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Genetic polymorphism of *Populus pruinosa* and *Populus euphratica* in Kazakhstan based on ISSR markers

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Abstract. *Akhatayeva D, Kutsev M, Vesselova P, Inelova Z, Osmonali B, Aitzhan M, Zaparina Y, Shadmanova L, Boros E. 2025. Genetic polymorphism of* Populus pruinosa *and* Populus euphratica *in Kazakhstan based on ISSR markers. Biodiversitas 26: 6120-6129.* The genus *Populus* (Salicaceae) in Kazakhstan is represented by 13 species, although the exact number of species, including varieties and hybrids, has not been clearly established. Among them, *Populus pruinosa* and *Populus euphratica* are native species with rapidly declining population sizes and shrinking habitats. Comprehensive studies on their biology and genetic diversity in Kazakhstan have not reported so far. This study aimed to assess genetic diversity, compare patterns of differentiation, evaluate the relationship between genetic variation and ecological conditions in natural populations of both species. Genetic variability was assessed using the ISSR method, and statistical analysis was performed using ANOVA. Samples from six natural populations in southern Kazakhstan (three populations of *P. pruinosa* and three of *P. euphratica*) showed distinct interspecific differentiation and variable genetic diversity. *P. pruinosa* populations had higher allelic diversity (h = 0.114-0.153) and expected heterozygosity (0.107-0.139) than *P. euphratica* (h = 0.041-0.085). Greater genetic diversity in *P. pruinosa* indicates stronger adaptive potential to arid environments. A moderate correlation was found between genetic and geographic distances (r = 0.597). The findings highlight *P. pruinosa* superior ecological adaptability. Both species are recommended for targeted conservation and restoration of arid ecosystems in Kazakhstan.

Keywords: Genetic polymorphism, ISSR markers, Kazakhstan, *Populus euphratica*, *Populus pruinosa*

INTRODUCTION

Kazakhstan is dominated by arid and semi-arid territories, which account for more than 75% of its area and are characterized by a sharply continental climate (Aishan et al. 2015; Vesselova and Kudabayeva 2017; Vesselova et al. 2022). These fragile ecosystems are highly vulnerable to anthropogenic pressures, such as overgrazing, industrial development, infrastructure expansion, and illegal logging. Such factors accelerate land degradation and biodiversity loss, underscoring the urgent need for restoration measures. Expanding green spaces is one of the most effective approaches to stabilizing landscapes and improving ecological resilience.

Poplars (*Populus* L., Salicaceae) are considered promising species for ecological restoration due to their rapid growth, vegetative propagation, and tolerance to abiotic stresses (Ling et al. 2019; Vdovina et al. 2024; Bruat et al. 2025). Globally, the genus *Populus* includes 61 species depending on taxonomic treatment, with 30 species recognized in Komarov's classification (Thomas et al. 2017a, 2017b; Rakhimzhanov et al. 2021; Stikhareva et al. 2021; Guleria et al. 2022). In Kazakhstan, 13 species occur naturally, including two desert poplars of ecological importance, namely *Populus pruinosa* Schrenk and *Populus euphratica* Olivier. These trees form tugai forests along rivers, stabilize soils, and provide key habitats, but their populations are declining due to habitat loss and exploitation (Kairova et al. 2023).

The genetic structure of *P. pruinosa* and *P. euphratica* in Kazakhstan remains poorly studied. Taxonomic identification in the genus is complicated by frequent interspecific hybridization and strong morphological variability (Hao et al. 2017; Yu et al. 2020; Zhai et al. 2020; Wu et al. 2023; Sun et al. 2025). Muellner-Riehl (2019) reported contradictory results regarding their genetic differentiation, while some haplotypes were species-specific, the most common ones were shared, and intraspecific variation often exceeded interspecific divergence. This highlights the need for more reliable molecular tools to clarify population structure and species boundaries.

Various molecular approaches, including RAPD, SSR, AFLP, SNP, and DNA barcoding, have been used to assess genetic diversity in *Populus* (Feng et al. 2013). However, markers from chloroplast and ribosomal regions often lack resolution to separate closely related species from sections

Aigeiros and *Tacamahaca* (Nasimovich et al. 2019; Du et al. 2024; Liu et al. 2025). Inter Simple Sequence Repeat (ISSR) markers provide an efficient alternative, as they are reproducible, cost-effective, and reveal genome-wide polymorphisms without prior sequence data. This makes ISSRs particularly suitable for species with complex evolutionary histories and hybridization events. ISSR markers have been successfully applied to study *Populus alba*, *Populus nigra*, and their hybrids, revealing patterns of population structure, differentiation, and gene flow (Chen et al. 2011; Mutegi et al. 2016; Zhou et al. 2018; Ahmed et al. 2019). They have also proven useful in closely related taxa for detecting intra- and interspecific variation and hybridization. Despite these successes, no ISSR-based analyses have yet been conducted on *P. pruinosa* and *P. euphratica* in Kazakhstan. This gap limits understanding of their adaptation to arid environments, their ability to maintain genetic diversity in fragmented habitats, and their potential for ecological restoration.

