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Research Article

Assessing genetic diversity and population structure in *Tulipa* species from Türkiye and Kazakhstan

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Abstract

Over the past two decades, genetic diversity within the *Tulipa* genus has been extensively studied using various morphological and molecular methods. However, the natural diversity of the *Tulipa* genus in Türkiye remains largely unexplored. The purpose of this study was to reveal the diversity of novel *Tulipa* species sampled from Türkiye and Kazakhstan, both of which have unique cultural histories and genetic diversity. In this study, iPBS (inter-primer binding site) markers were used for the first time in tulips grown naturally in Türkiye and Kazakhstan. The ability of iPBS markers to assess the genetic relationship between the preferred tulip varieties was revealed. According to the results of our study, it became clear that methods for determining iPBS markers can be easily used in studies of the genetic diversity of *Tulipa* species. To achieve the aforementioned aim, this study applied the iPBS method, which provides the theoretical novelty of this research. In addition, a total of 47 genotypes belonging to 14 *Tulipa* species that were selected based on their natural distribution in Türkiye and Kazakhstan were characterized morphologically and molecularly using 12 iPBS primers. The findings revealed significant variability in morphological traits among the *Tulipa* species. Notably, high variations were observed in flower size, leaf and stem characteristics, and bulb growth traits. Similarly, iPBS revealed high diversity with similarity indices ranging from 0.35 to 0.87 among genotypes. Principal component analysis (PCA) plots, both two- and three-dimensional, grouped the species into four distinct clusters, according to their origin. Structure analysis further confirmed the population structure, identifying four subpopulations. This study highlights the importance of species/genotypes with notable morphological and genetic traits within a highly variable population, providing insights into tulip breeding programs and utilization of natural genetic resources in sustainable agricultural production.

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Keywords: iPBS; ornamental plant; retrotransposon marker; *Tulipa*

Introduction

The genus *Tulipa* L. (Liliaceae) encompasses approximately 76 to 114 species worldwide (Christenhusz *et al*., 2013; Eker *et al*., 2016) and the centre of diversity for the *Tulipa* genus is Central Asia (Botschantzeva, 1982; Christenhusz *et al*., 2013; Eker *et al*., 2014). Most wild tulips are found in the mountainous regions of Central Asia (Sutula *et al*., 2024). Even some species extend as far as Mongolia (Kiran *et al*., 2016). The natural distribution of *Tulipa* species also spans Europe and North Africa, (Nikitina *et al*., 2021), In Central Asia, the genus *Tulipa* is represented by 63 wild species (Vvedensky and Kovalevskaja, 1971; Pechenitsyn *et al*., 2020), 37 of which are native to Kazakhstan and are distributed across the country, with 18 of them being endemic (Sutula *et al*., 2024). In Türkiye, the genus *Tulipa* is represented by 17 species, six of which are endemic (TarikahyaHacıoğlu and Eker, 2024).

In studies conducted so far, inter simple sequence repeat (ISSR) markers have been utilized to study *Tulipa* species from Iran, offering insights into their genetic diversity (Kiani *et al*., 2012). Earlier, Fay *et al*. (2006) explored interfamilial relationships within the order Liliales, including *Tulipa*, by analysing five DNA regions. Building on these efforts, Peterson *et al*. (2008), assessed both plastid and nuclear DNA regions to resolve phylogenetic relationships within GageaSalisb. (Liliaceae), a genus closely related to *Tulipa*.

Turktas *et al*. (2012) focused on the phylogenetic relationships within *Fritillaria* spp. (Liliaceae) by sequencing the trnL–trnF region. Around the same time, Clennett *et al*. (2012) examined two plastid loci and one nuclear locus to determine the phylogenetic systematics of *Erythronium* (Liliaceae), a sister lineage to *Tulipa*. These studies underscored the utility of trnL–trnF and ITS regions in understanding phylogenetic relationships within Liliaceae.

Turktas *et al*. (2013) specifically targeted the phylogenetic relationships of tulips in Türkiye by analyzing 11 species using the plastid trnL–trnF region and nuclear ITS regions. Their work expanded the understanding of the phylogenetic utility of these genomic regions for the *Tulipa* genus. Some studies have used molecular markers such as random amplified polymorphic DNA (RAPD), ISSR, and single nucleotide polymorphisms (SNPs) to assess genetic variation in tulips (Qi-fu *et al*., 2008). Furthermore, Tulips has been the focus of studies utilizing AFLP (Asgari *et al*., 2020), ISSR (Kiani *et al*., 2012), and SNP markers (Tang *et al*., 2013). Also, Christenhusz *et al*. (2013) investigated the phylogenetic relationships within the *Tulipa* genus using DNA sequences from five plastid regions (the trnL intron, trnL–trnF intergenic spacer, rpl16 intron, rps12–rpl20 intergenic spacer, and matK) as well as the nuclear ribosomal DNA internal transcribed spacer (ITS) region. Their analysis included 25 *Tulipa* taxa, 8 of which are distributed in Türkiye. Recent research by Pourkhaloee *et al*. (2018) examined the genetic diversity and population structure of wild and cultivated tulips from Iran and the Netherlands using EST–SSR markers. Similarly, Kutlunina *et al*. (2013) conducted AFLP analysis on four *Tulipa* taxa in Russia, revealing a low level of variability among *T. biebersteiniana*, *T. patens*, *T. scytica*, and *T. riparia* across 81 individuals from 13 populations. In a more recent study, Asgari *et al*. (2020) employed AFLP data to analyse the biodiversity and taxonomic relationships of 47 wild accessions representing nine *Tulipa* species from Iran. Their findings indicated a high level of genetic diversity within the genus. Continuing this effort, Tarikahya-Hacıoğlu and Eker (2024) assessed the genetic variation within 57 accessions from 19 taxa native to Türkiye using ISSR markers.

