 7        | 58      | <i>hsp3</i>                                                            |
|    |                | R: ATTGGTTGGTTCTTGCTTCCATTA    |           |          |         |                                                                        |
| 20 | <i>umc1941</i> | F: ACGACGAGACTCTGTTCTGGTTCT    | (CTG)10   | 5        | 58      | <i>umc1941</i> , kernel row number                                     |
|    |                | R: AGGAGGATTACGTCAATCTGTTCCG   |           |          |         |                                                                        |
| 21 | <i>umc2189</i> | F: CGTAAGTACAGTACACCAATGGGC    | (CAG)4    | 1        | 58      | <i>epf10</i>                                                           |
|    |                | R: ACACCGACTACAAGCCTCTCAACT    |           |          |         |                                                                        |

**Genetic diversity and Population structure analysis.** Genetic diversity among the 51 maize accessions was evaluated using a set of diversity indices calculated with GenAlEx version 6.5 [35]. The parameters analyzed for SSR data included: the number of alleles per locus ( $N_a$ ), the effective number of alleles ( $N_e$ ), Shannon's information index ( $I$ ), Nei's genetic diversity index ( $u_h$ ), and the proportion of polymorphic loci ( $%P$ ). In addition, levels of genetic differentiation among populations ( $F_{st}$ ), expected Heterozygosity ( $u_{He}$ ), and estimates of gene flow ( $N_m$ ) were determined. Also, polymorphic information content (PIC) was calculated as described by Botstein et al. [36]. According to PIC values, SSR markers were categorized as  $PIC > 0.5$  (highly informative),  $0.25 < PIC \leq 0.5$  (moderately informative), and  $PIC \leq 0.25$  (slightly informative), following the classification proposed by Botstein et al. [36].

Analysis of Molecular Variance (AMOVA) was conducted to quantify the distribution of genetic variation within and among populations. Genetic structuring among individuals and populations was visualized using Principal Coordinate Analysis (PCoA). Initial PCoA by country of origin was performed in GenAlEx 6.5 [35] in a reduced-dimensional space. Additionally, a PCoA of 51 accessions was conducted in RStudio [37] to provide a clearer visualization of genetic relationships.

Patterns of genetic relationships among maize accessions were further examined using both distance-based and multivariate analytical methods. Genetic distance matrices were used to construct classical dendrograms to illustrate the relationships among accessions and their geographical origins. The analysis was performed using PAST software, version 3.19 [38], and the tvBOT online web application for visualizing the results [39].

The robustness of the clusters was assessed using bootstrap analysis with 1000 replicates. The population genetic structure was inferred using a Bayesian clustering approach implemented in STRUCTURE version 2.3.4 [40] under an admixture model with correlated allele frequencies. Runs were performed for K values ranging from 1 to 10, and the most probable number of genetic clusters was identified using the  $\Delta K$  method of Evanno et al. [41], as implemented in the CLUMPAK platform [42]. STRUCTURE runs were performed using a burn-in period of 100,000 iterations followed by 100,000 MCMC repetitions. For each K value (K = 1-10), 10 independent runs were conducted to ensure consistency of clustering results. The STRUCTURE outputs were summarized and visualized using the CLUMPAK platform [42].

### Results and Discussion

*Genetic diversity across accessions.* A total of 51 maize accessions, five replicates per accession, were genotyped using 21 simple sequence repeat (SSR) markers. Genetic diversity parameters varied markedly among the analyzed maize accessions (Tab. 3). The observed number of alleles ( $N_a$ ) ranged from 1.0 to 2.7, with a mean value of  $1.517 \pm 0.023$ . The effective number of alleles ( $N_e$ ) ranged from 1.0 to 2.3, with an average of  $1.373 \pm 0.018$  across all accessions.

Table 3

Genetic diversity by 51 maize accessions

| №  | Accession | $N_a$ | $N_e$ | $I$   | $u_h$ | $u_{He}$ | %P       |
|----|-----------|-------|-------|-------|-------|----------|----------|
| 1  | ZM-001    | 2.6   | 2.3   | 0.833 | 0.638 | 0.567    | 95.24 %  |
| 2  | ZM-002    | 2.0   | 1.7   | 0.524 | 0.424 | 0.377    | 71.43 %  |
| 3  | ZM-003    | 2.2   | 1.9   | 0.652 | 0.514 | 0.457    | 80.95 %  |
| 4  | ZM-004    | 1.7   | 1.5   | 0.367 | 0.286 | 0.254    | 47.62 %  |
| 5  | ZM-005    | 2.4   | 2.1   | 0.744 | 0.586 | 0.521    | 85.71 %  |
| 6  | ZM-006    | 2.3   | 1.9   | 0.672 | 0.514 | 0.457    | 80.95 %  |
| 7  | ZM-007    | 2.5   | 2.1   | 0.741 | 0.552 | 0.491    | 85.71 %  |
| 8  | ZM-008    | 2.1   | 1.8   | 0.597 | 0.467 | 0.415    | 76.19 %  |
| 9  | ZM-009    | 2.4   | 2.1   | 0.771 | 0.614 | 0.546    | 100.00 % |
| 10 | ZM-010    | 2.2   | 2.0   | 0.710 | 0.586 | 0.521    | 95.24 %  |
| 11 | ZM-011    | 1.0   | 1.0   | 0.000 | 0.000 | 0.000    | 0.00 %   |
| 12 | ZM-012    | 1.0   | 1.0   | 0.000 | 0.000 | 0.000    | 0.00 %   |
| 13 | ZM-013    | 2.5   | 2.1   | 0.758 | 0.571 | 0.508    | 90.48 %  |
| 14 | ZM-014    | 1.0   | 1.0   | 0.032 | 0.029 | 0.025    | 4.76 %   |
| 15 | ZM-015    | 1.1   | 1.0   | 0.048 | 0.038 | 0.034    | 9.52 %   |
| 16 | ZM-016    | 1.2   | 1.1   | 0.104 | 0.086 | 0.076    | 19.05 %  |
| 17 | ZM-017    | 1.0   | 1.0   | 0.000 | 0.000 | 0.000    | 0.00 %   |
| 18 | ZM-018    | 1.2   | 1.1   | 0.127 | 0.105 | 0.093    | 23.81 %  |
| 19 | ZM-019    | 1.0   | 1.0   | 0.000 | 0.000 | 0.000    | 0.00 %   |
| 20 | ZM-020    | 1.0   | 1.0   | 0.000 | 0.000 | 0.000    | 0.00 %   |
| 21 | ZM-021    | 1.4   | 1.3   | 0.239 | 0.200 | 0.178    | 42.86 %  |
| 22 | ZM-022    | 2.4   | 2.0   | 0.688 | 0.524 | 0.466    | 85.71 %  |
| 23 | ZM-024    | 1.4   | 1.3   | 0.253 | 0.214 | 0.190    | 38.10 %  |
| 24 | ZM-025    | 1.2   | 1.1   | 0.104 | 0.086 | 0.076    | 19.05 %  |
| 25 | ZM-026    | 1.0   | 1.0   | 0.000 | 0.000 | 0.000    | 0.00 %   |
| 26 | ZM-027    | 1.1   | 1.1   | 0.045 | 0.033 | 0.030    | 4.76 %   |
| 27 | ZM-029    | 1.0   | 1.0   | 0.032 | 0.029 | 0.025    | 4.76 %   |
| 28 | ZM-030    | 1.3   | 1.2   | 0.167 | 0.133 | 0.119    | 33.33 %  |
| 29 | ZM-038    | 2.7   | 2.3   | 0.854 | 0.643 | 0.571    | 95.24 %  |
| 30 | ZM-039    | 1.9   | 1.7   | 0.519 | 0.433 | 0.385    | 80.95 %  |
| 31 | ZM-040    | 1.3   | 1.2   | 0.151 | 0.124 | 0.110    | 28.57 %  |
| 32 | ZM-041    | 1.8   | 1.4   | 0.389 | 0.314 | 0.279    | 76.19 %  |