Genes associated with salt tolerance have been identified in *P. pruinosa,* but are absent in *P. euphratica* (Sun et al. 2024), indicating evolutionary divergence and highlighting opportunities for selective breeding. Such traits may play a key role in developing strategies for restoring desert ecosystems and improving population resilience. The present study addresses this gap by conducting ISSR-based analysis of natural populations of *P. pruinosa* and *P. euphratica* in southern Kazakhstan. Therefore, this study aimed to assess the level of genetic diversity in natural populations of both species, compare patterns of genetic differentiation, and examine the relationship between genetic variation and ecological conditions. The results provide insights into the evolutionary divergence of desert poplars and inform strategies for their conservation and use in restoring fragile arid ecosystems.

MATERIALS AND METHODS

Plant materials

The plant materials consisted of samples from three populations of each species, *Populus pruinosa* and *Populus euphratica*, with 10 samples from each population. The minimum distance between trees was 20 m. Collections were made in the Kyzylorda and Almaty regions (Table 1), and the collection points are shown in Figure 1. The nomenclature of each taxon mostly follows Plants of the World Online (POWO 2025).

Table 1. Source of *Populus pruinosa* and *Populus euphratica* samples used in the study

Population	Species	Location	Coordinate (N, E)
1	*Populus pruinosa*	5691 Zhanakorgan district, Zhanakorgan forestry, along the Syrdarya River	43°52'43", 67°12'40"
2	*Populus pruinosa*	5694 Kazakhstan, Ili River valley, China border	43°41'15", 80°11'47"
3	*Populus pruinosa*	5695 Kyzylorda region, Shieli district, «Kargaly» nature reserves	44°44'17", 65°39'29"
4	*Populus euphratica*	5692 Kyzylorda region, Shieli district, turanga woodland	44°16'09", 66°34'17"
5	*Populus euphratica*	5697 Almaty region, Balkash district, Akkol forestry, quartile 19, allotment 14, area 540, appraisal-3	44°58'52", 75°49'59"
6	*Populus euphratica*	5696 Kazakhstan, Ili River valley, China border	43°41'15", 80°11'47"

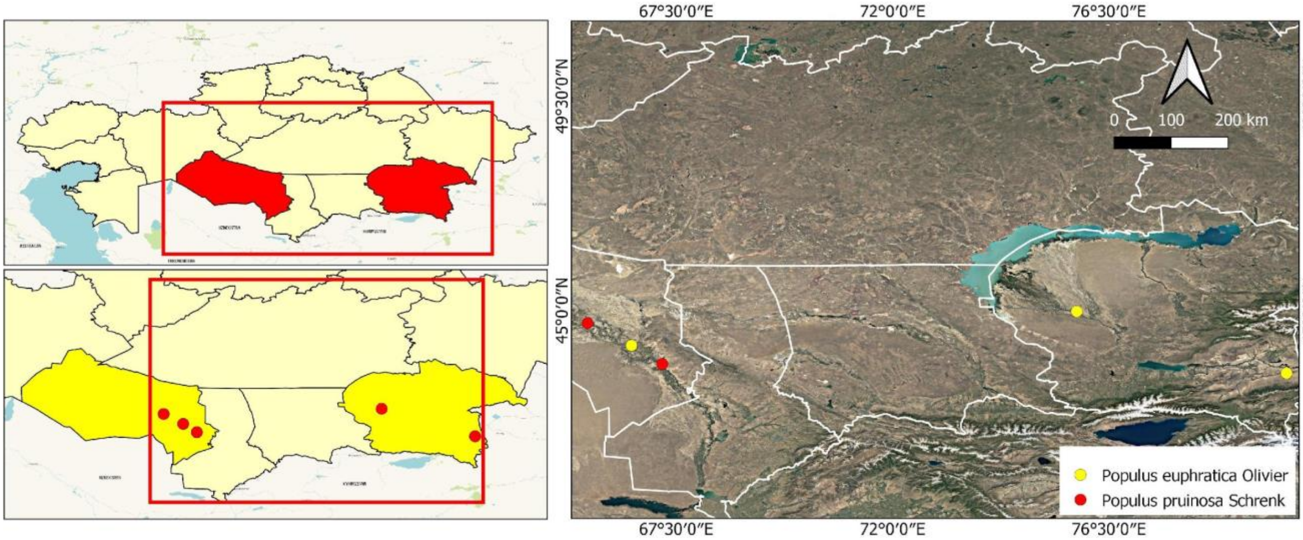

Figure 1. The populations of *Populus euphratica* (yellow) and *Populus pruinosa* (red) were used for molecular genetic analysis. Collections were made in the Kyzylorda and Almaty regions, Kazakhstan

Procedures

DNA isolation

We selected 10 leaves from each population for the analysis with several criteria as follows: the plant was not affected by any diseases, the distance between the individuals from whom the samples were taken should have been at least 20 m, and they were of different ages (Wang et al. 2011). Total DNA was isolated from dry silica gel leaves using the DimondDNA Plant kit (Altaibiotech, Russia) according to the manufacturer's protocol. The quality of the extracted DNA was analyzed using an Implan NanoPhotometer P360 spectrophotometer, with the A260/A280 ratio for different samples being 1.9-2.0. Samples with poor DNA quality were reextracted.

