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GENETIC DIVERSITY AND POPULATION STRUCTURE OF *TULIPA BUHSEANA* USING SIMPLE SEQUENCE REPEAT MARKERS

Understanding the genetic diversity and population structure of *Tulipa buhseana* Boiss. is essential for its conservation and management. We investigated 282 individuals from 15 populations of *Tulipa buhseana* collected in two regions (Almaty, Zhambyl) using SSR (Simple Sequence Repeat) markers. The results revealed that eight polymorphic SSR markers identified 31 alleles across the studied populations. The genetic diversity indices, including Nei's diversity index (uh) and Shannon Information Index (I), indicated considerable genetic variation within and among populations, with Population 9 from the Almaty region exhibiting the highest genetic diversity. Analysis revealed high genetic diversity within populations (66%) and significant differentiation among populations (34%) based on Analysis of molecular variance (AMOVA). PCoA identified two distinct groups, consistent with the dendrogram analysis, which grouped populations into two main clusters. Bayesian analysis suggested subtle genetic structuring, supporting findings of low population differentiation by STRUCTURE. These results underscore the complex genetic dynamics of *Tulipa buhseana* and emphasize the need for tailored conservation strategies to preserve its genetic diversity.

Key words: *Tulipa buhseana*, Kazakhstan, genetic diversity, population structure, SSR markers, principal coordinate analysis (PCoA), AMOVA, dendrogram.

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Қарапайым қайталанатын тізбек маркерлерін қолдану негізінде *Tulipa buhseana* популяцияларының генетикалық алуантүрлілігі мен құрылымы

Tulipa buhseana Boiss. популяциясының генетикалық алуантүрлілігі мен құрылымын анықтау оның популяцияларын сақтау және ұтымды пайдалану үшін өте маңызды. Бұл жұмыста SSR маркерлерін (қарапайым қайталанатын тізбектер) қолдану арқылы, екі аймақтан (Алматы және Жамбыл облыстары) жиналған *Tulipa buhseana* он бес популяцияның 282 үлгісі зерттелді. Нәтижесінде сегіз полиморфты SSR маркерлері зерттелген популяцияларда 31 аллельді анықтады. Генетикалық алуантүрлілік индекстері, соның ішінде Нейдің генетикалық алуантүрлілік индексі (uh) және Шеннонның ақпараттық индексі (I) популяциялар ішіндегі және популяциялар арасындағы маңызды генетикалық вариацияны көрсете отырып, оның ішінде Алматы облысынан жиналған 9 популяция ең жоғары генетикалық алуантүрлілікті анықтады. Молекулярлық дисперсия (AMOVA) негізіндегі талдау популяция ішіндегі жоғары генетикалық алуантүрлілікті (66 %) және популяциялар арасындағы айтарлықтай дифференциацияны (34 %) анықтады. PCoA популяцияларды екі негізгі кластерге топтастырған дендрограммалық талдауға сәйкес екі түрлі координаталарға бөлді. Байес талдауы популяцияның төмен құрылымдық дифференциациясы туралы қорытындыларды қолдайтын салыстырмалы генетикалық құрылымды анықтады. Бұл нәтижелер *Tulipa buhseana* күрделі генетикалық динамикасын және оның генетикалық алуантүрлілігін сақтаудың жеке стратегияларын әзірлеу қажеттілігін көрсетеді.

Түйін сөздер: *Tulipa buhseana*, Қазақстан, генетикалық алуантүрлілік, популяция құрылымы, SSR-маркерлер, негізгі координаттарды талдау (PCoA), AMOVA, дендрограмма.

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Генетическое разнообразие и структура популяции *Tulipa buhseana* с использованием маркеров простых повторяющихся последовательности

Понимание генетического разнообразия и структуры популяции *Tulipa buhseana* Boiss. имеет важное значение для его сохранения и рационального использования. Мы исследовали 282 особи из 15 популяций *Tulipa buhseana* в двух регионах (Алматинская и Жамбылская области) с использованием SSR (простые повторяющиеся последовательности) маркеров. Результаты показали, что восемь полиморфных маркеров SSR идентифицировали 31 аллель в изученных популяциях. Индексы генетического разнообразия, включая индекс разнообразия Нея (uh) и индекс информации Шеннона (I), указали на значительную генетическую изменчивость внутри и между популяциями, при этом популяция 9 из Алматинской области продемонстрировала самое высокое генетическое разнообразие. Анализ выявил высокое генетическое разнообразие внутри популяций (66%) и значительную дифференциацию между популяциями (34%) на основе анализа молекулярной дисперсии (AMOVA). PCoA выявил два различных кластера, что согласуется с анализом дендрограммы, который сгруппировал популяции в два основных кластера. Байесовский анализ выявил тонкую генетическую структурированность, подтверждающую выводы о низкой дифференциации популяции по STRUCTURE. Эти результаты подчеркивают сложную генетическую динамику *Tulipa buhseana* и необходимость разработки индивидуальных стратегий сохранения ее генетического разнообразия.

