DOI: 10.11134/btp.2.2026.10

UDC 631.523.5 Original Article

ASSESSMENT OF GENETIC DIVERSITY AND POPULATION STRUCTURE OF *IRIS KOLPAKOWSKIANA* REGEL FROM KAZAKHSTAN USING MICROSATELLITE MARKERS

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ABSTRACT

Iris kolpakowskiana Regel is a rare wild iris species distributed in southeastern and southern Kazakhstan. To assess its genetic diversity and population structure, 168 individuals from 9 natural populations in the Almaty, Zhambyl, and Turkestan regions were analyzed using expressed sequence tag – simple sequence repeat (EST-SSR) markers. Of the nine primer pairs tested, 6 polymorphic loci were selected for further analysis. The mean number of alleles per locus was 3.1, with an average effective number of alleles of 2.4. Polymorphic information content (PIC = 0.688) indicated moderately high genetic variation and high marker informativeness. The highest genetic diversity was observed in populations from the Zhambyl region. AMOVA revealed that 55% of genetic variation occurred within populations and 45% among populations. High genetic differentiation was detected (F_{ST} = 0.481, $p < 0.001$), accompanied by limited gene flow (N_m = 0.654). Principal Coordinate Analysis, Neighbor-Joining clustering, and STRUCTURE analysis consistently identified two major genetic groups corresponding to southeastern Kazakhstan (Almaty region) and southern Kazakhstan (Zhambyl and Turkestan regions). The Mantel test showed no significant correlation between genetic and geographic distances (R^2 = 0.0008, $p > 0.05$). These results revealed geographic structuring and restricted genetic exchange among populations, providing the first genetic assessment of *Iris kolpakowskiana* and valuable information for its further conservation and management.

Key words: *Iris kolpakowskiana,* Kazakhstan, EST-SSRs, genetic diversity, AMOVA, genetic structure.

INTRODUCTION

The genus *Iris* Tourn. ex L. belongs to the family Iridaceae Juss. and comprises 312 accepted species [1], distributed mainly across temperate regions of the Northern Hemisphere [2]. According to Mathew [2], the genus is divided into six subgenera: *Iris*, *Limniris* (Tausch) Spach, *Nepalensis* (Dykes) Lawr., *Xiphium* (Mill.) Spach, *Scorpiris* Spach, and *Hermodactyloides* Spach. The subgenus *Limniris* is further subdivided into 12 sections and 16 series, reflecting the high morphological and taxonomic diversity of the genus.

Species of *Iris* are known not only for their ornamental value, but also for their considerable medicinal importance. Phytochemical studies have shown that *representatives of Iris* contain diverse groups of biologically active compounds, including flavonoids, isoflavonoids, phenolic acids, quinones, terpenes, steroids, alkaloids, and other secondary metabolites [3-5]. In addition, the pleasant violet-like aroma of rhizomes has also made *Iris* an important raw material in perfumery and cosmetics [6]. Moreover, some *Iris* species are used in the food industry as flavoring agents in confectionery, baked products, alcoholic beverages, chewing gum, soft drinks, and traditional spice mixtures [5, 6].

Kazakhstan hosts approximately 30 species of *Iris* [7], nine of which are listed in the Red Book of Kazakhstan [8]. One of these is *I. kolpakowskiana* Regel, a bulbous perennial geophyte belonging to subgenus *Hermodactyloides*, section *Monolepsis* [9]. The species is native to Kazakhstan, Kyrgyzstan, and Uzbekistan [1]. *I. kolpakowskiana* is considered a valuable ornamental species. However, its populations are declining, and its range is decreasing due to habitat degradation and other anthropogenic pressures [8]). Therefore, molecular genetic studies of its wild populations are important for biodiversity conservation and the sustainable utilization of native genetic resources.

Simple sequence repeat (SSR) markers are highly informative molecular tools for assessing genetic diversity, population structure, and differentiation in wild plant populations, especially in rare and endangered species. Numerous studies have demonstrated the effectiveness of SSR markers for evaluating genetic variation within and among plant populations [10], and such data are widely used to develop conservation strategies, identify genetically valuable populations, and support the sustainable management of plant genetic resources [11-13].

In Kazakhstan, *I. kolpakowskiana* has so far been studied only in the context of *ex situ* cultivation [14, 15]. Other *Iris* species from Kazakhstan have been investigated using plastome sequences [7, 16]. However, SSR-based studies evaluating genetic diversity in natural populations of *Iris* species in Kazakhstan have not yet been conducted. Therefore, the application of SSR markers to wild populations of *I. kolpakowskiana* is particularly important, as it can provide essential information for the conservation of this rare species and help protect native *Iris* genetic resources in Kazakhstan. The aim of this study was to assess the genetic diversity, differentiation, and population structure of wild populations of *I. kolpakowskiana* collected in Kazakhstan using EST-SSR markers.