DNA amplification and electrophoresis

Polymerase Chain Reaction (PCR) was performed using the ready-to-use Biomaster HS-Taq PCR mix (Biolabmix, Russia) in a 50 µL reaction volume of containing 25 µL of ready-to-use PCR mix, 21 µL of H_2O, 1 µL of each corresponding 10 mM primer, and 2 µL of total DNA. Amplification was carried out in a BioRad MyCycler thermocycler following this program: initial denaturation at 95°C for 20 sec, denaturation at 95°C for 20 sec, primer annealing at 55°C for 45 sec, and elongation at 72°C for 90 sec (13 cycles), followed by 25 additional cycles with the following conditions: denaturation at 95°C for 20 sec, primer annealing at 44°C for 30 sec, and elongation at 72°C for 90 seconds, final extension at 72°C for 30 sec. After analysis, the five most informative primers were identified (Table 2). Prime primer selection was performed based on the repeatability of the amplified fragment pattern and the presence of polymorphic fragments among samples from different populations.

Amplification products were separated in a 2% agarose gel, with ethidium bromide used as a dye. The fragments were visualized using a gel documentation system (Quantum-ST5, France). Only clear and repetitive DNA fragments were selected for analysis.

Data analysis

The obtained results were transformed into a binary data matrix, where the presence or absence of a DNA fragment was coded as 1 or 0, respectively. The resulting matrix of amplified fragment lengths was analyzed using the GENEPOP 4.7.5 program. Initially, a genetic differentiation matrix was created in GENEPOP based on allele frequencies, calculated using Markov chains and the "^a" statistic (Rousset 2000). In addition, a population differentiation matrix based on F-statistics was constructed. The fixation index (Fst) was estimated using a "weighted" analysis of variance.

Further analysis of population genetic differentiation was carried out using cluster analysis in the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc), version 2.1. Expected and observed heterozygosity were determined with the GenAlEx6 Fmacro (ANOVA) extension for MS Excel. Bayesian probability analysis was performed using the STRUCTURE 2.3.1 program to investigate the population genetic structure. The analysis was conducted under an admixture model with correlated allele frequencies, assuming inheritance from a common ancestor via genetic drift. The number of clusters (K) was tested from 1 to 7, with ten replicates for each K value, using 10^5 iterations and a burn-in period of 10^5. The optimal number of clusters was determined as K = 3 using the STRUCTURE HARVESTER program (Earl and vonHoldt 2012).

Correlation analysis and heatmap visualization were performed in the ClustVis program to visualize the degree of population clustering and interpopulation relationships (Metsalu and Vilo 2015), where a graphical representation ("heat map") was generated to demonstrate the degree of inclusion of specific populations into conditional clusters. The population structure at pre-determined locations was assessed using two complementary approaches: (i) Multidimensional, Model-Free Analysis: Principal Component Analysis (PCA) was conducted in GenAlEx, based on a pairwise genetic distance matrix for individual samples. The analysis employed the covariance standardization method (Osmonali et al. 2023), enabling identification of the main axes of genetic variation; (ii) Bayesian clustering analysis: Population structure was further examined using STRUCTURE v2.3.4 under an admixture model, with a burn-in period of 10,000 and 100,000 iterations of the Markov Chain Monte Carlo (MCMC) algorithm. The number of clusters (K) ranged from 2 to 7, with ten repetitions performed for each K value. The resulting data were analyzed in STRUCTURE HARVESTER (Earl and vonHoldt 2012), and the optimal K value was determined using the Evanno method, which revealed a pronounced peak in the Delta K distribution, indicating the most likely number of genetic clusters.

RESULTS AND DISCUSSION

DNA polymorphisms

After individually analyzing each ISSR primer, the most informative ones were identified. The maximum number of amplified fragments was observed for UBC-825 primer, with 36 fragments, while the minimum number was observed for primer 17899A, with 21 fragments (Figure 2; Table 3).

Table 2. Sequence of primers used in ISSR

Name of primer	Sequence of primer (5'-3')	References
17899A	$(CA)_6$ AG	Hussein et al. (2008)
UBC-811	$(GA)_8$ C	Datta and Lal (2011)
UBC-827	$(AC)_8$ G	Datta and Lal (2011)
UBC-825	$(AC)_8$ T	Ratnaparkhe et al. (1998)
UBC-828	$(TG)_8$ A	Levi et al. (2004)

Table 3. Results of the used primers

Name of primer	Number of amplified DNA fragments	Number of polymorphic DNA fragments	Polymorphic DNA fragments (%)
17899A	21	18	85.71
UBC-811	25	22	88
UBC-827	32	30	93.75
UBC-825	36	36	100
UBC-828	23	20	86.96
Total	137	126	
Average	27.4	25	90.88