Ключевые слова: *Tulipa buhseana*, Казахстан, генетическое разнообразие, популяционная структура, SSR-маркеры, анализ главных координат (PCoA), AMOVA, дендрограмма.

Introduction

The Liliaceae Juss. family, is a perennial bulbous plant endemic to the regions of Central Asia, particularly Kazakhstan [1, 2]. One of the largest genera of the family is *Tulipa* L., genus comprises approximately 150 species distributed primarily across Europe, North Africa, and Asia, with a notable diversity center in Central Asia [3-7]. The genus is taxonomically complex and is typically divided into four subgenera: *Eriostemones* Raamsd., *Tulipa* L., *Clusianae* (Baker) Zonn., and *Orithyia* (D. Don) Baker [5]. These subgenera are further categorized into 12 sections based on morphological and genetic characteristics [5, 8].

In Kazakhstan, the genus *Tulipa* is represented by 42 species that exhibit significant ecological and morphological diversity [2, 9]. Among these, species *Tulipa buhseana* Boiss. from the subgenus *Eriostemones* [10] is a widely distributed in territory of Kazakhstan [2].

T. buhseana has a bulbs 1-1.5 cm in diameter with brown, leathery, thin-haired scales at the top. Its stem is 15-40 cm tall and typically bare, featuring two widely spaced, narrow linear leaves that do not reach the flower. It is characterized by its woolly filaments and relatively small, often brightly colored flowers. It bears 1 to 6-8 flowers, which wilt after blooming. The tepals are white with a distinct yellow spot at the base; the outer tepals are dirty-purple or pink-brown, slightly longer, and almost twice as long as the inner ones. The filaments are thin and yellow, with a thick, hairy ring at the base and sparser hairs along the thread, while the anthers are short, measuring 4-6 mm. The fruit is an oblong, sometimes nearly spherical capsule, up to 2 cm long and about 1 cm wide. It blooms from late March to April and bears fruit from late May to June [2].

The species is distributed in Kazakhstan's desert zone and the foothills of the Tien Shan, extending from the Aral Sea region to the Balkhash-Alakul depression and the Zhetysu Alatau. Outside Kazakhstan, it is found in neighboring regions of Turkmenistan and Uzbekistan, as well as in Northwest China and Iran [2, 10]. Renowned for its striking floral morphology and ecological significance, *T. buhseana* contributes not only to the biodiversity of the region but also holds potential ornamental and horticultural value. Despite its importance, limited genetic information is available on this species, which poses challenges for its conservation and breeding programs.

Molecular markers have become indispensable tools in plant genetics and breeding, offering insights into genetic diversity, population structure, and phylogenetic relationships [11]. Simple Se-

quence Repeats (SSRs), also known as microsatellites, are particularly valued for their high polymorphism, co-dominant inheritance, and reproducibility among the various types of molecular markers. SSR markers have been successfully used in many wild and cultivated plant species to analyses genetic diversity, phylogenetic relationships and molecular breeding [12].

The use of molecular markers in the study of tulips has advanced our understanding of genetic relationships, evolutionary history, and species differentiation within the genus [13-15]. Research utilizing SSR markers has been instrumental in uncovering genetic diversity in wild and cultivated tulip species, assisting in developing of conservation strategies and breeding programs. Studies on species such as *T. gesneriana* L. and *T. sylvestris* M.Bieb. have highlighted the genetic richness and complexity within the genus, aiding in the identification of distinct genetic lineages and hybridization events [5, 16].

In Kazakhstan, however, the application of molecular markers in tulip research remains limited. While some studies [17, 18] have begun to explore the genetic diversity of certain tulip species in the region, comprehensive genetic analyses particularly using SSR markers, are still scarce. This gap in research poses a significant challenge to the conservation and sustainable use of Kazakhstan's tulip species, including *T. buhseana*.

This study aims to analyze the genetic diversity and population structure of *T. buhseana* populations in Kazakhstan using SSR markers. By generating a comprehensive genetic profile, this research seeks to enhance our understanding of the genetic resources of *T. buhseana*, informing both conservation efforts and potential breeding initiatives. The findings will contribute to the broader knowledge of tulip genetics and support efforts to preserve this valuable genetic resource.