MATERIALS AND METHODS

Plant Material and Sample Collection

A total of 168 individuals of *I. kolpakowskiana* were collected from 9 natural populations distributed across Almaty (ALM), Zhambyl (ZH), and Turkestan (TUR) regions of Kazakhstan (Figure 1, Table 1). The number of sampled individuals per population varied from 10 (TUR1) to 21 (TUR2), ensuring representative coverage of the genetic diversity present within each population.

Young healthy leaves were collected from randomly selected flowering or vegetative individuals located at least 5-10

DOI: 10.11134/btp.2.2026.10

Figure 1. Locations of the collected *Iris kolpakowskiana* populations in Kazakhstan. Sampling sites are grouped by region: ALM (Almaty region, blue), ZH (Zhambyl region, purple), and TUR (Turkistan region, orange).

Table 1. Geographic information of the sampled *Iris kolpakowskiana* populations in Kazakhstan.

Population	Samples	Longitude (N)	Latitude (E)	Elevation (m a.s.l.)	Collected sites
ALM 1	20	43.292532	76.299698	773	Almaty Region
ALM 2	20	43.272982	75.781057	1086	Almaty Region
ALM 3	20	43.270445	75.777617	1043	Almaty Region
ZH 1	20	43.374328	75.045482	891	Zhambyl Region
ZH 2	19	44.266142	73.833595	706	Zhambyl Region
ZH 3	18	44.275303	73.889273	761	Zhambyl Region
ZH 4	20	43.466547	69.81272	729	Zhambyl Region
TUR 1	10	42.432782	70.37882	1259	Turkestan Region
TUR 2	21	43.594442	68.984452	793	Turkestan Region

m apart. Leaf samples were immediately dried in silica gel and transported to the laboratory for molecular analyses.

DNA Extraction

Total genomic DNA was isolated from silica-dried leaf tissues using the cetyltrimethylammonium bromide (CTAB) extraction protocol [17]. Approximately 20-30 mg of dried leaf material was ground into a fine powder using liquid nitrogen. The homogenized tissue was incubated in 700 µL of preheated CTAB extraction buffer containing 2% CTAB, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl, and 1% polyvinylpyrrolidone (PVP) at 65°C for 30 min. Following incubation, an equal volume of chloroform:isoamyl alcohol (24:1) was added, and samples were centrifuged at 12,000 rpm for 10 min. The aqueous phase was transferred to a new tube, and DNA was precipitated using cold isopropanol. The resulting DNA pellet was washed with 70% ethanol, air-dried, and dissolved in 100 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNA quality and concentration were assessed spectrophotometrically and by electrophoresis on 1% agarose gels.

SSR Amplification and Fragment Analysis

Nine microsatellite (EST-SSR) markers previously developed for *Iris* species [18-20] were selected for genotyping *I. kolpakowskiana* populations based on their reported polymorphism and amplification reliability (Table 2). Polymerase chain reactions (PCR) were performed in a final volume of 20 µL containing approximately 10 ng of genomic DNA, 10 µL of 2× PCR Master Mix (Thermo Scientific, USA), 0.5 µM of each forward and reverse primer, and nuclease-free water. PCR amplifications were carried out under the following

DOI: 10.11134/btp.2.2026.10

Table 2. Characteristics of EST-SSR markers [18-20] used for analysis of *Iris kolpakowskiana*

№	Primer	Sequence (5'– 3')	Expected size (bp)	Motif	Annealing T (°C)
1	IM123	F: GTTTCATGGAGGAGTTGCAGT R: TATCTTGCGGGTCTTCTTGG	156	(TCT)10	55
2	IM200	F: AAGCGAAATGGCGAATAAACT R: AGATCAGAAGTCCGAGCACCT	397	(GAA)23	55
3	IM327	F: AAAGAGGAAGTGAGTTCTGGAGAG R: GCTCCATTCTCAGGAGAGGTT	218	(GA)12	55
4	IM348	F: TCGAGTAGTCCCTCCCTCGT R: GGAGAAGACGATGCTGAACC	151	(AGG)7	55
5	I296	F: GGTCTGAAACCATCGAAAC R: AGTAAGGCGATTCCGAGAT	296	(AC) 24	51
6	IEST8	F: ATCAAGCTTTGCAATGGCCG R: CGGATCATGGTGTCGTTCTT	207	(AGC) 11	52
7	IEST5	F: ACTTTCCCTTTCCCACTTCA R: ATTCTTCCGCCAGGCTTTC	130	(GA) 16	51
8	IEST12	F: AGCTTATCACTCCATTCCC R: TCATCGGGAGGACAGAGAA	166	(CT)13	51
9	I23	F: CCAATGCAACAGGTTGACTC R: AGAATAGCACTGTGCAAGCC	166	(CT) 13 (CA) 19	60