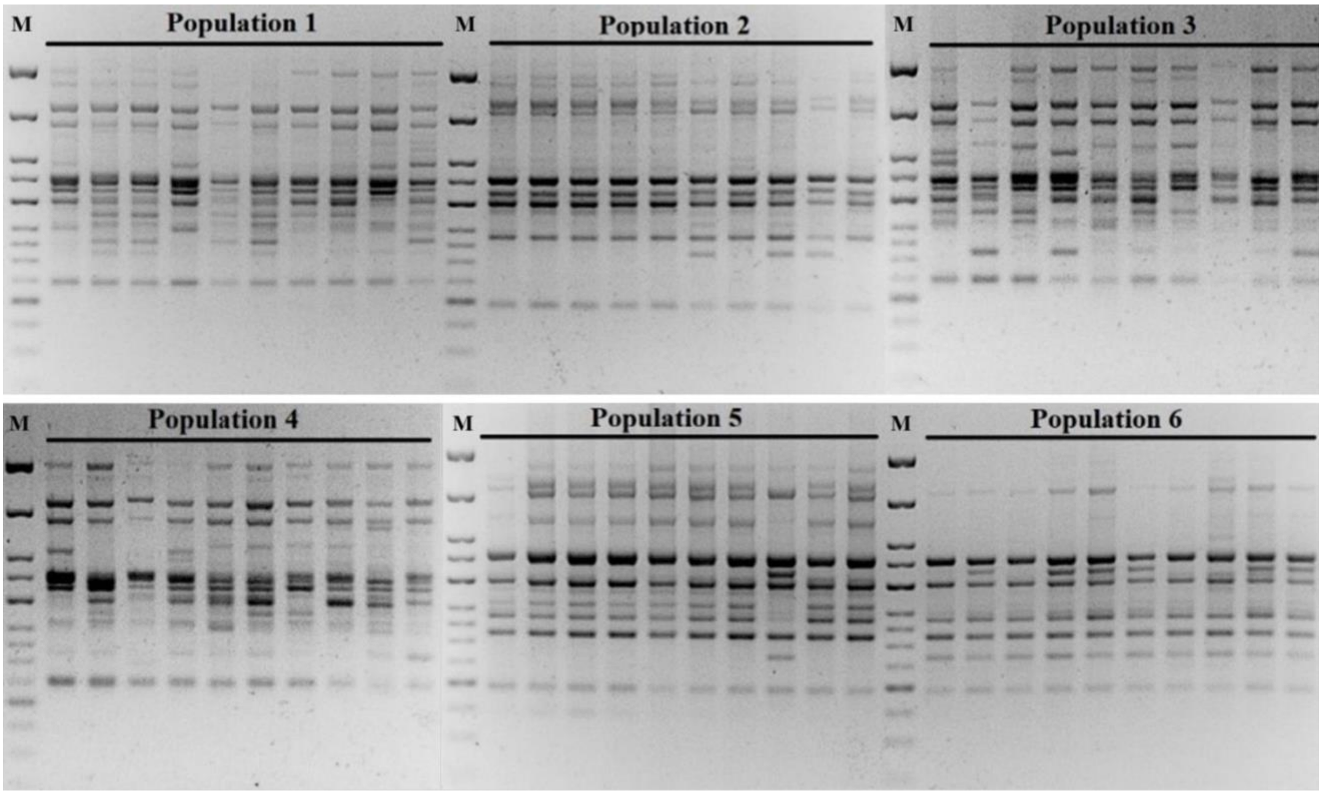

Figure 2. Amplified DNA fragments of *Populus pruinosa* and *Populus euphratica* populations generated by primer 17898A. M - DNA molecular weight marker

The ISSR amplification profiles of six natural populations of *Populus pruinosa* (Populations 1-3) and *P. euphratica* (Populations 4-6) obtained using primer 17898A are presented in Figure 2. Distinct banding patterns are visible among populations and between species, reflecting interspecific polymorphism. *P. pruinosa* populations generally display a higher number and intensity of amplified fragments, indicating greater allelic diversity compared with *P. euphratica*. In contrast, the banding profiles of *P. euphratica* populations appear less variable, consistent with their lower genetic diversity. The overall polymorphism revealed by this primer confirms its effectiveness in distinguishing both species and supports the observed differences in genetic variability derived from ISSR analysis.

A total of 137 ISSR fragments were registered, with an average of 27.4 amplified fragments per primer (Table 3). Each primer showed a high level of polymorphism, clearly distinguishing the two species studied. However, intraspecific variability was found to be lower, which may be attributed to the predominantly vegetative reproduction of the species.

An ANOVA analysis revealed that allelic diversity is higher among the *P. pruinosa* populations (Pop1-Pop3) compared to the *P. euphratica* populations (Pop4-Pop6) (Table 4). This is likely due to the greater adaptation of *P. pruinosa* to the growing conditions in the arid territories of Kazakhstan. This finding is also supported by Sun et al. (2024) on the greater drought resistance of *P. pruinosa*.

The expected heterozygosity of *P. pruinosa* ranged from 0.170 to 0.225, while that of *P. euphratica* was almost twice as low, ranging from 0.075 to 0.135, indicating greater genetic diversity within *P. pruinosa* populations. This finding aligns with data from earlier studies and can be attributed to the reproductive characteristics of these species. A study on the primary mode of reproduction in *P. euphratica* in Xinjiang Province (China) showed that, depending on growing conditions, the proportion of clones in different populations can be as high as 82%. In contrast, the primary method of reproduction for *P. pruinosa* is the formation of clones, which results in a decrease in genetic diversity within populations (Zheng et al. 2016). The greater allelic diversity in *P. pruinosa* relative to *P. euphratica* reflects its stronger adaptive capacity to arid environments in Kazakhstan, supporting previous reports of higher drought resistance in *P. pruinosa* (Sun et al. 2024).

Cluster analysis

According to the pairwise genetic distances matrix, the greatest genetic distance in *P. pruinosa* is observed between populations 1 and No. 2, with a value of 0.2007, while the lowest genetic distances is between populations 2 and 3, with a value of 0.0878 (Table 5). The average Fst value for all *P. pruinosa* populations was 0.1601. Sun et al. (2024) reported an analysis of single-nucleotide polymorphisms in the northern and southern groups of *P. pruinosa* from the Tien Shan yielded an Fst value of 0.07, which is slightly lower than the value obtained in this study for ISSR markers. In a similar study focused on *P. tremula*, the smallest genetic distance between populations was found to be 0.0338.