Materials and methods

Sample collection

A total of 282 individual plants of *Tulipa buhseana* were collected from 15 populations across Almaty and Zhambyl regions in Kazakhstan (Figure 1; Table 1).

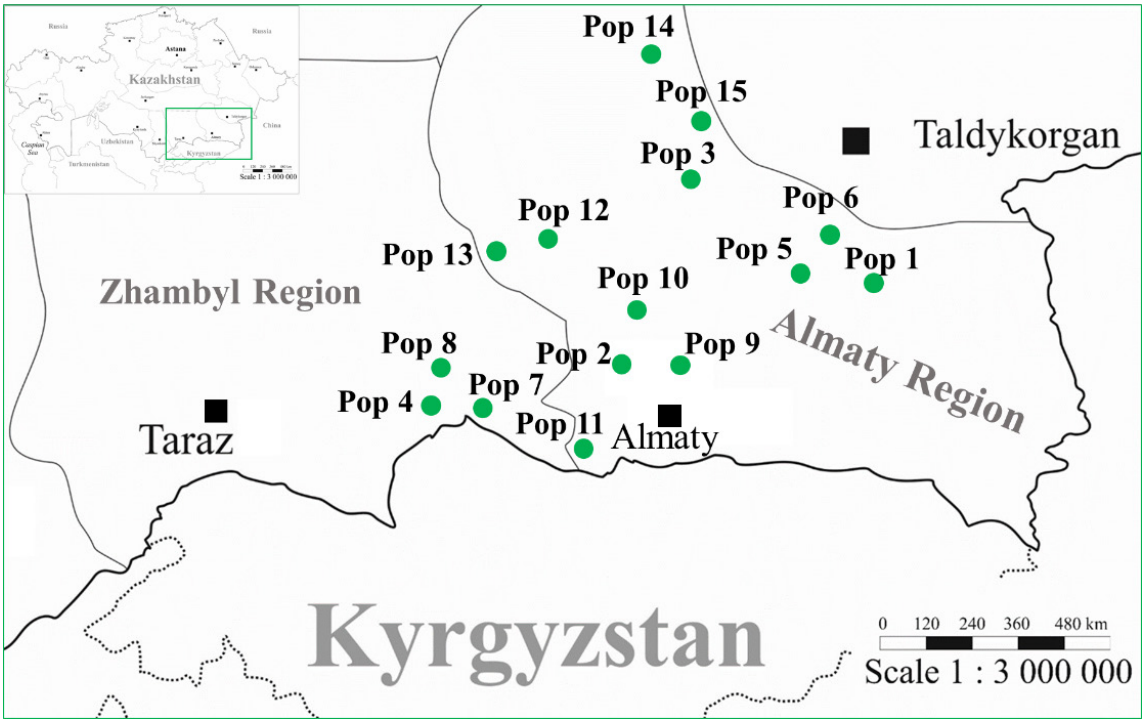


Figure 1 – Locations of the collected *Tulipa buhseana* populations in Almaty and Zhambyl regions

The sample size for each population ranged from 12 to 21 individuals (Table 1), ensuring adequate representation of genetic diversity within and among populations. Fresh leaves were collected from each plant, stored in silica gel, and transported to the laboratory for further analysis.

Table 1 – Collected information on *Tulipa buhseana* populations in Kazakhstan

Population number	Collected samples	Location
Population 1	21	Almaty region, left bank of Kapchagay sea
Population 2	21	Almaty region, right bank of Kurty River (Kurty River is the left tributary of the Ili River)
Population 3	18	Almaty region, right bank of the Ili River
Population 4	12	Zhambyl region, Kordai district, near the Tarylgan river (Tarylgan – river of the Shu River basin)
Population 5	21	Almaty region, northeast of Konaev city
Population 6	19	Almaty region, 5-6 km along the Ili River
Population 7	20	Zhambyl region, Kordai district, at the turn to the village of Sogandy
Population 8	20	Zhambyl region, Kordai district, right bank of the Tarylgan River
Population 9	20	Almaty region, near the Kurtinsky reservoir
Population 10	21	Almaty region, left bank of Kurty River
Population 11	20	Zhambyl region, near the village of Degeres
Population 12	21	Almaty region, Chu-Ili mountains, Tambaly tas
Population 13	21	Zhambyl region, near the village of Matybulak, Kyzylkainar
Population 14	15	Almaty region, Balkhash district
Population 15	12	Almaty region, Balkhash district, Malaysary