In light of previous studies, the present research aims to assess genetic diversity in tulips by employing a novel retrotransposon-based molecular marker method, iPBS (inter-primer binding sites) (Kalendar *et al*, 2011), which has not been previously used for this genus, but overall proved its usefulness during the last decade (Kalendar, 2014). Specifically, this study evaluates 47 tulip genotypes from 14 species sampled in Türkiye and Kazakhstan, with the goal of detailing the genetic diversity and comparing the genetic characteristics with those

of cultivated varieties to provide a comprehensive analysis of the gene pool. This research supports the future conservation activities for these species in their natural habitats.

Materials and Methods

Flow chart

The tulip genotypes were sampled from different regions of Türkiye and Kazakhstan. Morphological measurements and observations were conducted during the flowering period. DNA isolation for molecular studies was carried out from young leaves. The graphical abstract of the study is presented in Figure 1.

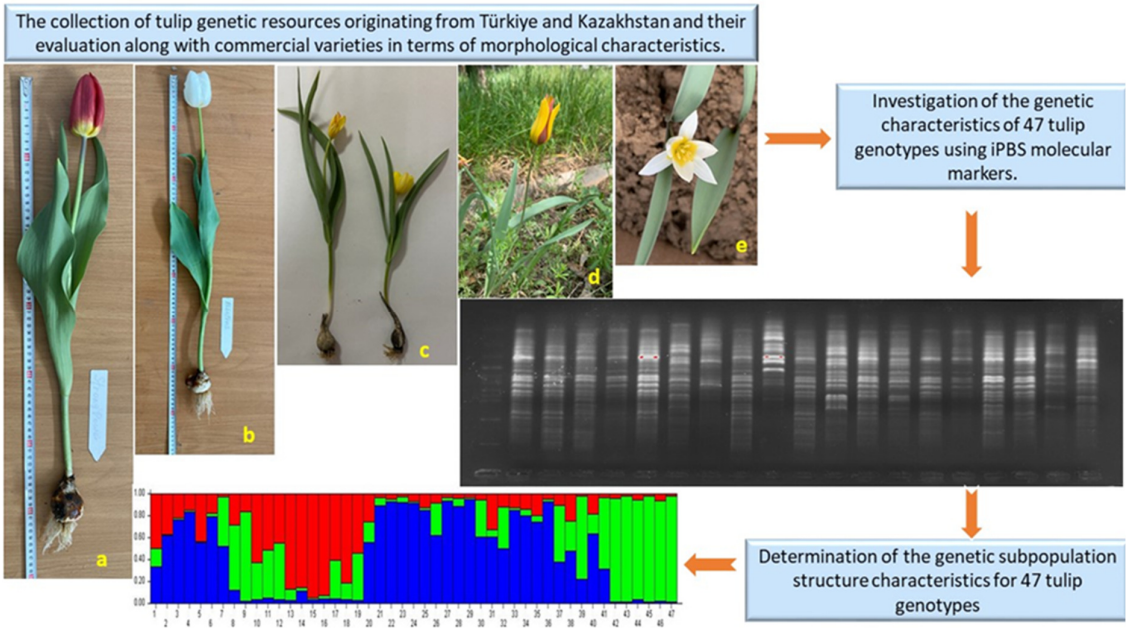


Figure 1. Graphical abstract for assessing genetic diversity and population structure in *Tulipa* genotypes (a: Commercial tulip cv. Strong Power; b: Commercial tulip cv. Albatros; c: *Tulipa lemmersii*; d: *Tulipa tetraphylla*; e: *Tulipa orthopoda*)

Plant materials

Measurement and analysis procedures were conducted in Kazakhstan and Türkiye between 2022 and 2023. A population comprising 47 accessions was established, including 41 genotypes from 14 species that are naturally distributed in Türkiye and Kazakhstan, along with five commercial varieties (Table 1). Genotypes sampled from Türkiye were recorded by on-site examinations and measurements. The genotypes of the commercial varieties were cultivated in a heated greenhouse in Kazakhstan, whereas the naturally occurring genotypes were identified, and their morphological characteristics were documented through in situ field studies. The germination of tulips under protected cultivation conditions was conducted in the greenhouse of the Botanical Garden at Khoja Akhmet Yassawi International Kazakh-Turkish University, located in Turkestan city. During the growing season, five exclusive varieties of tulips were selected for research that met high consumer requirements for decorative, economic, and biological indicators (height, shape, and bud, flowering period, adapted to the climate), depending on the degree of their decorative effect on the conditions of exposure to qualitative indicators of tulips.