Continuation of Table 3

| №    | Accession | <i>Na</i> | <i>Ne</i> | <i>I</i> | <i>uh</i> | <i>uHe</i> | % <i>P</i> |
|------|-----------|-----------|-----------|----------|-----------|------------|------------|
| 33   | ZM-042    | 2.3       | 1.9       | 0.693    | 0.543     | 0.483      | 90.48 %    |
| 34   | ZM-044    | 1.0       | 1.0       | 0.000    | 0.000     | 0.000      | 0.00 %     |
| 35   | ZM-045    | 1.0       | 1.0       | 0.000    | 0.000     | 0.000      | 0.00 %     |
| 36   | ZM-048    | 1.0       | 1.0       | 0.000    | 0.000     | 0.000      | 0.00 %     |
| 37   | ZM-049    | 1.0       | 1.0       | 0.000    | 0.000     | 0.000      | 0.00 %     |
| 38   | ZM-050    | 1.1       | 1.1       | 0.077    | 0.062     | 0.055      | 9.52 %     |
| 39   | ZM-051    | 1.0       | 1.0       | 0.024    | 0.019     | 0.017      | 4.76 %     |
| 40   | ZM-056    | 1.1       | 1.0       | 0.048    | 0.038     | 0.034      | 9.52 %     |
| 41   | ZM-057    | 1.6       | 1.4       | 0.353    | 0.295     | 0.262      | 57.14 %    |
| 42   | ZM-062    | 1.1       | 1.0       | 0.048    | 0.038     | 0.034      | 9.52 %     |
| 43   | ZM-063    | 1.1       | 1.1       | 0.071    | 0.057     | 0.051      | 14.29 %    |
| 44   | ZM-065    | 1.0       | 1.0       | 0.000    | 0.000     | 0.000      | 0.00 %     |
| 45   | ZM-066    | 1.1       | 1.1       | 0.056    | 0.048     | 0.042      | 9.52 %     |
| 46   | ZM-067    | 1.1       | 1.0       | 0.048    | 0.038     | 0.034      | 9.52 %     |
| 47   | ZM-069    | 1.9       | 1.7       | 0.481    | 0.390     | 0.347      | 61.90 %    |
| 48   | ZM-070    | 2.0       | 1.8       | 0.528    | 0.414     | 0.368      | 66.67 %    |
| 49   | ZM-072    | 1.5       | 1.4       | 0.290    | 0.248     | 0.220      | 42.86 %    |
| 50   | ZM-073    | 1.0       | 1.0       | 0.000    | 0.000     | 0.000      | 0.00 %     |
| 51   | ZM-074    | 1.1       | 1.1       | 0.080    | 0.067     | 0.059      | 14.29 %    |
| Mean |           | 1.517     | 1.373     | 0.273    | 0.216     | 0.192      | 36.79 %    |
| SE   |           | 0.023     | 0.018     | 0.012    | 0.009     | 0.008      | 5.09 %     |

*Note.* *Na* — number of alleles per locus; *Ne* — effective number of alleles; *I* — Shannon's Information Index; *uh* — Nei's genetic diversity index; *uHe* — expected heterozygosity; %*P* — level of polymorphism; SE — standard error.

Genetic diversity estimated using Shannon's information index (*I*) ranged from 0.000 to 0.854, with a mean value of 0.273. A similar pattern was observed for heterozygosity indices. The Nei's diversity index (*uh*) ranged from 0.000 to 0.643, while expected heterozygosity (*uHe*) varied from 0.000 to 0.571, with mean values of  $0.216 \pm 0.009$  and  $0.192 \pm 0.008$ , respectively.

The highest levels of polymorphism (%*P*) were observed in ZM-009 (100.00 %) and in ZM-001, ZM-010, and ZM-038 (95.24 %). In contrast, several accessions, including ZM-011, ZM-012, ZM-017, ZM-019, ZM-020, ZM-026, ZM-044, ZM-045, ZM-048, ZM-049, ZM-065, and ZM-073, exhibited no polymorphism at all and showed minimal values for all diversity indices.