conditions: an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer-specific annealing at 51°C–60°C for 30 s, and extension at 72°C for 1 min. A final extension step was performed at 72°C for 8 min. The amplified products were separated and analyzed using a QIAxcel Connect capillary electrophoresis system (QIAGEN, Germany) equipped with the QIAxcel DNA High Resolution Kit and QX Alignment Marker (15 bp-3 kb). Fragment sizes were determined automatically using QIAxcel ScreenGel software based on the internal alignment marker and size standards. Alleles were scored by fragment length and subsequently used in genetic diversity and population structure analyses.

Genetic Diversity and Population Structure Analyses

Genetic diversity parameters were calculated for each population and locus using GenAlEx version 6.5 [21]. The parameters included the observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), Nei's genetic diversity (Nei), and polymorphic information content (PIC). Population differentiation was evaluated using Wright's fixation index (F_{ST}), while gene flow (N_m) was estimated from genetic differentiation values [21]. The informativeness of SSR markers was assessed using PIC values and categorized as high (PIC > 0.50), moderate (0.25 < PIC ≤ 0.50), or low (PIC < 0.25) based on the classification system of Botstein et al. [22]. Relationships among populations were evaluated using Principal Coordinate Analysis (PCoA) based on genetic distance matrices. Analysis of Molecular Variance (AMOVA) was performed in GenAlEx 6.5 [21] to partition the genetic variation within and among populations. A Mantel test [23] was conducted in GenAlEx 6.5 [21] to assess the correlation between genetic and geographic distances among populations. A Neighbor-Joining (NJ) dendrogram was constructed using PAST software [24] to visualize genetic relationships among populations. The robustness of the clusters was assessed using bootstrap analysis with 1000 replicates. Population genetic structure was further assessed using STRUCTURE version 2.3.4 [25] based on a Bayesian clustering approach. Population structure was inferred by evaluating K values from 1 to 10 using multiple independent STRUCTURE runs. The optimal number of genetic clusters was determined using the ΔK statistic proposed by Evanno et al. [26], with results visualized and summarized with the CLUMPAK platform [27].

RESULTS AND DISCUSSION

Marker Informativeness

Nine EST-SSR primer pairs were employed to assess the genetic diversity of *I. kolpakowskiana* populations collected from different regions of Kazakhstan. All primer pairs produced clear and reproducible amplification products. Of the nine markers analyzed, six SSR markers (IM123, IM200, IM327, IM348, I296, and IEST8) were polymorphic, while the remaining three markers (IEST5, IEST12, and I23) were monomorphic across the studied populations of *I. kolpakowskiana*. Since monomorphic SSRs do not contribute to estimates of genetic variation, only the six polymorphic markers were retained for further statistical analyses (Table 3). The six polymorphic EST-SSR markers generated a total of 18.4 alleles, with an average of 3.1 alleles per locus (Table 3). The number of alleles (Na) varied from 2.1 for loci IM348 and I296 to 4.2 for IM200 and IM327. The effective number of alleles (Ne) ranged from 1.6 (IM348) to 3.3 (IM200), with

DOI: 10.11134/btp.2.2026.10

Table 3. Assessment of the genetic diversity parameters of six EST-SSR markers in *I. kolpakowskiana* populations study

EST-SSR Markers	Na	Ne	PIC
IM123	2.7	2.3	0.777
IM200	4.2	3.3	0.796
IM327	4.2	2.8	0.804
IM348	2.1	1.6	0.523
I296	2.1	1.7	0.521
IEST8	3.1	2.5	0.707
Mean	3.1	2.4	0.688
SE	0.2	0.1	0.13

Notes: Na – number of alleles per locus; Ne – effective number of alleles; PIC – polymorphic information content; SE – Standard error

a mean value of 2.4, indicating a moderate level of allelic diversity across the analyzed loci.

The polymorphic information content (PIC) values ranged from 0.521 to 0.804, with an average of 0.688. According to the classification of Botstein et al. [22], four loci (IM123, IM200, IM327, and IEST8) were highly informative (PIC > 0.50), whereas IM348 and I296 were moderately informative. The highest PIC value was recorded for IM327 (0.804), closely followed by IM200 (0.796) and IM123 (0.777), demonstrating their high discriminatory power and suitability for population genetic analyses of *I. kolpakowskiana*.