Table 1. Data of the locations where tulip genotypes were sampled

Geno-type no	Species name	Origin	Genotype no	Species name	Origin
1	*T. saxatilis*	Türkiye/Muğla	**25**	*T.agenensis*	Türkiye/Aydın
2	*T. armena var. armena*	Türkiye/Ankara	**26**	*T. sintenesii*	Türkiye/Muş
3	*T. armena var. armena*	Türkiye/Ankara	**27**	*T. sintenesii*	Türkiye/Erzurum
4	*T. armena var. armena*	Türkiye/Ankara	**28**	*T. sintenesii*	Türkiye/Ağrı
5	*T. armena var. armena*	Türkiye/Karaman	**29**	*T. sintenesii*	Türkiye/K.Maraş
6	*T. armena var. armena*	Türkiye/Tokat	**30**	*T.aleppensis*	Türkiye/Adıyaman
7	*T. armena var. armena*	Türkiye/Bayburt	**31**	*T.aleppensis*	Türkiye/Şanlıurfa
8	*T. armena var. armena*	Türkiye/Erzurum	**32**	*T.armena* Boiss. var. *lycica*	Türkiye/Antalya
9	*T. orphanidea*	Türkiye/İzmir	**33**	*T.armena* Boiss. var. *lycica*	Türkiye/Amasya
10	*T. orphanidea*	Türkiye/Aydın	**34**	*T.armena* Boiss. var. *lycica*	Türkiye/Karaman
11	*T. orphanidea*	Türkiye/Muğla	**35**	*T.armena* Boiss. var. *lycica*	Türkiye/Adıyaman
12	*T. orphanidea*	Türkiye/Muğla	**36**	*T. undulatifolia*	Türkiye/Çanakkale
13	*T. orphanidea*	Türkiye/Muğla	**37**	Strong fover	Commercial cv.
14	*T. orphanidea*	Türkiye/Muğla	**38**	Strong fire	Commercial cv.
15	*T. orphanidea*	Türkiye/İzmir	**39**	Albatros	Commercial cv.
16	*T. orphanidea*	Türkiye/Antalya	**40**	Dynasty	Commercial cv.
17	*T. orphanidea*	Türkiye/Antalya	**41**	Behmina	Commercial cv.
18	*T. julia*	Türkiye/Erzurum	**42**	Darwisnow	Commercial cv.
19	*T. julia*	Türkiye/Erzincan	**43**	*T.orthopoda chegmi*	Kazakhstan
20	*T. julia*	Türkiye/Bayburt	**44**	*T.orthopoda* 1WT	Kazakhstan
21	*T. julia*	Türkiye/Kars	**45**	*T. tetrafhylla*	Kazakhstan
22	*T. julia*	Türkiye/Hakkari	**46**	*T. lemmersii*	Kazakhstan
23	*T.agenensis*	Türkiye/Mersin	**47**	*T. ontario*	Kazakhstan
24	*T.agenensis*	Türkiye/Hatay			

Morphological characterization analysis

In this study, 47 tulip genotypes from 14 distinct species were evaluated for various phenotypic characteristics including plant height (cm), flower stalk length (cm), flower diameter (cm), flower length (cm), leaf number, leaf length (cm), leaf width (cm), and bulb circumference (cm). The experiments were conducted according to the parameters set by the tulips UPOV (International Union for the Protection of New Varieties of Plants, UK). Morphological measurements were performed in 3 replicates, with 3 plants in each replicate.

Molecular characterization analysis

Genomic DNA was extracted from young leaves of *Tulipa* species using a modified CTAB protocol (Doyle and Doyle, 1987), with purification procedures as described by Dalda-Sekerci (2023). The extracted DNA was then subjected to PCR amplification using 12 iPBS primers. The PCR mixture contained 1.5 µL of Taq buffer, 0.33 µL of 2.5 mM dNTPs, 1 µL (5 pM) of each iPBS primer, 0.2 µL of Taq DNA polymerase, and 2.0 µL (20 ng) of template DNA in a total volume of 15 µL. Amplified products were separated on a 1.5%

agarose gel using TBE (Tris-Boric acid-EDTA) buffer at 110 V for four hours. After staining with ethidium bromide, the gel was visualized under UV light, and the results were documented using a gel documentation system (Bio-Rad, GelDoc Go Imaging System, USA). DNA fragments were recorded as a binary matrix (1 for presence and 0 for absence of a band). Cluster analysis of the 47 tulip genotypes was performed using Dice's similarity coefficient (Dice, 1945) and the unweighted pair-group method with arithmetic average (UPGMA) SAHN clustering algorithm. Analyses were carried out with the NTSYS-pc software (Numerical Taxonomy Multivariate Analysis System, NTSYS, 2.11, USA). Key metrics such as total number of fragments, number of polymorphic fragments, mean polymorphism, allele frequency, effective number of alleles, Shannon's information index, expected heterozygosity, and the ratio of unbiased expected heterozygosity were assessed. A Mantel test was conducted to compare Dice and Jaccard similarity matrices. Principal Component Analysis (PCA) was performed based on the variance-covariance matrix. The STRUCTURE program (version 2.3.4) was utilized to analyse population structure, with K values ranging from 1 to 10. Each analysis was conducted 5 times with a burn-in length of 100,000 iterations.