When comparing the present study with our previous work on 21 maize accessions cultivated in Kazakhstan [32], the mean values of *Na*, *Ne*, and PIC are lower in the expanded dataset of 51 accessions. The mean number of alleles per locus ( $Na = 1.52 \pm 0.023$ ), effective alleles ( $Ne = 1.37 \pm 0.018$ ), and PIC ( $0.712 \pm 0.01$ ) were reduced compared to the earlier study ( $Na = 1.71 \pm 0.04$ ;  $Ne = 1.52 \pm 0.03$ ;  $PIC = 0.765 \pm 0.093$ ), despite the inclusion of a larger number of accessions. This decrease can be attributed to the broader sampling, which incorporated genetically diverse and geographically distant accessions, introducing rare alleles that lower the average allele frequency. At the same time, the larger sample size improved the reliability of estimates, as reflected by smaller standard errors, providing a more robust and statistically representative picture of population-wide diversity. Genetic diversity parameters calculated by origin are presented in Table 4.

Table 4

## Genetic diversity among the three groups of origin

| №    | Origin     | <i>N</i> | <i>Na</i> | <i>Ne</i> | <i>I</i> | <i>uh</i> | <i>uHe</i> | % <i>P</i> |
|------|------------|----------|-----------|-----------|----------|-----------|------------|------------|
| 1    | China      | 50       | 4.7       | 3.603     | 1.345    | 0.716     | 0.709      | 100.00 %   |
| 2    | Europe     | 120      | 5.9       | 3.804     | 1.430    | 0.710     | 0.708      | 100.00 %   |
| 3    | Kazakhstan | 85       | 4.3       | 2.848     | 1.121    | 0.611     | 0.607      | 100.00 %   |
| Mean |            | 85.000   | 4.952     | 3.419     | 1.299    | 0.679     | 0.674      | 100.00 %   |
| SE   |            | 3.629    | 0.200     | 0.152     | 0.043    | 0.016     | 0.016      | 0.00 %     |

*Note.* *N* — number of samples; *Na* — number of alleles per locus; *Ne* — effective number of alleles; *I* — Shannon's Information Index; *uh* — Nei's genetic diversity index; *uHe* — expected heterozygosity; %*P* — level of polymorphism; SE — standard error.

The Chinese group, represented by 50 individuals, showed that the observed number of alleles ( $N_a$ ) was 4.7, while the effective number of alleles ( $N_e$ ) reached 3.603. Shannon's information index ( $I$ ) was 1.345. The Nei's diversity index ( $u_h$ ) and expected heterozygosity ( $u_{He}$ ) were 0.716 and 0.709, respectively. The percentage of polymorphic loci (%P) was 100.00 %. The European group, comprising 120 individuals, had  $N_a$  and  $N_e$  values of 5.9 and 3.804, respectively. Shannon's information index ( $I$ ) reached 1.430. The values of  $u_h$  and  $u_{He}$  were 0.710 and 0.708, respectively, with %P also equal to 100.00 %.

The Kazakhstani group included 85 individuals and showed an observed number of alleles ( $N_a$ ) of 4.3 and an effective number of alleles ( $N_e$ ) of 2.848. Shannon's information index ( $I$ ) was 1.121. The Nei's diversity index ( $u_h$ ) and expected heterozygosity ( $u_{He}$ ) were 0.611 and 0.607, respectively. The percentage of polymorphic loci (%P) was 100.00 %. Mean values across all groups were  $4.952 \pm 0.200$  for  $N_a$ ,  $3.419 \pm 0.152$  for  $N_e$ , and  $1.299 \pm 0.043$  for Shannon's information index ( $I$ ), while  $u_h$  and  $u_{He}$  averaged  $0.679 \pm 0.016$  and  $0.674 \pm 0.016$ , respectively.

These findings suggest that the European population harbors greater overall genetic variability, likely due to historical selection, extensive gene flow, and cultivation across diverse environments. The Shannon's information index ( $I$ ), which reflects both allele richness and evenness, was also highest in European accessions ( $I = 1.430$ ), followed by Chinese ( $I = 1.345$ ) and Kazakhstani ( $I = 1.121$ ) samples, further supporting the conclusion that European maize possesses the most diverse genetic structure.

The diversity observed in Chinese maize accessions reflects earlier findings that inbred lines in China are influenced by environmental conditions and human selection, resulting in high genetic variability [22, 43]. Waxy maize is reported to have originated in Southwestern China, where it displays substantial diversity in agronomic traits, highlighting the region as a center of diversity [44]. Consequently, while some diversity measures are slightly lower than in European accessions, Chinese maize still exhibits considerable allelic richness and heterozygosity, making it an important resource for breeding and conservation.

Kazakhstani populations exhibited intermediate genetic positions, reflecting their role as a transition zone between East Asian and European germplasm. The relatively lower polymorphism and reduced diversity indices observed in Kazakhstani accessions may be attributed to several factors. Local breeding and selection practices, often focused on adaptation to arid continental climates and specific agronomic requirements [30], may have led to the fixation of favorable alleles and the reduction of rare variants. Limited gene flow from distant European or Chinese germplasm compared to local populations could have constrained allelic richness, resulting in fewer effective alleles ( $N_e$ ) and lower heterozygosity. Historical founder effects and population bottlenecks, common in geographically isolated or recently introduced germplasm, may also contribute to reduced genetic variability [45]. Together, these factors explain why Kazakhstani maize represents a genetically intermediate group: it shares allelic features with both European and East Asian populations but exhibits lower overall polymorphism due to local adaptation and limited gene exchange. A total of 51 maize accessions were evaluated using 21 SSR markers, all of which were polymorphic and included in analyses of genetic diversity and population structure (Tabl. 5).