The six polymorphic EST-SSR markers used in this study were relatively highly informative for assessing genetic diversity in populations of *I. kolpakowskiana*. The mean polymorphic information content (PIC = 0.688) and average Nei's genetic diversity (Nei = 0.518) indicate substantial marker informativeness. According to Botstein et al. [22], loci with PIC values above 0.50 are considered highly informative, and four of the six loci met this criterion.

Similar results have been reported for other *Iris* species. For instance, Tang et al. [18] demonstrated the high transferability and polymorphism of EST-SSR markers (IM327 (Na = 6.8, h = 0.750), IM123 (Na = 5.2, h = 0.680), IM200 (Na = 5.6, h = 0.574)) across multiple *Iris* taxa, while Sun et al. [19] reported successful amplification and polymorphism of EST-derived microsatellite loci (IEST8 (Na = 4.3, h = 0.630)) in *Iris laevigata* and related species. The expected heterozygosity (h) values were positively correlated with PIC values [22, 28]. Overall, the relatively high values of genetic diversity and PIC indicate that the selected EST-SSR markers were effective for detecting genetic variation and assessing population structure in *I. kolpakowskiana* populations.

Genetic Diversity of Iris kolpakowskiana Populations

Genetic diversity parameters varied among the nine studied populations of *I. kolpakowskiana* (Table 4). The mean number of alleles per locus (Na) ranged from 2.5 in population ALM3 to 3.5 in populations ALM2 and TUR2, with an overall average of 3.1 alleles per locus. The effective number of alleles (Ne) ranged from 1.9 to 2.8, with an average of 2.4 across all populations. The Shannon information index (I) ranged from 0.667 (ALM3) to 0.993 (ZH1), with a mean value of 0.863. Similarly, Nei's genetic diversity index (Nei) showed moderate variation among populations, ranging from 0.425 in ALM1 to 0.605 in ZH3, with an overall mean of

Table 4. Genetic diversity parameters of nine *Iris kolpakowskiana* populations based on six polymorphic EST-SSR markers

Population	N	Na	Ne	I	Nei	%P
ALM1	20	2.8	2.1	0.721	0.425	83,33%
ALM2	20	3.5	2.6	0.965	0.550	100,00%
ALM3	20	2.5	1.9	0.667	0.430	83,33%
Mean for ALM		2,9	2.2	0.784	0.468	88.89%
ZH1	20	3.3	2.8	0.993	0.582	83,33%
ZH2	19	3.0	2.5	0.898	0.536	83,33%
ZH3	18	3.3	2.5	0.986	0.605	100,00%
ZH4	20	3.0	2.2	0.823	0.493	83,33%
Mean for ZH 3,2 2.5				0.925	0.554	87.50%
TUR1	10	2.7	2.1	0.738	0.478	83,33%
TUR2	21	3.5	2.6	0.979	0.560	83,33%
Mean for TUR		3,1	2.3	0.858	0.519	83.33%
Total Mean		3,1	2.4	0.863	0.518	87.04%
SE		0,2	0.1	0.06	0.03	2.45%

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

DOI: 10.11134/btp.2.2026.10

0.518. The highest levels of genetic diversity were observed in populations ZH3, ZH1, TUR2, and ALM2, whereas populations ALM1 and ALM3 exhibited comparatively lower diversity values. The percentage of polymorphic loci (%P) ranged from 83.33% to 100%. Complete polymorphism was detected in populations ALM2 and ZH3, whereas the remaining populations exhibited polymorphism at five of the six loci analyzed. On average, 87.04% of loci were polymorphic across all populations, indicating a relatively high level of genetic variation within *I. kolpakowskiana.*

When populations were grouped according to geographic regions, all Zhambyl populations (ZH1, ZH2, ZH3, and ZH4) exhibited the highest genetic diversity (Na = 3.2, Ne = 2.5, I = 0.925, Nei = 0.554), followed by the Turkestan populations (Na = 3.1, Ne = 2.3, I = 0.858, Nei = 0.519). The Almaty populations (ALM1, ALM2, and ALM3) displayed slightly lower diversity estimates (Na = 2.9, Ne = 2.2, I = 0.784, Nei = 0.468). These results suggest that populations from the Zhambyl region (ZH1, ZH2, ZH3, and ZH4) harbor a greater proportion of the species' genetic variation and may represent important reservoirs of genetic diversity for the conservation of *I. kolpakowskiana.*

The overall level of genetic diversity detected in *I. kolpakowskiana* populations was moderately high (Nei = 0.518), indicating that substantial genetic variation is maintained within natural populations despite the species' fragmented distribution. Comparable levels of genetic diversity have been reported in other perennial *Iris* species. For example, Zhang et al. [20] reported high genetic diversity in *I. loczyi* populations from the Qinghai–Tibet Plateau, with genetic diversity values reaching 0.699. Thus, the moderately high diversity observed in *I. kolpakowskiana* is generally consistent with patterns reported for *I. loczyi*, although its lower value may reflect differences in geographic range, population size, or reproductive biology.