Results

Morphological results

In the present study, 47 tulip genotypes representing 14 distinct species and five commercial varieties were evaluated for a range of phenotypic characteristics. The genotypes exhibited a significant morphological diversity. Analysis of plant height revealed that commercial varieties generally exhibited greater heights than naturally occurring species, with the 'Darwisnow' variety reaching 50.23 cm. Among the naturally occurring species, *T. undulatifolia* from Türkiye (genotype 36, 36.9 cm) and *T. armena* (genotypes 2-8, approximately 33 cm) had higher plant heights. In contrast, the Kazakhstani species *T. tetraphylla* (genotype 45, 11.16 cm) had the lowest plant height. The length of the flower stem is a crucial parameter for both species identification and the evaluation of cut flowers. In the current population, there was substantial variation in the flower stem length, ranging from 2.23 cm to 48 cm. Similar to plant height, commercial varieties exhibited notably longer flower stems, whereas naturally occurring tulip species generally had shorter stems. However, the genotypes of *T. undulatifolia* and *T. argenensis* were found to have flower stem lengths of approximately 26 cm (Table 2).

Regarding flower size, measurements of flower width and length indicate that naturally occurring species show promising traits. Notably, *T. orphanidea* genotypes exhibited flower sizes surpassing those of commercial varieties, with flower diameters of approximately 6 cm and lengths of approximately 7 cm. In terms of petal count, *T. armena* genotypes generally possessed four to five petals, whereas *T. orthopoda* genotypes typically had two petals.

Leaf size analysis revealed that *T. aleppensis* had the longest leaves, measuring approximately 22 cm, whereas *T. tetraphylla* exhibited the shortest leaves, at 6.56 cm. Additionally, there was considerable variation in bulb circumference among the genotypes, ranging from 4.1 cm to 11.46 cm (Table 2).

Table 2. Morphological characteristics measured in 47 tulip genotypes

Genotype code	Plant height (cm)	Flower stalk length (cm)	Flower diameter (cm)	Flower length (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Bulb circumference (cm)
1	22.33gh	16.43l	4.5h-n	5.26ıj	2.63e-g	19.23f-k	3.5e-k	5.3j-q
2	33.10c-f	26.46e-h	5.76a-e	6.5d-h	4.01a-e	14.98m-s	3.35g-k	7.5b-e
3	31.46d-f	26.08e-g	5.6b-f	6.58c-h	3c-g	13.50p-t	3.15h-k	7.7bc
4	30.06ef	24.5e-ı	5.4c-f	6.41d-h	4.01a-e	15.03m-s	3.37g-k	7.5b-e
5	32.26c-f	26.33e-h	6.03a-c	6.46d-h	4.66ab	14.4o-t	3.41f-k	7.5b-e