Table 5

## Genetic diversity by markers

| Nº | Locus           | No | $N_a$ | $N_e$ | $I$   | $u_h$ | $u_{He}$ | $F_{st}$ | $N_m$ | PIC   |
|----|-----------------|----|-------|-------|-------|-------|----------|----------|-------|-------|
| 1  | <i>bnlg1520</i> | 10 | 1.7   | 1.6   | 0.361 | 0.269 | 0.738    | 0.727    | 0.094 | 0.786 |
| 2  | <i>phi002</i>   | 5  | 1.6   | 1.5   | 0.346 | 0.278 | 0.583    | 0.633    | 0.145 | 0.607 |
| 3  | <i>phi021</i>   | 6  | 1.4   | 1.3   | 0.232 | 0.190 | 0.719    | 0.795    | 0.065 | 0.742 |
| 4  | <i>phi027</i>   | 5  | 1.5   | 1.4   | 0.276 | 0.222 | 0.690    | 0.742    | 0.087 | 0.686 |
| 5  | <i>phi042</i>   | 5  | 1.4   | 1.3   | 0.208 | 0.171 | 0.720    | 0.813    | 0.058 | 0.729 |
| 6  | <i>phi047</i>   | 12 | 1.6   | 1.5   | 0.318 | 0.249 | 0.826    | 0.775    | 0.073 | 0.884 |
| 7  | <i>phi058</i>   | 4  | 1.7   | 1.5   | 0.374 | 0.290 | 0.633    | 0.641    | 0.140 | 0.647 |
| 8  | <i>phi061</i>   | 7  | 1.5   | 1.3   | 0.261 | 0.210 | 0.706    | 0.785    | 0.068 | 0.782 |
| 9  | <i>phi070</i>   | 5  | 1.4   | 1.3   | 0.236 | 0.190 | 0.691    | 0.795    | 0.065 | 0.741 |
| 10 | <i>phi076</i>   | 7  | 1.5   | 1.4   | 0.283 | 0.222 | 0.549    | 0.710    | 0.102 | 0.611 |
| 11 | <i>phi079</i>   | 5  | 1.4   | 1.3   | 0.210 | 0.173 | 0.714    | 0.816    | 0.056 | 0.750 |
| 12 | <i>phi116</i>   | 6  | 1.6   | 1.4   | 0.298 | 0.237 | 0.701    | 0.737    | 0.089 | 0.722 |
| 13 | <i>phi127</i>   | 5  | 1.6   | 1.4   | 0.305 | 0.237 | 0.692    | 0.751    | 0.083 | 0.761 |
| 14 | <i>umc1060</i>  | 6  | 1.6   | 1.5   | 0.330 | 0.253 | 0.759    | 0.747    | 0.085 | 0.800 |
| 15 | <i>umc1265</i>  | 6  | 1.6   | 1.4   | 0.323 | 0.247 | 0.724    | 0.741    | 0.087 | 0.764 |

Continuation of Table 5

| №    | Locus          | No  | Na    | Ne    | I     | uh    | uHe   | Fst   | Nm    | PIC   |
|------|----------------|-----|-------|-------|-------|-------|-------|-------|-------|-------|
| 16   | <i>umc1327</i> | 4   | 1.3   | 1.2   | 0.145 | 0.116 | 0.455 | 0.792 | 0.066 | 0.446 |
| 17   | <i>umc1403</i> | 7   | 1.5   | 1.4   | 0.276 | 0.216 | 0.640 | 0.747 | 0.085 | 0.683 |
| 18   | <i>umc1446</i> | 4   | 1.4   | 1.3   | 0.208 | 0.161 | 0.635 | 0.814 | 0.057 | 0.692 |
| 19   | <i>umc1545</i> | 8   | 1.5   | 1.4   | 0.280 | 0.225 | 0.705 | 0.771 | 0.074 | 0.787 |
| 20   | <i>umc1941</i> | 7   | 1.3   | 1.2   | 0.188 | 0.157 | 0.516 | 0.766 | 0.076 | 0.536 |
| 21   | <i>umc2189</i> | 8   | 1.5   | 1.4   | 0.273 | 0.218 | 0.766 | 0.784 | 0.069 | 0.808 |
| Mean |                | 6.3 | 1.517 | 1.373 | 0.273 | 0.216 | 0.674 | 0.756 | 0.082 | 0.712 |
| SE   |                | 2.0 | 0.023 | 0.018 | 0.012 | 0.009 | 0.016 | 0.011 | 0.005 | 0.100 |

Note. No — number of observed alleles; Na — number of alleles per locus; Ne — effective number of alleles; I — Shannon's Information Index; uh— Nei's genetic diversity index; uHe — expected heterozygosity; Fst — pairwise fixation index; Nm — gene flow value; PIC — polymorphic information content; SE — standard error.

The number of observed alleles (*No*) varied noticeably across the SSR markers analyzed, ranging from 4 to 12, for a total of 132 alleles. The highest allelic richness was detected at locus *phi047* (*No* = 12), followed by *bnlg1520* (*No* = 10) and several loci, including *umc1545* and *umc2189* (*No* = 8). Intermediate values (*No* = 6–7) were observed for loci such as *phi021*, *umc1060*, *umc1265*, *umc1403*, and *umc1941*. In contrast, the lowest number of alleles (*No* = 4–5) was recorded for loci including *phi058*, *umc1327*, *umc1446*, *phi002*, *phi027*, and *phi079*. The loci also showed variation in allelic richness, with the number of alleles per locus (*Na*) ranging from 1.2 to 1.7. The highest values were observed for *bnlg1520* and *phi058* (1.7), while the lowest were recorded for *umc1327* and *umc1941* (1.2). Effective alleles (*Ne*) ranged from 1.2 to 1.6, with the greatest values at *bnlg1520*, *phi047*, *phi127*, *umc1060*, and *umc1265*.

Genetic diversity across loci also varied according to Shannon's Information Index (*I*), which spanned from 0.145 (*umc1327*) to 0.374 (*phi058*). Nei's diversity index (*uh*) ranged between 0.116 (*umc1327*) and 0.290 (*phi058*), whereas expected heterozygosity (*uHe*) extended from 0.455 (*umc1327*) to 0.826 (*phi047*). Population differentiation, measured by the fixation index (*Fst*), ranged from 0.056 (*phi079*) to 0.145 (*phi002*), while gene flow (*Nm*) varied from 0.446 (*umc1327*) to 0.884 (*phi047*). Polymorphic information content (*PIC*) values ranged from 0.446 (*umc1327*) to 0.884 (*phi047*), with a mean of 0.712±0.1. According to common criteria (*PIC* > 0.5 = highly informative markers), the most informative loci included *phi047* (0.884), *bnlg1520* (0.786), *phi021* (0.742), *phi058* (0.647), *phi127* (0.761), *umc1060* (0.800), and *umc2189* (0.808).