Among the studied regions, all analyzed populations from the Zhambyl region (ZH1–ZH4) exhibited the highest diversity estimates (Nei = 0.554), whereas populations from the Almaty region (ALM1, ALM2, and ALM3) showed comparatively lower diversity (Nei = 0.468). Such differences may reflect variation in population size, habitat connectivity, or historical demographic processes [29]. The high percentage of polymorphic loci (87.04%) further suggests that most populations have maintained a broad spectrum of genetic variation, which is essential for long-term adaptation and persistence under changing environmental conditions [30].

Analysis of Molecular Variance (AMOVA)

The distribution of genetic variation among and within populations of *I. kolpakowskiana* was evaluated using AMOVA (Table 5). The results revealed that the majority of the genetic variation was within populations, accounting for 55% of the total variance, whereas 45% was attributable to differences among populations. Similar patterns of genetic variation, in which a substantial proportion of total diversity is maintained within populations, have been reported for various plant species analyzed using molecular markers [31, 32].

The fixation index (F_{ST}) was estimated at 0.481 and was highly significant ($p < 0.001$), indicating substantial genetic differentiation among populations. According to Wright's criteria [33], F_{ST} values greater than 0.25 reflect very high levels of population differentiation, suggesting limited genetic connectivity among the studied populations (Table 5). The estimated gene flow (N_m) was 0.654 migrants per generation (Table 5), which is below the threshold value of 1.0 generally considered sufficient to counteract the effects of genetic drift [33, 34]. This result indicates restricted gene exchange among populations and suggests that genetic drift and geographic isolation have played important roles in shaping the genetic structure of *I. kolpakowskiana.*

Overall, the AMOVA results demonstrate a pronounced population structure, characterized by considerable genetic differentiation among populations despite the slightly higher proportion of variation maintained within populations. The observed pattern is consistent with fragmented distribution, restricted dispersal, and habitat isolation, which are common features of many rare and endemic plant species [35, 36].

Population Structure Analysis

The genetic relationships and population structure of *I. kolpakowskiana* were investigated using PCoA, NJ cluster analysis, and Bayesian clustering implemented in STRUCTURE (Figure 2).

The PCoA explained 87.70% of the total genetic variation, with the first and second coordinates accounting for 71.66% and 16.04% of the variation, respectively (Figure 2A). The analysis revealed a clear separation of populations into two major groups corresponding to their geographic distribution. Group I comprised populations from southeastern Kazakhstan, specifically the Almaty region (ALM1–ALM3), whereas Group II included populations from southern Kazakhstan, represented by the Zhambyl (ZH1–ZH4) and Turkestan (TUR1 and TUR2) regions. The close clustering of populations within each group indicates substantial genetic similarity among geographically proximate populations.

The Neighbor-Joining dendrogram (Figure 2B) supported the pattern observed in the PCoA (Figure 2A). Two major clusters were identified with strong bootstrap support (100). The first cluster comprised all Almaty populations, while

Table 5. Analysis of molecular variance (AMOVA) and population differentiation parameters for nine populations of *Iris kolpakowskiana* based on six polymorphic EST-SSR markers

Source	df	SS	MS	Est. Var.	%	F_{st}	N_m
Among Pops	8	661.207	82.651	4.165	45%		
Within Pops	159	824.841	5.188	5.188	55%		
Total	167	1486.048		9.352	100%	0.481*	0.654*

Notes: df – degrees of freedom; SS – sum of squares; MS – mean squared; Est.var. – estimates of variance; % – percentage of variation; F_{st} – fixation index; N_m – gene flow value; * $p < 0.001$; $Nm = (1 - F_{st})/4F_{st}$.