6	32.7c-f	25.56e-ı	5.9a-e	6.33d-h	5.1a	15.96m-r	3.45e-k	6.8c-h
7	32.03d-f	27.07d-f	5.3c-h	6.73c-h	4.01a-e	13.84p-t	3.55d-k	7.4b-e
8	33.36c-e	28.13b-e	5.68a-f	6.93c-h	5.1a	14.84m-s	3.46e-k	7.2b-f
9	30.6ef	23.51f-ı	5.73a-e	7.06c-f	3.76a-e	19.28f-k	3.64c-k	4.96m-q
10	28.6f	23.93e-ı	6.35ab	6.78c-h	3.5b-f	17.29ı-n	3.11ı-k	5.3j-q
11	29.53ef	23.91e-ı	5.46b-g	6.93c-h	3.53b-f	18.06h-l	3.5e-k	4.93n-q
12	30ef	24.33e-ı	5.5b-g	7.06c-f	3.76a-e	18h-l	3.7c-k	5.5ı-f
13	30.56ef	23.73e-ı	5.7a-f	7.06c-f	3.93a-e	19g-l	3.73c-j	4.1q
14	30.8ef	24.06e-ı	5.9a-e	7.2c-e	3.93a-e	19.53e-j	3.74c-j	4.5o-q
15	31.10ef	24.4e-ı	6a-d	7.28c-e	3.8a-e	20b-ı	3.86c-h	4.33pq
16	29.66ef	23.3f-ı	6.33ab	7.06c-f	4.01a-e	20.73c-h	3.67c-k	4.86n-q
17	34.06c-e	21.71ı-k	6.53a	7.08c-f	4.03a-d	22.41cd	3.82c-ı	5.2k-q
18	21.4gh	17.81j-l	5.10d-ı	6.83c-h	3.06c-g	18.11h-l	3.04j-l	7.5b-e
19	22.6g	18.2j-l	5.26c-h	6.73c-h	3.6b-f	21.8c-g	4.13c-f	8.23b
20	20.73gh	16.45l	5.63a-f	6.96c-g	3.5b-f	22.45cd	3.48e-k	7.16b-f
21	21.56gh	17.43kl	4.81f-l	6.46d-h	2.93d-g	17.59ı-n	2.98k-l	7.76b-d
22	21.53gh	17.73j-l	5.33c-h	7.2c-e	3.33b-g	17.61ı-n	3.12h-k	7.1b-g
23	32.6c-f	26.44e-h	1.63u	7.2c-e	3.66a-f	18.3h-l	3.18h-k	6.5c-j
24	32.7c-f	25.98e-ı	1.53u	6.8c-h	3.66a-f	18.2h-l	3.14h-k	6.4d-k
25	32.63c-f	26.53e-h	1.65u	6.8c-h	4.03a-d	18.38h-l	3.34g-k	6.8c-h
26	19.61gh	15.66l	4.1k-p	4.6j	4.01a-e	13.17r-t	2.27mn	5.26j-q
27	19.53gh	15.41l	3.95l-p	4.3j	3.93a-e	12.89st	2.09n	5m-q
28	19.6gh	15.50l	4.01l-p	4.43j	3.96a-e	13.24r-t	2.17n	5.2k-q
29	19.86gh	15.85l	4.2ı-o	4.41j	4.03a-d	13.35r-t	2.35l-n	5.5ı-f
30	30.6ef	23.8e-ı	4.63g-l	7.2c-e	4.06a-d	20.66c-h	4.26cd	5.96fn
31	32.48c-f	23.03g-ı	4.33ı-n	7.16c-e	3.7a-f	22.14c-e	4.19c-e	5.86g-n
32	29.61ef	23.95e-ı	5.75a-e	5.96gı	4.01a-e	16.4l-p	3.16h-k	5.6h-p
33	29.56ef	23.91e-ı	5.46b-g	6.1f-ı	3.93a-e	16.33l-p	3.04j-l	5.4ı-f
34	29.66ef	24.1e-ı	5.86a-e	5.93hı	4.06a-d	16.6k-p	3.22h-k	5.7h-o
35	29.81ef	24.26e-ı	6.06a-c	6.26e-ı	5.03a	16.2l-p	3.36g-k	5.6h-p
36	36.9bc	31.41cd	5.03e-j	7.16c-e	3.06c-g	22.23c-e	3.64c-k	5.5ı-f
37	35.93b-d	28.06be	3.6n-r	7.53bc	2.33fg	32.5a	3.46e-k	11.46a
38	53.5a	46.93a	5e-k	7.33cd	4.01a-e	16.83j-o	3.96c-g	11.03a
39	54.5a	48a	4.26ı-o	6.6c-h	4.01a-e	23.26c	5.23b	10.63a
40	39.56b	33.86bc	3.76m-p	8.4ab	3.66a-f	22c-f	6.13a	10.6a
41	14.16ıj	4.43m	4.16j-p	4.56j	4.33a-c	15.03m-s	2no	6.26e-m
42	50.23a	37.83b	3.36o-r	8.83a	2g	28d	4.36c	11.33a
43	17.66hı	2.23	3.23p-s	2.23k	2.33fg	12t	1.7m-p	4.86n-q
44	17.83hı	2.83m	3.26p-r	2.26k	2g	11.76t	1.2p	5.63h-o
45	11.16j	3m	2.2t-u	3.13k	4.33a-c	6.56u	1.3op	5.83g-n
46	13.23ıj	3.53m	2.7r-t	2.96k	5.1a	15.83m-r	1.2p	6.73c-ı
47	29.9ef	22.1h-j	2.36s-u	4.76j	3c-g	31.26a	5.56ab	6.3e-l
	<.0001	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**

Molecular data results

In this study, 12 primers that successfully generated amplifications were utilized to assess the genetic diversity of 47 tulip genotypes across 14 species. The use of these 12 iPBS primers resulted in 150 distinct DNA band profiles, with 148 identified as polymorphic and 2 as monomorphic. The overall polymorphism rate was determined to be 98.6%. The band sizes produced by the iPBS primers ranged from 125 to 1250 base pairs.

Specifically, primer iPBS-2379 yielded the highest number of bands, whereas primers iPBS-2219 and iPBS-2277 produced the fewest, with 19 and 7 bands, respectively (Table 3).

The expected and observed allele frequencies (p, q) derived from the iPBS primers varied between 0.185 and 0.489, and 0.511 and 0.815, respectively. The number of effective alleles (Ne) ranged from 1.502 for primer iPBS-2219 to 1.806 for primer iPBS-2239. The Shannon's information index (I) values ranged from 0.423 to 0.626. The expected heterozygosity (He) values varied from 0.269 to 0.437, while the unbiased expected heterozygosity (uHe) values ranged from 0.272 to 0.440 (Table 3).