Despite these differences, the uniformly high level of polymorphism across all groups indicates that the SSR markers used in this study are highly informative and suitable for detecting genetic variation in maize. This is further supported by the high mean *PIC* value (0.712±0.1), confirming the strong discriminatory power of the selected loci. These findings are consistent with previous reports emphasizing the effectiveness of SSR markers in analyzing maize genetic structure [46]

Particular attention should be given to the most polymorphic markers identified in this study, such as *phi047*, *bnlg1520*, *umc1060*, and *umc2189*, which demonstrated the highest *PIC* values and allelic richness. These markers are not only effective for differentiating genotypes but are also associated with genes controlling important agronomic traits (Tab. 2). For example, markers such as *phi058* and *umc1265* are linked to traits including plant height, tassel architecture, and leaf angle, while *phi002* and *umc1403* are associated with kernel weight [47]. Similarly, markers such as *phi027* and *phi061* are connected to genes involved in starch biosynthesis (*wx1*) and carbohydrate metabolism, which directly influence grain quality [48]. In addition, the marker *phi047* has been reported to be associated with the *mybri17* gene, a transcription factor implicated in the regulation of photosynthetic processes and in enhancing photosynthetic efficiency in maize [49]. This highlights the potential of these SSR markers for marker-assisted selection, particularly to improve yield components, adaptation, and stress tolerance in maize breeding programs.

#### Analysis of Molecular Variance (AMOVA)

The distribution of genetic variation among and within maize accessions was assessed using analysis of molecular variance (AMOVA, Tab. 6). The results indicated that 70 % of the total genetic variation was attributable to differences among accessions, while the remaining 30 % was attributable to differences within accessions. The sum of squares (*SS*) for variation among accessions was 1445.608, with a mean square (*MS*) of 28.912 and an estimated variance of 5.329. In contrast, variation within accessions had an *SS* of 462.000, an *MS* of 2.265, and an estimated variance of 2.265. The total estimated variance across all accessions was 7.594. The fixation index (*Fst*) calculated from AMOVA was 0.702, reflecting the proportion of total genetic

variation attributable to differences among accessions. Correspondingly, the estimated number of migrants per generation ( $Nm$ ) was 0.212, representing the relative level of gene flow among accessions.

According to population genetics theory,  $Nm$  values below 1 indicate that gene flow is insufficient to counteract genetic drift, leading to substantial differentiation among populations [50]. In our collection, the high among-accession variation can largely be explained by the fact that it includes maize varieties from multiple countries: Germany, Switzerland, France, Russia, Türkiye, Greece, China, and Kazakhstan. Because these accessions come from different breeding programs, climates, and agricultural traditions, they carry distinct allelic compositions, which lead to much greater genetic differences between accessions than within them.

Table 6

## Results of Analysis of Molecular Variance

| Source            | df  | SS       | MS     | Est. Var. | %     | Fst    | Nm     |
|-------------------|-----|----------|--------|-----------|-------|--------|--------|
| Among accessions  | 50  | 1445,608 | 28,912 | 5,329     | 70 %  | 0.702* | 0.212* |
| Within accessions | 204 | 462,000  | 2,265  | 2,265     | 30 %  |        |        |
| Total             | 254 | 1907,608 |        | 7,594     | 100 % |        |        |

Note. df — degrees of freedom; SS — sum of squares; MS — mean squared; Est.var. — estimates of variance; % — percentage of variation; Fst — pairwise fixation index; Nm — gene flow value; \*  $p < 0.001$ .

To assess the genetic relationships among countries of origin and 51 maize (*Zea mays*) accessions, a dendrogram was constructed using the classical method based on SSR marker data. The phylogenetic analysis by country of origin revealed a clear division into three distinct clusters. Accessions from China formed a highly discrete clade, a pattern robustly supported by a bootstrap value of 100. In contrast, samples from Europe and Kazakhstan clustered together within a common clade (bootstrap = 58), suggesting that these two groups share a closer genetic affinity with each other than with the Chinese lineage (Fig. 2A).

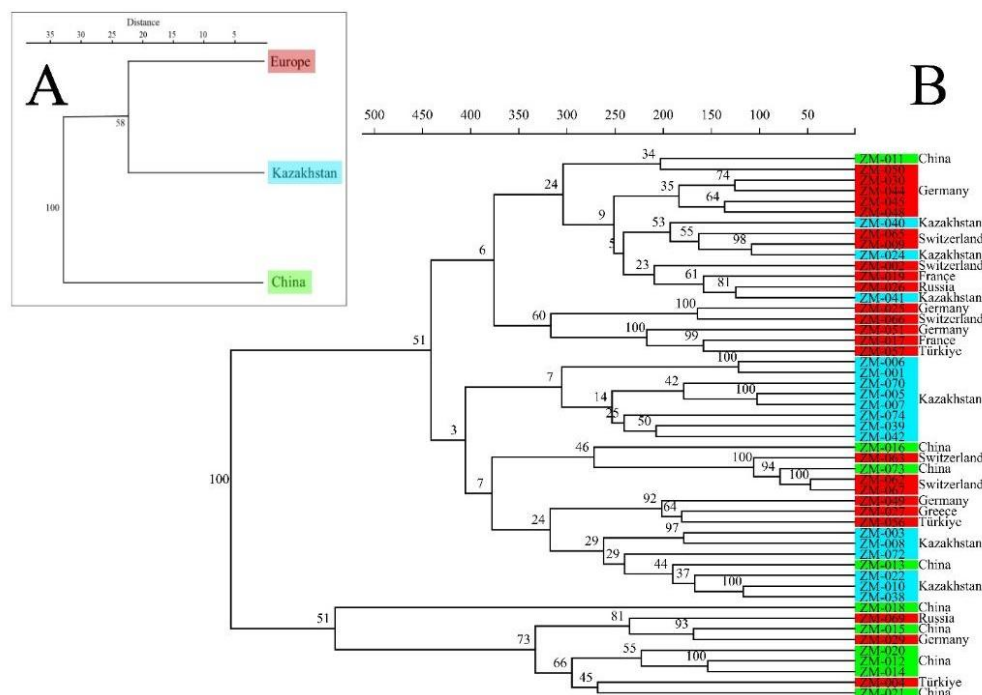


Figure 2. Dendrogram illustrating genetic relationships among *Zea mays* L.:  
A — by origin, B — by each accession (color-coded according to the country of origin)