DNA Extraction

Genomic DNA was extracted from the dried leaf samples using the CTAB (cetyltrimethylammonium bromide) method with minor modifications to optimize yield and purity [19]. Approximately 25 mg of leaf tissue was ground to a fine powder using liquid nitrogen. The powder was then mixed with 700 µL of CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 1% polyvinylpyrrolidone) and incubated at 65°C for 30 minutes. Following incubation, an equal volume of chloroform alcohol (24:1) was added, and the mixture was centrifuged at 12,000 rpm for 10 minutes. The supernatant was transferred to a new tube, and DNA was precipitated with isopropanol. The DNA pellet was washed with 70% ethanol, air-dried, and resuspended in 100 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

PCR Amplification and Visualization

Fifteen SSR markers specific to *Tulipa* species were selected [20] for this study based on their high polymorphism and reproducibility (Table 2). The PCR reactions were carried out in a total volume of 20 µL, containing 10 ng of genomic DNA, 10 µL of 2X PCR Master Mix (Thermo Scientific, USA), 0.5 µM of each primer, and nuclease-free water.

Table 2 – Characteristics of simple sequence repeat (SSR) primers [20] used in genetic diversity analysis of *Tulipa buhseana* populations

№	Primer	Sequence (5'– 3')	Expected size (bp)	Motif	Annealing T (°C)
1	Ca-2572	F:TGCACAGAGCCAAAGAAGTA R:TCTCCTTTCCATGTTTCCTC	213	(GAGAAG)4	54
2	Ca-3952	F:ACTCAATTCACTTGCAGCAG R:GTCGTTGCAGTTGTTGTGAT	189	(CAG)4	54
3	Ca-5526	F:TTTACGGGAATTACTTCGAG R:ACATGGATTCCAAACAAGAG	242	(GAG)6	54
4	Ca-5553	F:CCGATAATTGAGGTCAGGTT R:CCGAACTCCTCGCATATAAC	168	(TTG)9	54
5	Ca-6950	F:ATGCAATCTTGGGAACTGAT R:CACTGTCGTCATCTTCTCCA	198	(GAT)4	54
6	Ca-7862	F:AATCAACGCATCATGTCAAC R:TACTGGAGGTACGCCTCCTT	131	(CGC)4	54
7	Ca-8508	F:AGAATTTGTCTTGCGACAGT R:TAGGGGTACCAATTTGTGTT	325	(GTT)10	54
8	Ca-13333	F:ATGGTTGGAAGAGGAGACTG R:AGTCATTCGATCCTCGAGTC	242	(GAT)4	56
9	Ca-15730	F:CATCAAAACCGACAACACC R:CGGTCAACATCATTCAAGAG	213	(CGC)8	56
10	Kn-834	F:TCAGAAGGCTCTTCTTTCAG R:CTTTACATGGAGATAATGTTAACAA	221	(AT)8	54
11	Kn-1412	F:GTCCTTTGTACGGTGATGTT R:TAGCTTCCGGAGTTCAATAG	242	(GGA)10	54
12	Kn-2291	F:GAAGACGAAGATGATTCGAG R:TGGGTTTCACTTAAACAGCT	275	(GAGAAG)4	54
13	Kn-7108	F:TTGCTGCTTCGACTACTTTG R:GGTCATGCAACATAAACTGC	231	(TTTC)4	57
14	Kn-7480	F:GCAACTTAGGTCAACAGAGG R:CTCCTACCAACAAAGCATTC	268	(GAC)9	57
15	Kn-30956	F:TGAAGCTCCTCCACTCTACC R:ACAAGGGCACTCATTCTGTT	237	(CTC)6	57

The PCR cycling conditions were as follows: an initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 95°C for 30 seconds, 54-57C (depending on the primer) for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The QIAxcel Connect System for capillary electrophoresis (QIAGEN, Germany) was employed, utilizing the QIAxcel DNA High-Resolution Kit and the QX Alignment Marker (15 bp/3 kb) to separate PCR products. The SSR alleles were scored based on their size relative to the 100 bp DNA ladder (Thermo Scientific, USA).

Data Analysis

Genetic diversity parameters, including the number of alleles per locus (na), number of effective alleles (ne), Nei's genetic diversity index (uh) and the Shannon Information Index (I), and the polymorphic information content (PIC), genetic differentiation (Fst), and gene flow (Nm) were calculated using GenAlEx 6.5 software [21]. Additionally, variation among populations was studied using Principal Coordinate Analysis (PCoA), and Analysis of Molecular Variance (AMOVA) was conducted using GenAlEx 6.5 software [21] to partition ge-

netic variance within and among populations. The unrooted dendrogram for fifteen *T. buhseana* populations based on the neighbor-joining (NJ) method was constructed using PAST software [22]. Population structure was assessed using STRUCTURE 2.3.4 software [23], which employs a Bayesian clustering method to infer the number of genetic clusters (K) within the sample set. The optimal K value was determined by running the program with K values ranging from 1 to 15 and using the ΔK method described by Evanno et al. [24].