Table 3. Polymorphism values of studied iPBS primers

Primers	Sequence 5'-3'	Bp	TNF	NPF	MP %	p	q	Ne	I	He	uHe
iPBS-2230	TCTAGGCGTC TGATACCA	250-1180	14	13	92.86	0.406	0.594	1.676	0.557	0.383	0.387
iPBS-2379	TCCAGAGATC CA	175-1200	19	19	100	0.406	0.594	1.779	0.615	0.427	0.432
iPBS-2075	CTCATGATGC CA	150-1250	17	16	94.12	0.434	0.566	1.718	0.572	0.395	0.400
iPBS-2077	CTCACGATGC CA	125-900	11	11	100	0.465	0.535	1.790	0.625	0.435	0.440
iPBS-2393	TACGGTACGC CA	150-1175	17	17	100	0.347	0.653	1.685	0.562	0.385	0.389
iPBS-2243	AGTCAGGCTC TGTTACCA	350-1150	13	13	100	0.185	0.815	1.425	0.423	0.269	0.272
iPBS-2231	ACTTGGATGC TGATACCA	200-1175	11	11	100	0.262	0.738	1.608	0.547	0.365	0.369
iPBS-2272	GGCTCAGATG CCA	125-1000	13	13	100	0.364	0.636	1.777	0.607	0.422	0.426
iPBS-2239	ACCTAGGCTC GGATGCCA	125-1175	13	13	100	0.393	0.607	1.806	0.626	0.437	0.442
iPBS-2219	GAACTTATGC CGATACCA	300-1175	7	7	100	0.269	0.731	1.502	0.442	0.290	0.293
iPBS-2277	GGCGATGATA CCA	150-1000	7	7	100	0.489	0.511	1.786	0.591	0.413	0.418
iPBS-2251	GAACAGGCGA TGATACCA	150-1175	8	7	100	0.331	0.669	1.725	0.599	0.411	0.416
Total	**-**	**125-1250**	**150**	**148**		**-**	**-**	**-**	**-**	**-**	**-**

*TNF: Total Number of Fragments, NPF; Number of Polymorphic Fragments, MP: Mean Polymorphism, p, and q: Allele Frequency, Ne: Number of Effective Alleles, I: Shannon's Information Index, He: Expected Heterozygosity and uHe: Unbiased Expected Heterozygosity

Cluster analysis was performed using the UPGMA method with the Dice similarity index based on data obtained from 12 iPBS primers applied to 47 tulip genotypes. The resulting dendrogram is shown in Figure 2. According to the Dice similarity matrix, the genetic similarity among the 47 tulip genotypes, representing 14 different species, ranged from 0.35 to 0.87 (Figure 2).

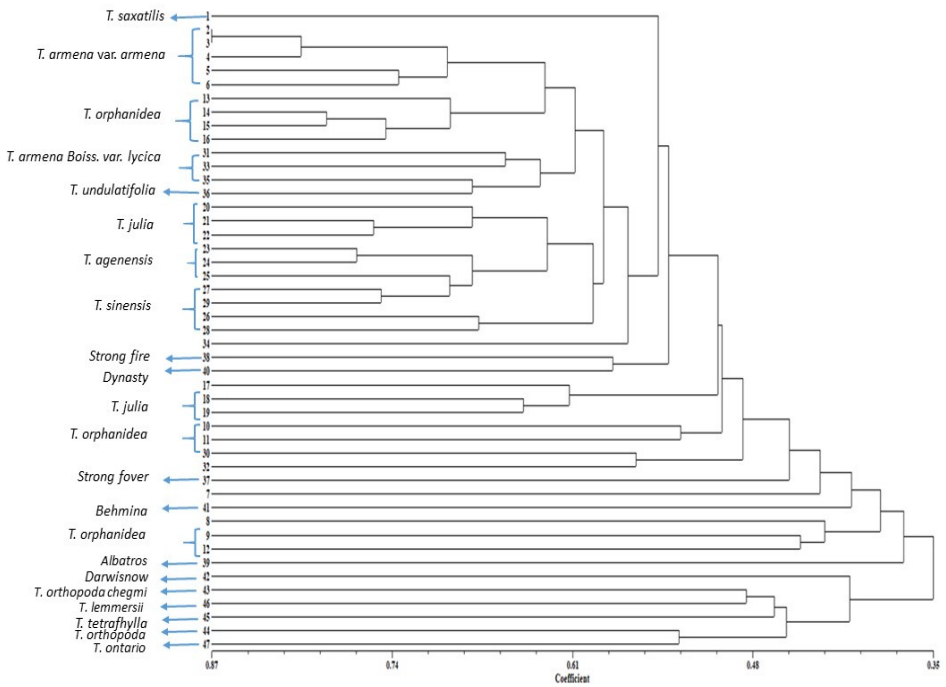


Figure 2. The UPGMA analysis based on Dice coefficients of iPBS markers from the 47 tulip genotypes

The dendrogram revealed that the genotypes sampled from Türkiye and Kazakhstan exhibited high genetic diversity. It is evident from the dendrogram that genotypes from Kazakhstan and Türkiye are positioned in distinct branches, whereas genotypes of the same species are clustered together. The highest similarity was observed among the genotypes of *T. orphanidea*, with these genotypes sharing 87% genetic similarity. This suggests that *T. orphanidea* genotypes are relatively close to commercial varieties. However, even among genotypes of the same species, there were notable differences in genetic characteristics, with intra-species genetic similarity varying between 48% and 74%, indicating the richness of the genetic pool (Figure 2).

Additionally, the distinct clustering of genotypes from *T. saxatilis* (Türkiye), *T. lemmersi* (Kazakhstan), *T. orthopoda* (Kazakhstan), *T. tetraphylla* (Kazakhstan), and *T. ontario* (Kazakhstan), with low similarity (approximately 50%) to other genotypes, highlights a high level of genetic variation. This emphasizes the importance of incorporating natural species into breeding programs and identifying new genetic traits (Figure 2).