The phylogenetic analysis of the accessions reveals a similar pattern (Fig. 2B), dividing the studied accessions into three clusters based on genetic similarity coefficients and branching patterns, distinguishing European (Cluster I) and Kazakhstani (Cluster II) accessions from Chinese (Cluster III) accessions. Cluster I, located in the upper section of the dendrogram, consists primarily of European accessions and includes sam-

ples from Germany (ZM-050, ZM-030, ZM-044, ZM-045, ZM-048, ZM-025, ZM-051), Switzerland (ZM-065, ZM-009, ZM-002, ZM-066), France (ZM-019), Russia (ZM-026), and Türkiye (ZM-057). Also, this cluster consists of one Chinese (ZM-011) and a few Kazakhstani accessions (ZM-040, ZM-024, ZM-041). Cluster II represents a larger, more complex meta-cluster, subdivided into multiple subclades. The upper portion of Cluster II contains a stable Kazakhstani sub-group (ZM-001, ZM-005, ZM-006, ZM-007, ZM-039, ZM-042, ZM-070, ZM-074) supported by bootstrap values up to 100. Other mixed-origin subclades are present within Cluster II. One sub-clade (bootstrap 100) unites Swiss accessions (ZM-062, ZM-063, ZM-067) with a Chinese accession (ZM-073), while another highly supported sub-clade (bootstrap 97–100) groups Kazakhstani accessions (ZM-003, ZM-008, ZM-072, ZM-022, ZM-010, ZM-038) together with the Chinese accession ZM-013. Cluster III, located in the lower section of the dendrogram, is predominantly Chinese and includes accessions ZM-018, ZM-015, ZM-020, ZM-014, ZM-012, and ZM-021. In addition, individual accessions from Germany (ZM-029), Russia (ZM-069), and Türkiye (ZM-004) were also localized within this cluster.

The observed clustering pattern is consistent with the patterns detected in PCoA: the dendrogram revealed three main clusters that largely correspond to the geographic origin of the accessions. European accessions formed a distinct cluster, reflecting their relatively high genetic diversity and shared breeding history. Chinese accessions were grouped into a separate cluster, indicating moderate differentiation from both European and Kazakhstani accessions. The Kazakhstan accessions, while showing some internal substructuring, tended to cluster together, reflecting their closer genetic relatedness and narrower gene pool. Some accessions, however, exhibited intermediate positions between clusters, suggesting historical gene flow or shared ancestry. For example, certain European accessions (ZM-004, ZM-029, ZM-069) clustered near the Chinese group, suggesting shared alleles due to past germplasm exchange or common parental lines. Similarly, a few Kazakhstan accessions (ZM-024, ZM-040, and ZM-041) showed affinity with European accessions, suggesting limited introgression of exotic germplasm or residual heterogeneity within local populations.

#### Principal Coordinate Analysis (PCoA)

To further explore the genetic relationships among the three groups by origin and 51 maize (*Zea mays*) accessions, principal coordinate analysis was performed based on SSR marker data (Fig. 3).

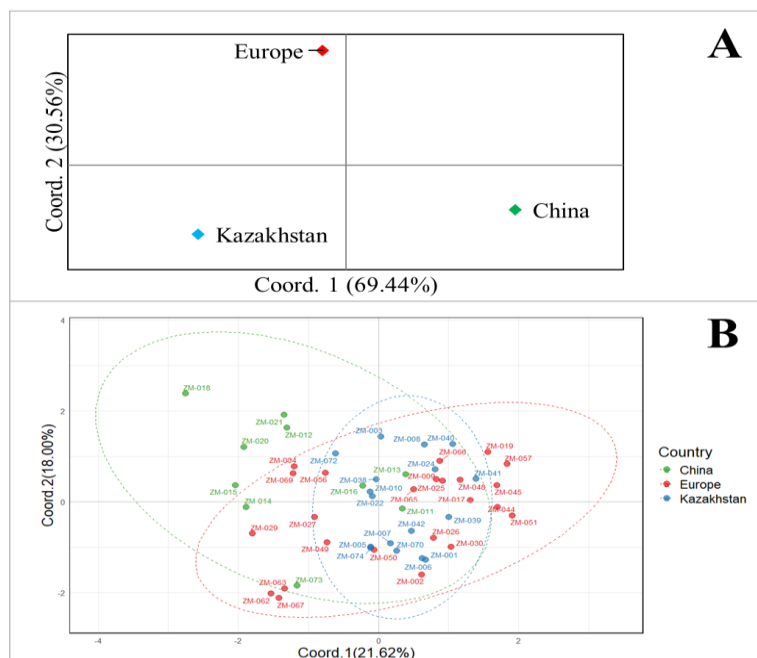


Figure 3. Principal Coordinate analysis plots based on SSR data:

A — by origin, B — by accession, each dot represents one accession, color-coded according to the country of origin

The Principal Coordinate Analysis (PCoA) revealed a distinct genetic separation by the samples' geographical origin. The first coordinate (Coord. 1), which accounted for 69.44 % of the total genetic variation, primarily differentiated the Chinese accessions from the Kazakhstani and European groups. The second co-

ordinate (Coord. 2), explaining an additional 30.56 % of the variance, provided further resolution by separating the European samples from those of Kazakhstani origin (Fig. 3A).

A similar analysis was performed among accessions. The first two principal coordinates accounted for 21.62 % and 18.00 % of the total genetic variation, respectively, together explaining 39.62 % of the observed variation (Fig. 3B). The PCoA plot revealed a relatively clear separation of the accessions into several groups, which is generally consistent with the clustering observed in the dendrogram (Fig. 2).