DOI: 10.11134/btp.2.2026.10

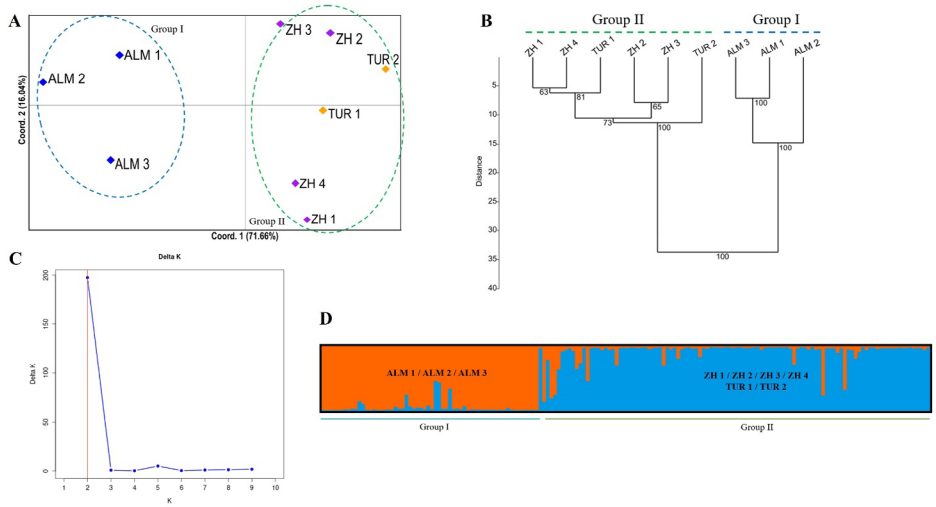

Figure 2. Genetic relationships and population structure of nine *Iris kolpakowskiana* populations based on six polymorphic EST-SSR markers. (A) Principal Coordinate Analysis (PCoA); (B) Neighbor-Joining (NJ) dendrogram showing genetic relationships among populations, with bootstrap values indicated at the nodes; (C) ΔK values from STRUCTURE analysis used to determine the optimal number of genetic clusters; (D) Bayesian clustering of individuals inferred by STRUCTURE at K = 2.

the second cluster included populations from the Zhambyl and Turkestan regions. Although some differentiation was observed within the latter cluster, the primary division remained consistent with the geographic grouping revealed by the PCoA.

Bayesian clustering analysis further confirmed the existence of two major genetic groups. The ΔK method identified K = 2 as the most likely number of genetic clusters, with a pronounced peak of ΔK = 197.47 (Figure 2C; Table 6). The corresponding STRUCTURE bar plot (Figure 2D) clearly separated the Almaty populations from those of Zhambyl and Turkestan. Most individuals exhibited high membership coefficients within their respective clusters, indicating strong genetic differentiation and limited admixture among regional groups.

A Mantel test showed no significant relationship between genetic and geographic distances among populations of *I. kolpakowskiana* ($R^2 = 0.0008$, $p > 0.05$), indicating the absence of a clear isolation-by-distance pattern. The combined results of AMOVA, PCoA, Neighbor-Joining clustering, and STRUCTURE analyses revealed pronounced genetic differentiation among populations of *I. kolpakowskiana*. The AMOVA showed that 45% of the total genetic variation was attributable to differences among populations, while the fixation index ($F_{ST} = 0.481$) indicated very high population differentiation according to Wright's classification. Such values are considerably higher than those typically reported for widespread outcrossing plant species [20] and suggest restricted gene flow among populations. The Bayesian clustering analysis identified two major genetic groups corresponding to their geographic distribution: populations from southeastern Kazakhstan (Almaty region) and populations from southern Kazakhstan (Zhambyl and Turkestan regions). This geographic pattern was consistently recovered by PCoA and Neighbor-Joining analyses, indicating the presence of distinct regional gene pools within the species (Figure 2).

Table 6. Results of Bayesian clustering analysis and estimation of the optimal number of genetic clusters (K) in populations of *Iris kolpakowskiana*

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln"(K)	Delta K
1	3	-1395.56667	1.09697	NA	NA	NA
2	3	-1216.40000	1.08167	179.16667	213.60000	197.47327
3	3	-1250.83333	49.10014	-34.43333	38.63333	0.78683
4	3	-1323.90000	13.45697	-73.06667	3.06667	0.22789
5	3	-1393.90000	30.81704	-70.00000	153.76667	4.98966
6	3	-1617.66667	190.73176	-223.76667	72.96667	0.38256
7	3	-1768.46667	147.08696	-150.80000	142.93333	0.97176
8	3	-1776.33333	140.59418	-7.86667	179.20000	1.27459
9	3	-1605.00000	117.95334	171.33333	202.80000	1.71932
10	3	-1636.46667	32.83509	-31.46667	NA	NA

Notes: K – assumed number of genetic clusters; Reps – number of independent STRUCTURE runs; Mean LnP(K) – mean log-likelihood of the data for a given K; Stdev LnP(K) – standard deviation of log-likelihood values; Ln′(K) – first-order rate of change of the likelihood function; |Ln″(K)| – second-order rate of change of the likelihood function; ΔK – Evanno's statistic used to identify the most probable number of genetic clusters. The highest ΔK value indicates the optimal K.