The greatest genetic distances in *P. euphratica* is observed between populations 5 and 6, with a value of 0.6531, while the lowest genetic distances is between populations 4 and 5, with a value of 0.6013. The average Fst value for all *P. euphratica* populations was 0.6311. The average fixation index between *P. euphratica* and *P. pruinosa* populations was 0.7323, with a minimum Fst value of 0.5054 and a maximum value of 0.7676. We found that the interspecific differentiation at ISSR loci was higher than that of the SSR analysis reported by Wang et al. (2011). However, a combination of all SSR loci produced better differentiation in two clusters between species in China. At ISSR loci, it is probable that these two species in Kazakhstan shared numerous ancestral polymorphisms. We associate this with the higher genetic variability of ISSR markers compared to SSR, which was described for genus *Populus* (Jianming et al. 2006).

The dendrogram constructed using the UPGMA method in the NTSYSpc21 program is shown in Figure 3. The dendrogram divides the studied species into two clusters. The first cluster unites the populations of *P. pruinosa* (populations 1, 2, 3). Populations from the Kargaly Reserve (Kyzylorda region) and from the river valley near the China border were found to be converging, while the population from the Syrdarya River (Zhanakorgan District, forestry) was isolated. The second cluster unites the populations of *P. euphratica* (populations 4, 5, 6). Within this cluster, the populations from the Akkol forest area (Almaty region) and from the river valley near the China border were the closest, while the population from the Turanga woodlands (Kyzylorda Region) was isolated. The Mantel isolation test was also conducted using the GENEPOP program. The correlation between genetic and geographical distances was moderate (0.597), even though the greatest similarity in *P. pruinosa* was observed between the most distant populations. In contrast, in *P. euphratica*, genetic similarity correlated with the distance between populations.

According to Broeck et al. (2005), many species of the genus *Populus* hybridize with one another. An analysis of the populations of *P. pruinosa* (Pop 3) and *P. euphratica* (Pop 5), which grow in close proximity, showed an Fst value of 0.7410. This value is significantly higher than the average for all populations, indicating that hybridization between these two species does not occur. Meanwhile, the low level of intrapopulation genetic diversity could be a significant concern for the conservation of these species, as both *P. pruinosa* and *P. euphratica* are rare, listed in the Red Books (Baitulin and Sitpayeva 2014) and require additional conservation efforts.

Table 4. Poplar population variability parameters based on ANOVA data

Population	n	Na	I	h	uh
Populus pruinosa Pop 1	10	0.920	0.170	0.114	0.127
Populus pruinosa Pop 2	10	1.000	0.225	0.153	0.170
Populus pruinosa Pop 3	10	0.978	0.181	0.121	0.134
Populus euphratica Pop 4	10	0.818	0.135	0.092	0.102
Populus euphratica Pop 5	10	0.657	0.065	0.041	0.046
Populus euphratica Pop 6	10	0.606	0.075	0.051	0.057

Note: n: Sample size, Na: Number of haplotypes, h: Allelic diversity, uh: Unbiased allelic diversity

Table 5. Pairwise genetic distances matrix of *Populus euphratica* and *Populus pruinosa* populations based on the Fst statistic

	P. pruinosa	***P. pruinosa***	***P. pruinosa***	***P. euphratica***	***P. euphratica***	***P. euphratica***
Populus pruinosa	-					
Populus pruinosa	0.2007	-				
Populus pruinosa	0.1918	0.0878	-			
Populus euphratica	0.8156	0.5054	0.7670	-		
Populus euphratica	0.7923	0.7626	0.7410	0.6013	-	
Populus euphratica	0.7676	0.7393	0.7086	0.6389	0.6531	-

Principal component analysis

The Principal Component Analysis (PCA) illustrates the genetic relationships among six populations of the genus *Populus* based on the first two Principal Components (PC1 and PC2), which account for 81.35% and 17.51% of the total genetic variance, respectively (Figure 4). Populations 1, 2, and 3, which belong to *P. pruinosa*, form a close cluster on the PC1 graph, indicating a high level of genetic similarity between them. This close clustering is likely due to both their taxonomic identity and their occurrence in the same geographical region. In contrast, Pop4, Pop5, and Pop6, identified as *P. euphratica*, are clearly separated from the *P. pruinosa* cluster along PC1, which explains 81.35% of the total variation. Within the *P. euphratica* group, Pop5 and Pop6 form a close cluster, while Pop4 is located more distantly along PC2, suggesting intraspecific genetic divergence, potentially related to geographical separation.

This overall pattern confirms differentiation at the species level and highlights the influence of geographical factors on intraspecific genetic variation. The ellipses represent 95% confidence intervals for each population.

The clear species separation confirms that genetic differentiation aligns with taxonomic identity. Tight clustering of *P. pruinosa* populations suggests stronger cohesion, potentially reflecting historical gene flow or less geographic isolation. By contrast, Pop 4's divergence within *P. euphratica* highlights how geographic distance and habitat heterogeneity shape genetic structure. Such differentiation underscores the importance of local adaptation processes and aligns with isolation-by-distance models in plant populations.