Accessions of Chinese origin (ZM-012, ZM-020, ZM-021, ZM-015, ZM-014) clustered together predominantly in the higher left quadrant. Notably, Chinese accessions ZM-018 and ZM-073 diverged from the core cluster, shifting toward the upper and lower regions of the plot, respectively. Also, accessions ZM-016, ZM-013, and ZM-011 settled in the central region of the plot, blending with samples from Kazakhstan and Europe. Kazakhstan accessions (ZM001, ZM-003, ZM-005, ZM006, ZM007, ZM010, ZM-074, ZM-070, ZM-024, and others) were mainly distributed in the middle of the plot, admixturing mainly with accessions of European origin. Accessions from European countries are split into two sides of the Coord. 1, and form two groups. The first group, to the left, consisted of Turkish (ZM-004, ZM-069), Russian (ZM-056), Greek (ZM-027), German (ZM-029, ZM-049, ZM-050), and Swiss (ZM-062, ZM-063, ZM-067) accessions. The second group, to the right, was composed of various accessions of European origin: Germany (ZM-025, ZM-030, ZM-044, ZM-045, ZM-048, ZM-051), Switzerland (ZM-002, ZM-009, ZM-065, ZM-066), Russia (ZM-026), Türkiye (ZM-057), and France (ZM-017, ZM-019).

Kazakhstani samples positioned between the European and Chinese clusters. This intermediate placement corroborates their role as a transition zone between East Asian and European germplasm, consistent with the reduced polymorphism observed in these populations. European accessions formed a relatively tight and distinct cluster, reflecting their high genetic diversity and structured allelic composition, while Chinese accessions showed greater spread within the cluster, indicating both high diversity and variability in allele distribution. Overall, the PCoA confirms the genetic distinctness of European, Chinese, and Kazakhstani maize populations, and visually supports the conclusions drawn from allele-based diversity indices.

These results are consistent with previous studies on crop and tree species, in which European accessions often exhibit higher genetic diversity than local or introduced populations. For example, analyses of walnut (*Juglans regia* L.) demonstrated that European genotypes had higher allelic richness and Shannon indices than Kazakhstani accessions, whereas Chinese populations often showed intermediate or variable values depending on the loci examined [51]. Similarly, population genetic studies in cultivated apples (*Malus x domestica* Borkh.) have reported that broader geographic distribution and historical gene flow in European populations contribute to higher  $N_a$ ,  $N_e$ , and heterozygosity values [52].

#### Population Structure Analysis

The population structure of the 51 maize accessions was analyzed using a model-based Bayesian clustering approach (STRUCTURE analysis) (Fig. 4). The highest  $\Delta K$  value was observed at  $K = 2$  ( $\Delta K = 238.49111$ ). The results indicated the presence of two distinct genetic clusters ( $K=2$ ). The first cluster (represented in red) comprised predominantly accessions from Europe and Kazakhstan. The second cluster (green) was composed of Chinese, Kazakhstani, and a few European accessions.

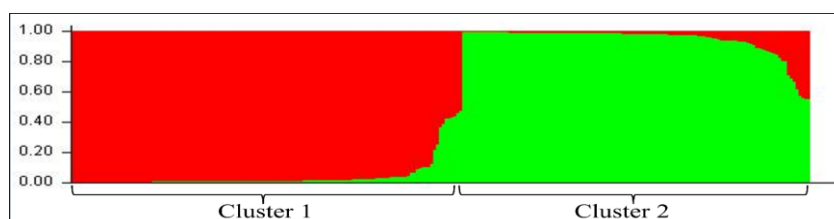


Figure 4. Population structure of 51 maize accessions. Distribution of maize accessions by clusters at  $K=2$

This consolidation of European and Kazakhstani accessions into a single cluster suggests a high degree of shared ancestry or historical gene flow between these regions, which is consistent with known patterns of germplasm exchange and breeding practices. A similar pattern was observed in soybean (*Glycine max* L.), where Kazakhstani accessions clustered together with European genotypes, indicating close genetic relatedness and likely historical germplasm exchange between regions [53]. Examination of individual membership coefficients highlights both clear assignments and intermediate cases. For example, accessions such as ZM-024, ZM-029, ZM-065, and ZM-073 exhibited strong affiliation with the red cluster (>95 %), while

ZM-003, ZM-009, and ZM-014 were almost entirely assigned to the green cluster (>95 %), reflecting distinct lineage separation. Interestingly, some individuals displayed mixed ancestry, including ZM-002-1 (57 % red, 42 % green) and ZM-010-5 (44 % red, 56 % green), indicating historical admixture or residual heterogeneity within populations. Similarly, certain Kazakhstan samples, such as ZM-022-1 (63 % red, 37 % green) and ZM-022-3 (57.5 % red, 42.5 % green), showed partial assignment to both clusters, supporting the idea that local varieties have retained alleles from European germplasm.

Overall, this study confirms that SSR markers are highly effective for evaluating genetic diversity and population structure in *Zea mays* L. The most informative markers identified here offer valuable tools for characterizing additional maize accessions and can be directly applied in breeding programs, supporting both the conservation of genetic resources and the development of improved, locally adapted varieties.

### Conclusion

The present study demonstrates substantial genetic diversity among 51 maize (*Zea mays* subsp. *mays*) accessions from Kazakhstan, China, and Europe, as revealed by analysis of 21 SSR markers. Significant genetic differentiation was observed among groups, largely corresponding to their geographic origin: European and Chinese accessions formed distinct clusters, while Kazakhstani landraces displayed unique genetic profiles. Accessions from geographically or breeding-similar backgrounds exhibited higher genetic similarity, whereas more distantly related lines showed greater differentiation, indicating that limited gene flow and breeding history contribute to the observed population structure. Several SSR markers exhibited high polymorphism and informativeness (*phi047*, *umc2189*, *bnlg1520*), confirming their suitability for evaluating genetic diversity and relationships among maize accessions. Importantly, many of the SSR markers used in this study are associated with key genes and agronomic traits, including kernel weight, starch composition, flowering time, and plant architecture, thereby enabling their potential use in marker-assisted selection and in identifying beneficial alleles for breeding programs. Thus, this study provides valuable insights into the genetic composition and structure of maize accessions in Kazakhstan and beyond. The findings provide a foundation for the preservation of local germplasm, the strategic selection of parental lines in hybrid development, and the broader use of genetic resources to enhance maize breeding, adaptability, and resilience across diverse agroecological conditions.