Results and discussion

Fifteen SSR primer pairs were utilized to assess genetic diversity within and among fifteen populations of *T. buhseana* collected in Almaty and Zhambyl regions. Based on the analysis of sample profiles across all fifteen populations using 15 SSR markers, eight SSR markers (Ca-2572, Ca-3952, Ca-5526, Ca-5553, Ca-6950, Ca-7862, Kn-2291, and Kn-7108) were found to be polymorphic (Table 3), while rest seven SSR markers were monomorphic.

Thirty-one alleles were identified among the 8 polymorphic microsatellite loci. The number of alleles (Na) per locus ranged from 2 (Ca-3952, Ca-6950) to 8 (Ca-3952), with an average of 3.9. The effective number of alleles (Ne) varied from 1.4 to 3.4, averaging 2.0 (Table 3). The most polymorphic loci were Ca-3952, Ca-5553, and Kn-2291, while Ca-6950 was the least polymorphic marker. Populations 8 and 15 exhibited the largest number of unique alleles with low frequencies at locus Ca-3952. The overall Nei's genetic diversity index (uh) was 0.460. The Shannon information index ranged from 0.387 to 1.315, with an average of 0.728 (Table 3). The mean polymorphism information content (PIC) index for the SSR markers was 0.592, ranging from 0.449 (Ca-6950) to 0.822 (Ca-3952). The range of PIC values, from 0.449 to 0.822, illustrates the variability in informativeness among the SSR markers. An SSR marker is considered informative when its PIC value is 0.5 or higher [25]. The marker Ca-3952, with a PIC value of 0.822, is particularly noteworthy as it indicates a high degree of allele diversity and utility in distinguishing genetic differences within the population. Conversely, Ca-6950, with a PIC value of 0.449, is less polymorphic but still useful for certain applications. The average fixation index (Fst) was 0.287. Gene flow (Nm) ranged from 0.333 to 1.090, with an average of 0.690 (Table 3). In our study, the Nei's and Shannon's genetic diversity indices are higher than the results obtained for *Tulipa* accessions using the CDDP method as reported by Haerinasab et al. [26], which showed these indices of 0.23 and 0.38, respectively. This demonstrates that SSR markers are highly informative compared to other types of molecular markers.

Table 3 – Assessment of the genetic diversity of simple sequence repeat markers in populations of *Tulipa buhseana*

№	Primers	Na	Ne	I	uh	PIC	F_{st}	N_m
1	Ca-2572	4	2.0	0.772	0.515	0.518	0.221	0.881
2	Ca-3952	8	3.4	1.315	0.708	0.822	0.187	1.090
3	Ca-5526	2	1.6	0.533	0.376	0.450	0.209	0.945
4	Ca-5553	4	1.9	0.688	0.419	0.650	0.390	0.391
5	Ca-6950	2	1.4	0.387	0.272	0.449	0.428	0.333
6	Ca-7862	4	1.8	0.687	0.432	0.580	0.297	0.592
7	Kn-2291	4	1.9	0.703	0.471	0.645	0.310	0.557
8	Kn-7108	3	1.9	0.742	0.487	0.619	0.256	0.726
Mean		3.9	2.0	0.728	0.460	0.592	0.287	0.690
SE		0.104	0.071	0.033	0.018	0.123	0.031	0.095

Notes: Na – number of alleles per locus; Ne – effective number of alleles; I – Shannon's Information Index; uh – Nei's genetic diversity index; PIC – polymorphism information content; F_{st} – fixation index; N_m – gene flow value; SE – Standard error

The analysis of 15 populations of *T. buhseana* revealed a range in the number of alleles (Na) from 2.1 in Populations 7 and 15 to 3.5 in Population 9, with an overall average of 2.7. The number of effective alleles ranged from 1.7 to 2.4, with an average of 2.0. These results indicate a higher genetic diversity within the populations of *T. buhseana* studied in our research compared to previous findings by Pourkhaloee et al. [20] reported an average number of alleles of 1.8 and an average number of effective alleles of 1.3 in their study of *Tulipa* accessions. The Shannon diversity index (I) ranged from 0.519 (in Population 15) to 0.994 (in Population 9), with a mean of 0.728. The percentage of polymorphic loci (PPL) spanned from 75% to 100%, with an average of 95%. The genetic diversity (Nei) across *T. buhseana* populations averaged 0.460, ranging from 0.348 (in Population 15) to 0.600 (in Population 9). The evaluation of all indices indicates that the highest values were observed for population 9 from the Almaty region (Table 4). Population 9's elevated genetic diversity suggests that the Almaty region may serve as a genetic hotspot for *T. buhseana*. This region's unique environmental conditions and ecological factors might contribute to the high genetic variation observed.