DOI: 10.11134/btp.2.2026.10

Interestingly, the Mantel test did not reveal a significant correlation between genetic and geographic distances (R^2 = 0.0008, $p > 0.05$). This finding indicates that geographic distance alone is insufficient to explain the observed population structure. Instead, the genetic differentiation observed among populations is likely associated with historical isolation, habitat fragmentation, and the complex mountainous landscape of Kazakhstan. Populations of *I. kolpakowskiana* occur in geographically separated mountain systems, including the Trans-Ili Alatau in southeastern Kazakhstan and the Karatau, Korday, and Western Tien Shan regions in southern Kazakhstan [1, 8, 37]. These landscape features may serve as effective barriers to gene flow, promoting independent evolutionary trajectories among regional populations. An influence of geographic barriers on genetic structure has been reported for *I. loczyi*, where strong regional differentiation was detected among populations distributed across the Qinghai-Tibet Plateau [20]. Together, these findings suggest that geographic isolation represents one of the principal factors shaping population genetic structure in wild *Iris* species inhabiting fragmented mountain ecosystems.

The present study represents the first assessment of genetic diversity and population structure in *I. kolpakowskiana* populations using EST-SSR markers. The generated dataset provides an important baseline for future conservation genetic studies and contributes to a better understanding of the evolutionary processes that shape genetic variation in wild *Iris* species of Central Asia. However, this study is limited by the use of a restricted number of markers and sampled populations, which may not fully capture the species-wide genetic variation. Therefore, future studies should include broader geographic sampling and additional molecular markers to obtain a more comprehensive understanding of genetic diversity in *I. kolpakowskiana*.

CONCLUSION

This study provides the first assessment of genetic diversity and population structure in *I.kolpakowskiana* using EST-SSR markers. The six polymorphic loci revealed relatively high genetic diversity within natural populations, with the highest diversity detected in the Zhambyl region, highlighting its importance as a reservoir of genetic resources. AMOVA, PCoA, Neighbor-Joining, and STRUCTURE analyses consistently revealed strong genetic differentiation among populations and identified two major genetic groups corresponding to southeastern Kazakhstan (Almaty region) and southern Kazakhstan (Zhambyl and Turkestan regions). The high F_{ST} value and low gene flow estimates indicate restricted genetic exchange among regions. In addition, the lack of a significant correlation between genetic and geographic distances suggests that population structure is shaped not only by distance but also by historical isolation, habitat fragmentation, and mountain barriers. The genetic data generated in this study provide an important foundation for future research on the genetic diversity and population structure of *I. kolpakowskiana* and other representatives of *Iris* in Kazakhstan.

ACKNOWLEDGMENTS

We thank Aruzhan Alikhanova, a junior researcher in the Molecular Genetics laboratory at the Institute of Plant Biology and Biotechnology (IPBB), for her assistance with the technical aspects of this study.

AUTHOR CONTRIBUTIONS

Conceptualization: Y.K.T., S.S.A., S.I.A.; Formal Analysis: M.M.Y., S.I.A.; Funding Acquisition: S.S.A.; Investigation: M.M.Y., S.I.A., S.S.A.; Project Administration: S.S.A.; Software: M.M.Y., S.S.A.; Supervision: S.S.A.; Visualization: M.M.Y.; Writing – Original Draft Preparation: M.M.Y., S.S.A.; Writing – Review & Editing: S.S.A., S.I.A., Y.K.T.

FUNDING

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP23483825).

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DOI: 10.11134/btp.2.2026.10

УДК 631.523.5

ОЦЕНКА ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ И ПОПУЛЯЦИОННОЙ СТРУКТУРЫ *IRIS KOLPAKOWSKIANA* REGEL ИЗ КАЗАХСТАНА С ИСПОЛЬЗОВАНИЕМ МИКРОСАТЕЛЛИТНЫХ МАРКЕРОВ