### Funding

This research was funded by the Science Committee of the Ministry of Science and Higher Education (Program No.BR24992903).

### Conflict of Interest

The authors declare no conflict of interest.

### Author contribution

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Ibraimov A.M.** — formal analysis, investigation, writing — original draft, writing — review & editing; **Yermagambetova M.M.** — data curation, investigation, methodology, validation, writing — original draft, writing — review & editing; **Makhmadzhanov S.P.** — formal analysis, investigation, resources; **Ortaev A.K.** — formal analysis, investigation, resources; **Turuspekov Y.K.** — conceptualization, data curation, funding acquisition, methodology, project administration, supervision, validation, writing — original draft, writing — review & editing.

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### Микросателлиттік маркерлерге негізделген әртүрлі географиялық аймақтардан алынған *Zea mays* L. үлгілерінің генетикалық алуантүрлілігін бағалау

Зерттеу жұмысының мақсаты — микросателлиттік (SSR) маркерлерді пайдалана отырып, Қазақстаннан, Қытайдан және Еуропадан алынған жүгерінің 51 сорттары мен гибридерінің (*Zea mays* L.) генетикалық алуантүрлілігі мен құрылымын қарастыру. Барлығы 255 үлгіні генотиптеу үшін 21 жоғары полиморфты SSR маркері қолданылды, бұл аллельдік алуантүрлілік пен генетикалық қатынастарды кешенді бағалауға мүмкіндік берді. Локустағы аллельдердің саны 1,517 орташа мәнімен 1,0-ден 2,7-ге дейін өзгерді, ал күтілетін гетерозиготалық (*uHe*) орташа есеппен 0,192 құрады, бұл үлгілер арасындағы генетикалық алуантүрліліктің орташа деңгейін көрсетеді. Географиялық шығу тегі бойынша генетикалық алуантүрлілікті талдау еуропалық үлгілердің ең үлкен аллельдік байлығымен ( $N_a = 5,9$ ) және генетикалық алуантүрлілігімен, одан кейін қытайлық және қазақстандық топтармен сипатталатынын көрсетті. Осы айырмашылықтарға қарамастан, барлық топтар 100 % полиморфизмді көрсетті, бұл тандалған SSR маркерлерінің жоғары ақпараттылығын растайды. Полиморфты ақпараттық мазмұнының (PIC) мәндері 0,446-дан 0,884-ке дейін, орташа мәні 0,712-ге дейін өзгерді, бұл *phi047*, *bnlg1520* және *umc2189* маркерлерінің жоғары ақпараттылығын көрсетті. Молекулалық дисперсияны (AMOVA) талдау жалпы генетикалық вариацияның 70 % үлгілер арасындағы айырмашылықтарға байланысты екенін көрсетті, бұл жоғары генетикалық дифференциацияны ( $F_{st} = 0,702$ ) және шектеулі ген ағынын ( $N_m = 0,212$ ) түсіндіреді. Кластерлік талдау, негізгі координаттарды талдау (PCoA) және STRUCTURE нәтижелері қытайлық үлгілердің нақты генетикалық бөлінуін дәйекті түрде анықтады, ал еуропалық және қазақстандық үлгілер жақынырақ генетикалық байланыстар мен ішінара араласуды көрсетті. Жалпы алғанда, нәтижелер жүгері үлгілері арасында айтарлықтай генетикалық алуантүрлілікті және айқын популяция құрылымын көрсетеді, бұл генетикалық ресурстарды сақтау және селекциялық бағдарламаларда генетикалық әртүрлі ата-аналық линияларды таңдау үшін құнды дерек көзі болады.

*Кілт сөздер:* *Zea mays*, Қазақстан, молекулалық маркерлер, SSR, генетикалық алуантүрлілік, полиморфизм, генетикалық құрылым, генотиптеу.

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### Оценка генетического разнообразия образцов *Zea mays* L. из разных географических регионов на основе микросателлитных маркеров

Целью данного исследования было изучение генетического разнообразия и структуры 51 сорта и гибрида кукурузы (*Zea mays* L.), происходящих из Казахстана, Китая и Европы, с использованием маркеров простых повторяющихся последовательностей (SSR). Для генотипирования 255 образцов был применен 21 высокополиморфный SSR-маркер, что позволило провести всестороннюю оценку аллельной изменчивости и генетических взаимосвязей. Количество аллелей на локус варьировалось от 1,0 до 2,7, в среднем составляя 1,517, а ожидаемая гетерозиготность (*uHe*) в среднем составляла 0,192, что указывает на умеренное генетическое разнообразие среди образцов. Анализ генетического разнообразия по географическому происхождению показал, что европейские образцы демонстрируют наибольшее аллельное богатство ( $N_a = 5,9$ ) и генетическое разнообразие, за ними следуют китайские и казахстанские группы. Несмотря на эти различия, все группы показали 100 % полиморфизм, подтверждая высокую информативность выбранных SSR-маркеров. Значения PIC варьировались от 0,446 до 0,884 со средним значением 0,712, что указывает на высокую информативность таких маркеров, как *phi047*, *bnlg1520* и *umc2189*. Анализ AMOVA показал, что 70 % общей генетической изменчивости обусловлено различиями между образцами, что указывает на сильную генетическую дифференциацию ( $F_{st} = 0,702$ ) и ограниченный генный поток ( $N_m = 0,212$ ). Кластерный анализ, анализ PCoA

и результаты STRUCTURE выявили четкое разделение китайских образцов, в то время как европейские и казахстанские образцы показали более тесные генетические связи и частичное смешение. В целом, полученные результаты подчеркивают генетическое разнообразие и различную популяционную структуру среди образцов кукурузы, предоставляя ценную информацию для сохранения генофонда и отбора генетически разнообразных родительских линий в селекционных программах.

*Ключевые слова:* *Zea mays*, Казахстан, молекулярные маркеры, SSR, генетическое разнообразие, полиморфизм, генетическая структура, генотипирование.

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