Table 4 – Genetic diversity indices of studied *Tulipa buhseana* populations

Populations	Na	Ne	I	uh	PPL, %
Population 1	3.0	2.1	0.782	0.476	100
Population 2	3.0	2.2	0.812	0.506	100
Population 3	2.6	2.0	0.699	0.431	87.5
Population 4	2.6	2.0	0.741	0.498	100
Population 5	2.6	1.9	0.696	0.454	100
Population 6	2.6	1.9	0.706	0.449	100
Population 7	2.1	1.7	0.565	0.388	87.5
Population 8	3.1	1.8	0.705	0.411	100
Population 9	3.5	2.4	0.994	0.600	100
Population 10	2.9	2.2	0.846	0.540	100
Population 11	2.5	1.9	0.664	0.419	87.5
Population 12	3.0	2.1	0.761	0.461	100
Population 13	2.8	2.1	0.746	0.465	87.5
Population 14	2.5	1.9	0.687	0.451	100
Population 15	2.1	1.7	0.519	0.348	75
Mean	2.7	2.0	0.728	0.460	95
SE	0.10	0.07	0.033	0.018	2.04

Notes: Na – number of alleles per locus; Ne – effective number of alleles; I – Shannon's Information Index; uh – Nei's genetic diversity index; PPL – the percentage of polymorphic loci; SE – Standard error

The AMOVA results revealed that most of the genetic diversity (66%) in *T. buhseana* was found within individual populations, while a significant portion (34%) was attributed to differences among populations (Table 5). This indicates substantial genetic variability both within and between populations. The gene flow (Nm) value of 0.754 suggests a moderate level of genetic exchange among the populations (Table 5). This moderate gene flow may be sufficient to prevent complete genetic isolation but is not high enough to homogenize the populations entirely [27, 28]. Consequently, while some degree of genetic exchange occurs, it is not enough to override the differentiation caused by factors such as geographic barriers, ecological variations, or limited seed and

pollen dispersal [29]. These findings underscore the importance of considering both within-population and among-population genetic diversity in conservation and management strategies for *T. buhseana*.

The PCoA plot separated the *T. buhseana* populations using two distinct principal coordinates: Coordinate 1 and Coordinate 2, which accounted for 66.5% and 17.2% of the total variation among populations, respectively (Figure 2).

Table 5 – AMOVA analysis of the 15 studied populations of *Tulipa buhseana*, using eight simple sequence repeat markers

Source	df	SS	MS	Est. Var.	%	Fst	Nm
Among Populations	14	821.944	58.710	2.839	34		
Within Populations	267	1452.676	5.441	5.441	66		
Total	281	2274.621		8.280	100	0.249	0.754
Notes: df – degrees of freedom; SS – sum of squares; MS – mean squared; Est.var. – estimates of variance; % – percentage of variation; FST – fixation index; Nm – gene flow (Nm) value; * $p < 0.001$; Nm = (1 – FST)/4FST.							

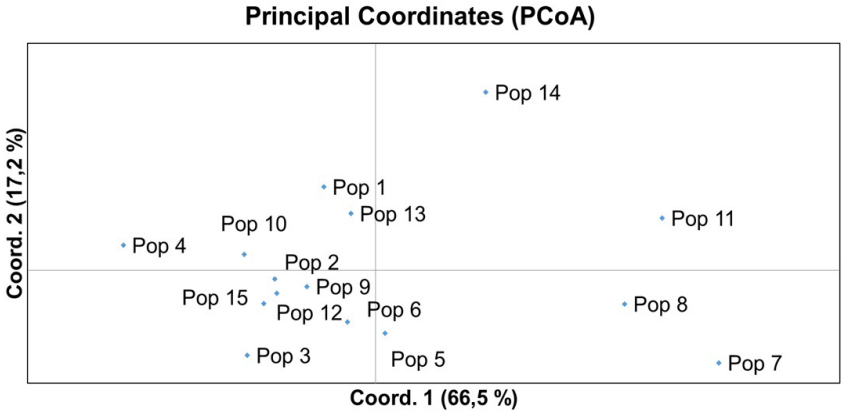


Figure 2 – Principal coordinates analysis (PCoA) of 15 *Tulipa buhseana* populations from Kazakhstan, based on pairwise population PhiPT values