DNA matrix data were subjected to correlation analysis using the Mantel test in the NTSYS software, incorporating primers and other similarity matrices. A significant mantel correlation was also observed (r = 0.92177). Principal Component Analysis (PCA) was employed to create both two-dimensional and three-dimensional visualizations in NTSYS. The cumulative sum of the first three eigenvalues in PCA accounted for 90% of the total variation. The two-dimensional PCA plot revealed that most genotypes were dispersed, rather than forming distinct clusters. However, genotypes with similar physical traits and those belonging to the same species exhibited close genetic proximity (Figure 3).

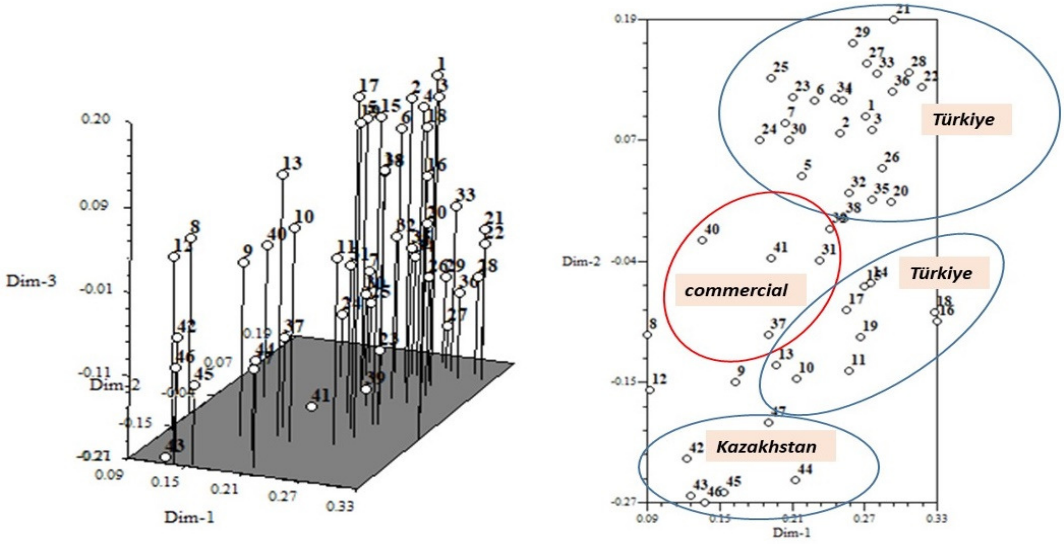
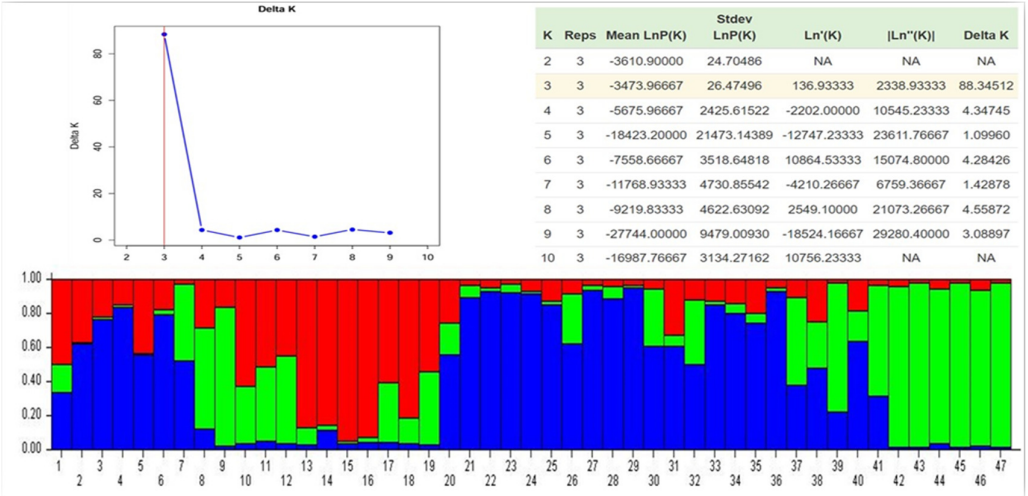


Figure 3. Two and three-dimensional graphs generated by principal component analysis with iPBS data on 47 tulip genotypes

The STRUCTURE program analyses the structure of the population, dividing the individuals into subpopulations, and creating a Q matrix. The number of subpopulations (K) was between 1 and 10, with 10 independent calculations performed for each K value. Subsequently, the subpopulations were identified using the Structure Selector program. Based on the K value obtained from iPBS data, the tulip genotypes consisted of four subpopulations (Figure 5). Analysis using the STRUCTURE program revealed the membership coefficients of the tulip genotypes for each population. Individuals with a membership coefficient of 0.80 or higher are considered pure and are a significant resource for breeding programs planned for hybridization. Those with a membership coefficient of 0.79 or lower, were considered hybrids. The membership status of tulip genotypes in the subpopulations is graphically presented in Figure 4. Accordingly, 25 genotypes were found to have high purity.