The PCA results obtained in this study clearly demonstrate the genetic differentiation between *P. pruinosa* and *P. euphratica*, which is consistent with earlier molecular investigations in the genus *Populus* across Central Asia and other arid regions. Similar patterns of interspecific divergence have been reported by Ma et al. (2018), who showed distinct clustering between *P. euphratica* and *P. pruinosa* based on chloroplast and nuclear markers, confirming their independent evolutionary lineages despite ecological overlap. In our study, the first two principal components explained 98.86% of the total variance, with PC1 (81.35%) representing the major axis of interspecific divergence, aligning with previous findings that this axis typically captures species-level separation within the *Populus* section *Turanga*. The tight clustering observed among *P. pruinosa* populations (Pop 1-Pop 3) supports a relatively high degree of genetic cohesion, which may be attributed to their limited geographic range and possible historical gene flow along connected riparian systems.

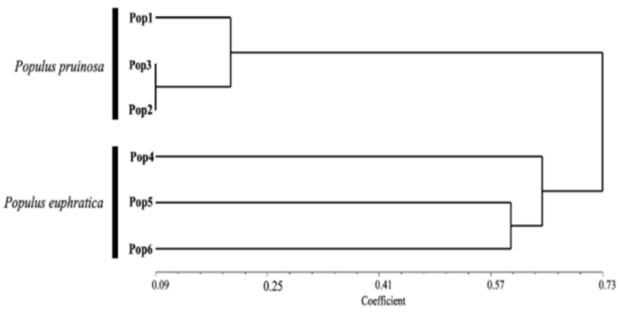

Figure 3. A dendrogram among 6 populations of *Populus euphratica* and *Populus pruinosa* generated by UPGMA cluster analysis based on ISSR polymorphism

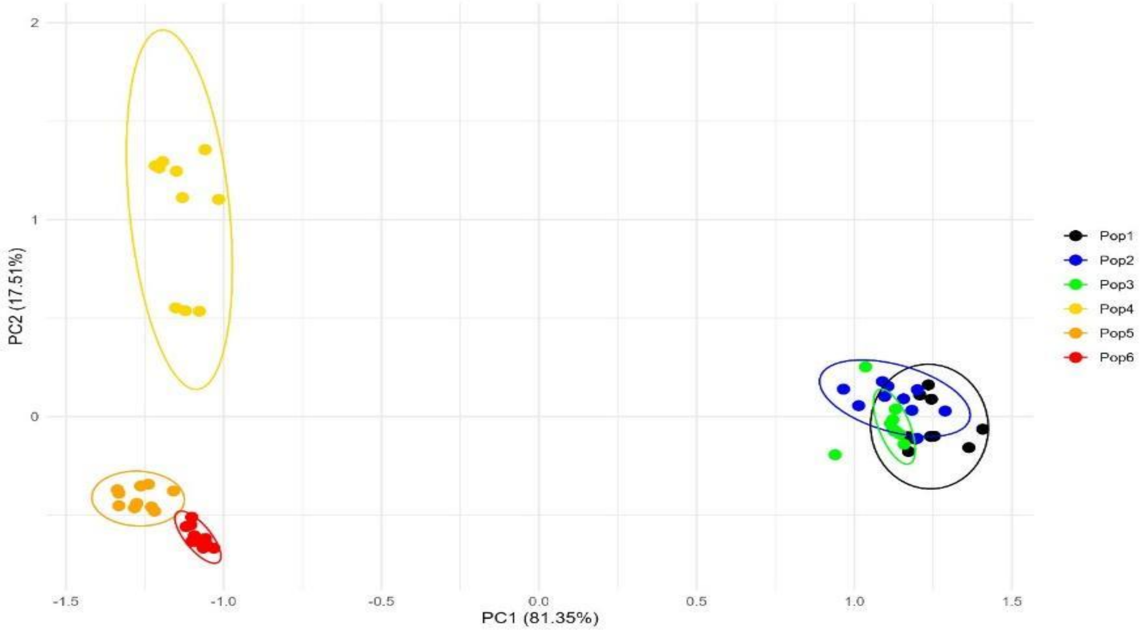

Figure 4. Principal component analysis among 6 populations of *Populus euphratica* and *Populus pruinosa*

Comparable results were obtained by Liu et al. (2025), who reported low intra-population differentiation and high genetic similarity in *P. pruinosa* populations from the Tarim Basin, indicating that hydrochory and clonal propagation maintain genetic homogeneity. Conversely, the wider dispersion of *P. euphratica* populations (Pop 4-Pop 6) along PC2 suggests a more pronounced intraspecific structure, a pattern also described by Yusup et al. (2022) for *P. euphratica* populations across the lower reaches of the Tarim River. Their study demonstrated that geographic isolation, hydrological fragmentation, and salinity gradients contribute significantly to the observed differentiation. The clear separation between the two species in the PCA diagram further supports their taxonomic distinction and reduced interspecific gene flow, despite partial sympatry in arid riparian zones. Vanden Broeck et al. (2005) have suggested that while hybridization events occasionally occur between *P. euphratica* and *P. pruinosa*, strong ecological and reproductive barriers, such as flowering time shifts and habitat preferences, maintain species integrity. The divergence detected in our results, especially the distinct position of Pop4 within *P. euphratica* may reflect local adaptation to heterogeneous environments, as observed by Wu et al. (2023), who linked genetic structure in *P. euphratica* to climatic and edaphic variables. Overall, the genetic differentiation revealed by PCA indicates that both species maintain distinct gene pools shaped by evolutionary isolation and ecological specialization. The stronger genetic cohesion in *P. pruinosa* may reflect its narrower distribution and adaptation to more stable habitats, whereas the broader genetic variance in *P. euphratica* points to higher ecological plasticity and adaptation to variable environmental conditions. These results collectively highlight how habitat heterogeneity and geographic barriers influence population structure in arid-adapted *Populus* species, supporting the isolation-by-distance and local adaptation models widely recognized in plant population genetics.