Based on the PCoA plot, it is evident that the *T. buhseana* populations exhibit significant genetic differentiation. Coordinate 1, which explains 66.5% of the total variation, has successfully separated populations 7, 8, 11, and 14 from the majority of other populations (Figure 2). This substantial separation along Coordinate 1 suggests that these four populations possess unique genetic characteristics that distinguish them from the rest. Coordinate 2, accounting for 17.2% of the variation, further refines the genetic distinctions among the populations but with less impact than Coordinate 1. Together, these two principal coordinates elucidate 83.7% of the total genetic variation, highlighting the robust nature of the genetic diversity within *T. buhseana*. This differentiation could be attributed to various ecological, geographical, or evolutionary factors that warrant further investigation to understand the underlying causes of this genetic structuring.

The SSR analysis for fifteen *T. buhseana* populations, as evaluated using an unrooted dendrogram based on the neighbor-joining (NJ) method, provides further insights into the genetic relationships among these populations (Figure 3). The dendrogram distinctly separated the populations into two groups: Group I and Group II (Figure 3). Group I formed the first clade and included populations 7, 8, 11, and 14, while Group II consisted of the remaining populations clustered into a second clade. Although populations 4, 7, and 8 of *T. buhseana* are distributed almost on one lace geographically, genetic analysis reveals a significant difference between population 4 and the closely related populations 7 and 8. Interestingly, population

14, which is distributed far from populations 7 and 8, shows genetic similarity to them. This genetic divergence in population 4 and the unexpected genetic similarity between the distant population 14 and populations 7 and 8 could be attributed to varying environmental factors such as climate conditions or soil composition. Different microclimates might result in distinct selective pressures, leading to genetic differentiation. Additionally, variations in soil properties, such as pH, nutrient availability, and moisture content, could influence the genetic makeup of these populations by affecting their growth and reproductive success [30, 31]. Gene flow and historical seed dispersal patterns might also play a role in these genetic similarities and differences [12, 32]. These clustering results align with the findings from the PCoA plot, reinforcing the genetic distinctiveness of populations 7, 8, 11, and 14 from the others (Figure 2).

This consistency between the dendrogram and the PCoA plot supports the robustness of the genetic differentiation observed. The clear separation into two groups indicates that there are significant genetic differences within *T. buhseana* populations, potentially driven by geographical, ecological, or evolutionary factors. Understanding these genetic relationships is crucial for conservation efforts, as it highlights the need to preserve the genetic integrity of both distinct groups to maintain the species' overall genetic diversity.

The Bayesian analysis of 282 individuals of *T. buhseana* revealed that the log-likelihood value (LnP(K)) continued to decrease with the decrease in K-value, without showing a significant inflection point (Figure 4A, B). This suggests that the data did not distinctly support a specific n umber of genetic clusters as K decreased, implying potential genetic admixture or interconnectedness among individuals [33]. The delta K plot indicated a peak at K = 2, suggesting that the optimal number of genetic groups within *T. buhseana* is two. The genetic structure analysis depicted in Figure 1C illustrates the clustering of 282 *T. buhseana* individuals. However, the results of STRUCTURE analysis indicated a low level of population structure among the 15 studied populations (Figure 4C), implying that the genetic variation observed predominantly reflects a single cluster encompassing all analyzed accessions.