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АННОТАЦИЯ

Iris kolpakowskiana Regel – редкий дикий вид ирисов, распространенный на юго-востоке и юге Казахстана. Для оценки его генетического разнообразия и популяционной структуры были проанализированы 168 особей из 9 природных популяций в Алматинской, Жамбылской и Туркестанской областях с использованием EST-SSR маркеров. Из девяти протестированных пар праймеров для дальнейшего анализа было выбрано 6 полиморфных локусов. Среднее число аллелей на локус составило 3,1, при среднем эффективном числе аллелей 2,4. Информационное содержание полиморфизма (PIC = 0,688) указывает на умеренно высокую генетическую изменчивость и высокую информативность маркеров. Наибольшее генетическое разнообразие наблюдалось в популяциях Жамбылской области. AMOVA показала, что 55% генетической изменчивости приходится на внутрипопуляционную структуру и 45% на межпопуляционную. Была обнаружена высокая генетическая дифференциация (F_{ST} = 0,481, $p < 0,001$), сопровождающаяся ограниченным генным потоком (N_m = 0,654). Метод анализа главных координат, кластеризация методом Neighbor-Joining и анализ STRUCTURE последовательно выявили две основные генетические группы, соответствующие юго-восточному Казахстану (Алматы) и южному Казахстану (Жамбылская и Туркестанская области). Тест Мантеля показал отсутствие значимой корреляции между генетическим и географическим расстоянием (R^2 = 0,0008, $p > 0,05$). Эти результаты выявили географическую структурированность и ограниченный генетический обмен между популяциями, предоставив первую генетическую оценку *Iris kolpakowskiana* и ценную информацию для ее дальнейшего сохранения и управления.

Ключевые слова: *Iris kolpakowskiana*, Казахстан, EST-SSR, генетическое разнообразие, AMOVA, генетическая структура.

ӘОЖ 631.523.5

МИКРОСАТЕЛЛИТТІК МАРКЕРЛЕРДІ ҚОЛДАНУ АРҚЫЛЫ ҚАЗАҚСТАНДАҒЫ *IRIS KOLPAKOWSKIANA* REGEL ТҮРІНІҢ ГЕНЕТИКАЛЫҚ АЛУАНТҮРЛІЛІГІ МЕН ПОПУЛЯЦИЯЛЫҚ ҚҰРЫЛЫМЫН БАҒАЛАУ

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АННОТАЦИЯ

Iris kolpakowskiana Regel – Қазақстанның оңтүстік-шығысы мен оңтүстігінде таралған сирек кездесетін жабайы құртқашаш түрі. Оның генетикалық алуантүрлілігі мен популяциялық құрылымын бағалау мақсатында Алматы, Жамбыл және Түркістан облыстарында 9 табиғи популяциядан жиналған 168 үлгі EST-SSR маркерлері көмегімен зерттелді. Сыналған тоғыз праймер жұбының ішінен әрі қарайғы талдау үшін 6 полиморфты локус таңдалды. Бір локусқа шаққандағы аллельдердің орташа саны 3,1 болса, ал тиімді аллельдердің орташа саны 2,4 құрады. Полиморфизмнің ақпараттық индексі (PIC = 0,688) генетикалық өзгергіштіктің орташа жоғары деңгейін және қолданылған маркерлердің жоғары ақпараттылығын көрсетті. Ең жоғары генетикалық алуантүрлілік Жамбыл облысы популяцияларында анықталды. AMOVA талдауы генетикалық өзгергіштіктің 55% популяция ішіндегі құрылымға, ал 45% популяциялар арасындағы айырмашылықтарға тиесілі екенін көрсетті. Жоғары генетикалық дифференциация (F_{ST} = 0,481, $p < 0,001$) анықталды, ол ген ағынының шектеулі болуымен (N_m = 0,654) қатар жүрді. Бас координаттар әдісімен жүргізілген талдау (PCoA), Neighbor-Joining кластерлеуі және STRUCTURE бағдарламасы арқылы жүргізілген талдау нәтижесінде екі негізгі генетикалық топ айқындалды. Олар географиялық тұрғыдан Қазақстанның оңтүстік-шығыс аймағына (Алматы) және оңтүстік аймағына (Жамбыл және Түркістан облыстары) сәйкес келді. Мантель тесті генетикалық және географиялық қашықтықтар арасында маңызды корреляцияның жоқ екенін көрсетті (R^2 = 0,0008, $p > 0,05$). Алынған нәтижелер популяциялар арасындағы генетикалық алмасудың шектеулі екенін және олардың географиялық құрылымдануын айқындап, *Iris kolpakowskiana* түрінің генетикалық жағдайына алғашқы бағалау берді және болашақта сақтау мен басқару шараларын жоспарлауда құнды ақпарат болып табылады.

Кілт сөздер: *Iris kolpakowskiana*, Қазақстан, EST-SSR, генетикалық алуантүрлілік, AMOVA, генетикалық құрылым.