Structure analysis

The STRUCTURE analysis determined the most likely number of clusters (K) by calculating the logarithmic probability of the data for each K value. The Evanno method, implemented in STRUCTURE Harvester, was used to identify the optimal K value for the genotyped *Populus*. Based on the clustering model, the Evanno test indicated that K = 5, with ΔK = 21.488, was the most informative value. This suggests that the genetic structure of the population is best explained by the five assumed clusters (Figure 5).

Bayesian clustering analysis was performed in the STRUCTURE program to further investigate the genetic structure of *P. pruinosa* and *P. euphratica*. At K = 2, all six populations separated into two distinct genetic clusters, i.e., Pop 1, Pop 2, and Pop 3 corresponding to *P. pruinosa*, while Pop 4, Pop 5, and Pop 6 corresponding to *P. euphratica*.

Some changes appeared in the clusters at K = 3 and K = 4, mainly affecting Pop 4, while the remaining five populations remained stable in their cluster assignments. Although Pop 4, Pop 5, and Pop 6 belong to the same species, Pop 4 was collected from a geographically distant region compared to Pop 5 and Pop 6. However, despite the geographic distance between Pop 1, Pop 2, and Pop 3, no similar changes were observed, as evidenced by the clustering at all values of K. Still, Pop 2 showed slight differences that were consistent across clusters. Genetic differentiation became more pronounced with an optimal K = 5. Pop 1, Pop 2, and Pop 3 remained mostly homogeneous and were grouped in the same cluster (shown in blue), while Pop 5 and Pop 6 formed a separate cluster (shown in orange). Pop 4 displayed a mixed genetic composition with contributions from several clusters, reflecting its transitional position in the population structure and potential gene flow between groups (Figure 6.A). The «heat map» of population similarity, constructed on the basis of the population belonging to a particular cluster in the ClustVis program, also indicates some isolation of Pop4 from other populations of *P. euphratica* (Figure 6.B). We associate all this with both the geographic remoteness of population 4 from the main range and possible hybridization processes between *P. pruinosa* and *P. euphratica*.

The STRUCTURE findings reinforce the species-level split but also highlight fine-scale structuring within *P. euphratica*. The genetic admixture in Pop4 can be explained by its geographic remoteness from the main distribution, which increases differentiation, or by past introgression events. The stable clustering of *P. pruinosa* populations demonstrates their genetic cohesion despite environmental variation. Together, these results illustrate the combined influence of ecological adaptation, geography, and reproductive strategy on population structure.

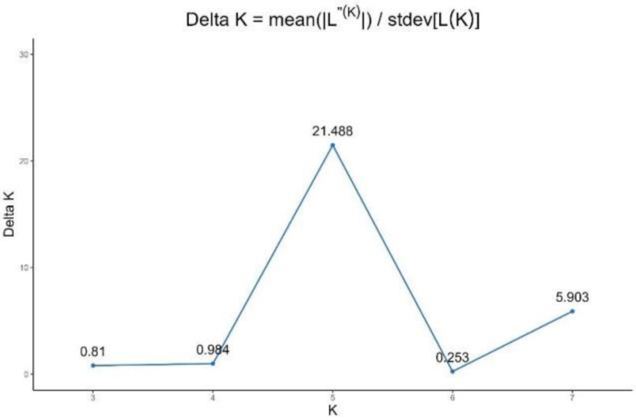

Figure 5. Determination of the optimal number of genetic clusters (K). The x-axis represents the number of clusters (K), and the y-axis shows the corresponding ΔK values