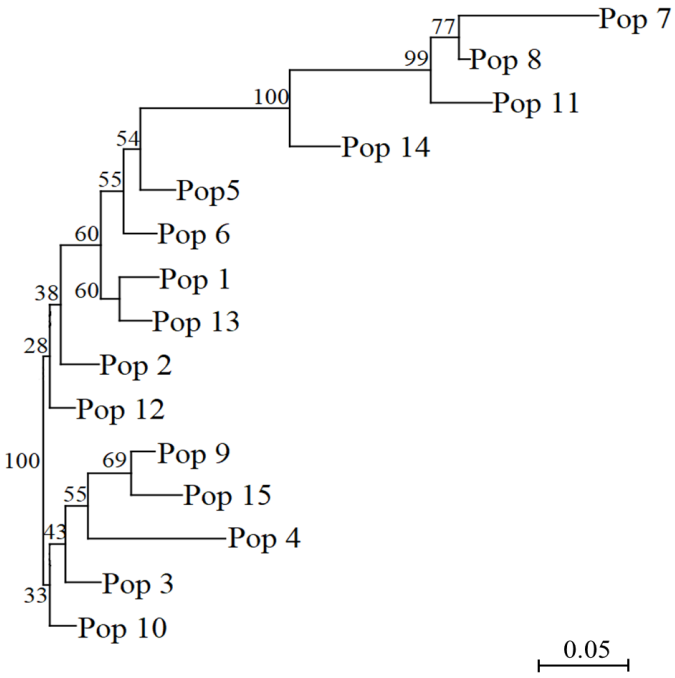
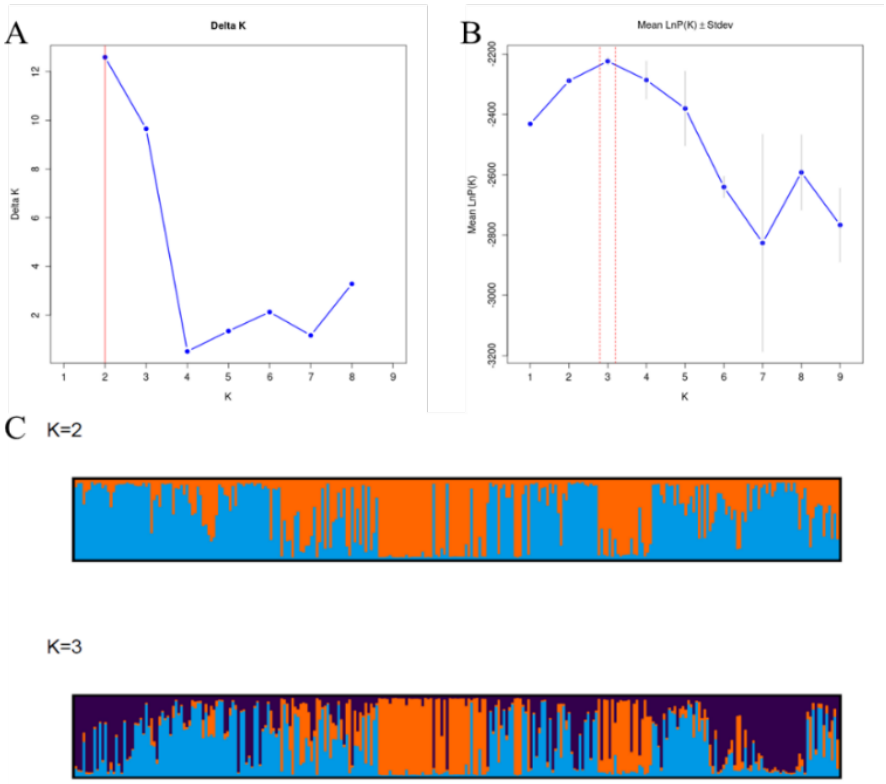


Figure 3 – The neighbor-joining dendrogram of 15 *Tulipa buhseana* populations was constructed based on Nei's distance, calculated from 15 simple sequence repeat markers



A – Delta K, B – LnPK, C – population structure among the 15 studied populations

Figure 4 – Genetic structure of 282 individuals from 15 *Tulipa buhseana* populations

The analysis of the STRUCTURE output indicates that the clustering of the 15 studied populations collected from different regions is not well-defined. This finding is consistent with the AMOVA results, which also showed how genetic variation is divided within and between populations (Table 5).

Conclusion

This study employed fifteen SSR primer pairs to evaluate the genetic diversity within and among fifteen populations of *T. buhseana* from the Almaty and Zhambyl regions. The analysis revealed that eight SSR markers were polymorphic, identifying of thirty-one alleles across these loci. Notably, the SSR marker Ca-3952 exhibited the highest polymorphism, highlighting its significant allele diversity. Genetic diversity indices, such as Nei's genetic diversity index (uh) and Shannon's information index, averaged 0.460 and 0.728, respectively. The mean polymorphism information content (PIC) was 0.592, indicating the SSR markers' high informativeness. Population 9 from the Almaty region exhibited the highest genetic diversity, suggesting that this region may be a genetic hotspot for *T. buhseana*. The AMOVA results indicated substantial genetic variability within populations (66%) and among populations (34%), with a moderate gene flow (Nm) of 0.754. Bayesian and STRUCTURE analyses supported the existence of two main genetic clusters within *T. buhseana*, although the overall population structure was low. These findings underscore the importance of preserving genetic diversity within *T. buhseana* populations, particularly in the Almaty region, to ensure the species' long-term survival and adaptability.

Conflict of interest

All authors declare that they have no conflicts of interest.

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