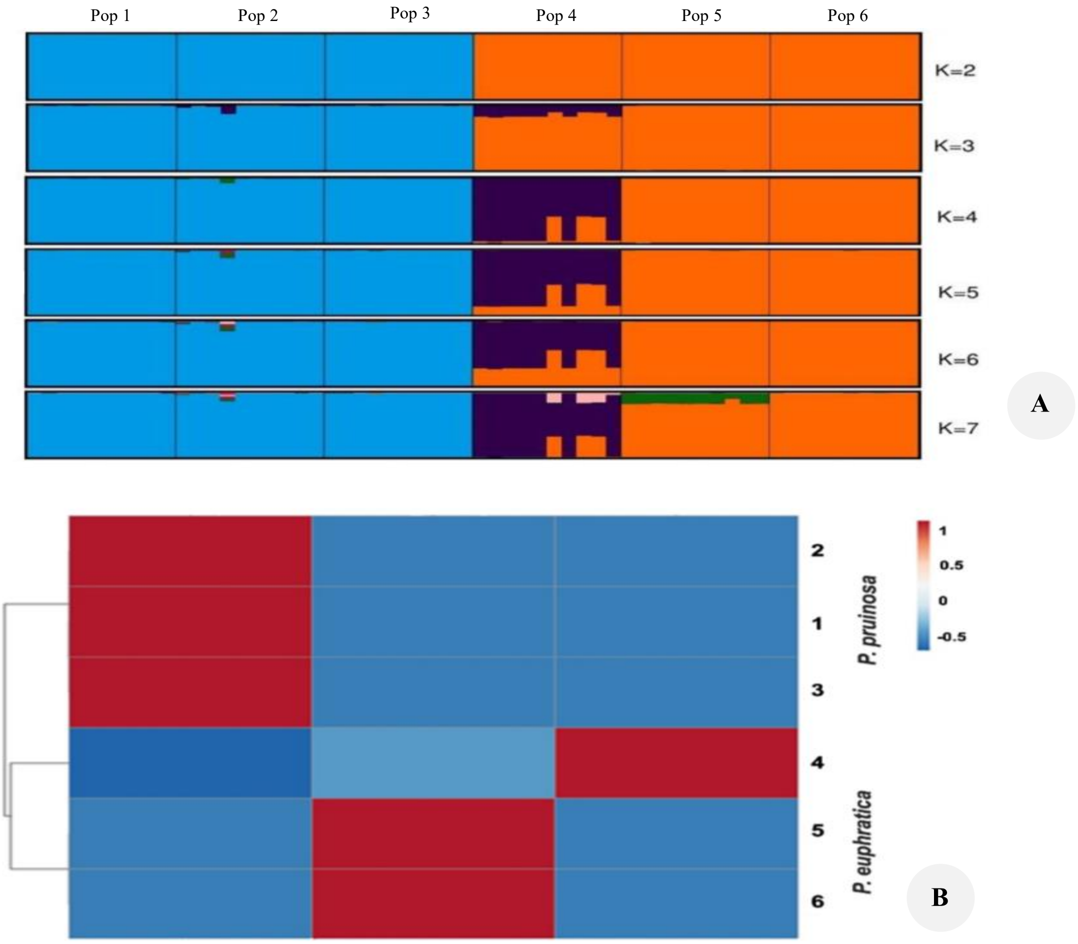

Figure 6. Genetic structure of the *Populus* populations based on: A. The STRUCTURE program, and B. "Heat map" of population similarity generated in the ClustVis program. The population numbers correspond to those listed in Table 1

Implications for conservation and ecological adaptation

The results collectively demonstrate that *P. pruinosa* maintains higher genetic diversity and stronger adaptive potential, making it particularly valuable for restoration in degraded desert ecosystems. By contrast, *P. euphratica* exhibits reduced diversity and strong structuring, which limit its resilience to climate change and habitat fragmentation. The conservation of both species is critical, given their listing in the Red Books (Baitulin and Sitpayeva 2014). The findings show that geographic isolation and ecological conditions are the main factors driving genetic divergence. Despite their close distribution, *P. pruinosa* and *P. euphratica* maintain distinct genetic structures, indicating limited hybridization. Among the two, *P. pruinosa* exhibited higher levels of genetic diversity, whereas *P. euphratica* showed stronger spatial structuring influenced by geographic distance. These results underline the adaptive potential of *P. pruinosa*, including tolerance to drought and salinity, which makes it especially valuable for restoration of degraded desert ecosystems. The study also provides a foundation for genetic passportization and targeted conservation planning.

In the Republic of Kazakhstan, a number of governmental and regional measures have been implemented to ensure the conservation and sustainable management of natural populations of *P. pruinosa* and *P. euphratica*, which represent key genetic and ecological components of riparian and desert ecosystems in the arid regions of Central Asia. *P. pruinosa* is listed under category 3 (R - Rare) in the Red Data Book of Kazakhstan (2014) and also included in the Catalogue of Rare and Endangered Plant Species of the Almaty Region (2023). *P. euphratica* is registered in the Catalogue of Rare and Endangered Plant Species of the Mangystau Region (2006), as well as in the Red Data Book: Wild-Growing Rare and Endangered Plant Species/State Cadastre of Plants of Zhambyl Region (2007).

The conservation of these species is supported by the Law of the Republic of Kazakhstan "On the Plant World" (2023) and the Law "On Amendments and Additions to Certain Legislative Acts of the Republic of Kazakhstan on Issues of the Plant World and Specially Protected Natural Areas*"* (2023). These legal frameworks aim to promote in situ and ex situ conservation, maintain the genetic diversity of native *Populus* species, and ensure the long-term stability of riparian and desert ecosystems in Kazakhstan. The findings show that geographic isolation and ecological conditions are the main factors driving genetic divergence between *P. pruinosa* and *P. euphratica*. Despite their close distribution, these two species maintain distinct genetic structures, indicating limited hybridization. Among the two, *P. pruinosa* exhibited higher levels of genetic diversity, whereas *P. euphratica* showed stronger spatial structuring influenced by geographic distance. These results underline the adaptive potential of *P. pruinosa*, including its tolerance to drought and salinity, which makes it especially valuable for the restoration of degraded desert and riparian habitats in arid regions.

In conclusion, the ISSR analysis revealed a high level of polymorphism across all primers, confirming their suitability for population genetic studies in the genus *Populus*. The allelic diversity and expected heterozygosity values demonstrated that *P. pruinosa* possesses greater genetic variability than *P. euphratica*, suggesting its stronger adaptive capacity to the arid and saline conditions of Kazakhstan's desert ecosystems. The PCA and STRUCTURE analyses consistently indicated clear species-level separation, with *P. pruinosa* populations forming a cohesive genetic cluster, while *P. euphratica* populations exhibited stronger genetic structuring and partial isolation influenced by geography and habitat heterogeneity. The moderate correlation between genetic and geographical distances supports the isolation-by-distance model, emphasizing the role of spatial separation and limited gene flow in shaping the observed genetic patterns.

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