K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
2	3	-3610.90000	24.70486	NA	NA	NA
3	3	-3473.96667	26.47496	136.93333	2338.93333	88.34512
4	3	-5675.96667	2425.61522	-2202.00000	10545.23333	4.34745
5	3	-18423.20000	21473.14389	-12747.23333	23611.76667	1.09960
6	3	-7558.66667	3518.64818	10864.53333	15074.80000	4.28426
7	3	-11768.93333	4730.85542	-4210.26667	6759.36667	1.42878
8	3	-9219.83333	4622.63092	2549.10000	21073.26667	4.55872
9	3	-27744.00000	9479.00930	-18524.16667	29280.40000	3.08897
10	3	-16987.76667	3134.27162	10756.23333	NA	NA

Figure 4. Graphical representation of subpopulation membership coefficients obtained from STRUCTURE analysis with iPBS data in 47 tulip genotypes

Discussion

The findings of this study reveal significant variation across several characteristics of tulip genotypes, including plant height, leaf morphology, flower size and bulb dimensions. This study observed a high degree of variation, consistent with previous research, which has also documented considerable diversity in plant traits among *Tulipa* species.

Numerous studies have been conducted to determine the taxonomic characteristics of *Tulipa* species from various regions. According to Khaleghi *et al*. (2018), six species of wild tulips were identified in Markazi Province and two areas of Isfahan Province, Iran, based on traits related to bulbs, flowers, and leaves. Additionally, the Flora Iranica reports 34 *Tulipa* species, with 18 occurring in Iran, including three endemic species, and eight species recorded from the Khorasan provinces (Kiani *et al*., 2012). In a related study, Kiani *et al*. (2012) collected 39 *Tulipa* accessions from the Khorasan and Yazd provinces in Iran and classified them into seven species. Among these, *T. stapfii* and *T. montana* var. *chrysantha* were noted for their broad distribution and high adaptability to varying environmental conditions, including extremes of temperature and drought. Based on geographical distribution, *T. edulis* (Miq.) Baker. has been identified as the most widely distributed species with significant adaptability across China (Xing *et al*., 2017). This species demonstrates considerable versatility in various environmental conditions, further highlighting the diverse adaptive strategies within the *Tulipa* genus.

In tulip breeding, the introgression of desirable traits such as unique flower colors, varied flower shapes, short forcing ability, vigorous growth, and disease resistance into cultivars is of considerable value (Khaleghi *et al*., 2018). For ornamental plants, flower colour is a critical quality determinant that impacts both the aesthetic appeal and commercial value of the plant. Consequently, enhancing flower color has long been a major objective for breeders (Zhao and Tao, 2015). Wild tulips exhibit a broad spectrum of flower colours, including white, red, orange, pink, purple, and yellow, which are useful for breeding programs. These colors are primarily due to specific pigments, namely anthocyanin and carotenoids (Shoji *et al*., 2007). Petal traits, including color, size, and shape, have been integral to tulip taxonomy. However, tepal color can be highly variable, even within populations of a single species (Xing *et al*., 2017). For example, T. *montana* var. *chrysantha* displays a range of petal colours from yellow to orange, while *T. humilis* shows variability from white to deep purple. This variation highlights the genetic diversity within tulip species and underscores the potential for incorporating these traits into breeding programs to achieve desired ornamental qualities (Xing *et al*., 2017).

In addition to flower colour, flower size is also a significant focus in tulip breeding. A study conducted on 208 accessions from six different *Tulipa* species identified 44 distinct morphological traits. This research highlighted considerable variation in flower size across the six species. Such variability in flower dimensions underscores the importance of selecting for size as a key trait in breeding programs aimed at enhancing ornamental qualities (Khaleghi *et al*., 2018). In the same study, the largest flower size was observed in *T. systola*, which also had the largest bulb size. The second largest bulb was found in *T. stapfii*, with a diameter ranging from 16.47 to 44.10 mm. Notably, within each species, accessions with the largest bulbs also exhibited the largest flower sizes.

Given the extended juvenile period and slow propagation rate of tulips, significant emphasis is placed on pre-selection. One of the primary objectives of pre-selection is to optimize bulb production, which can be assessed by measuring the annual increase in main bulb diameter and the number of bulbs (Van Eijk *et al*., 1983).

Further research on the growth of tulip plants, using bulbs of varying sizes, has demonstrated that leaf area, shoot dry weight, and new bulb weight are positively correlated with the size of the bulb. It has been reported that bulbous flower cultivars with larger bulbs tend to exhibit more vigorous growth and achieve higher yields (Akand *et al*., 2016; Khaleghi *et al*., 2018).

Flower stem length is another important trait in tulip breeding, particularly for ornamental purposes. In one study, the stem lengths of genotypes from six different *Tulipa* species ranged from 24.30 to 39.80 cm, highlighting the variability in this characteristic (Khaleghi *et al*., 2018). Another study, referencing the Flora of Iran, reported that *T. biebersteiniana* typically has a stem length of 15–30 cm and usually possesses two leaves. However, the present study found that accessions of *T. biebersteiniana* exhibited stem lengths exceeding 30 cm, with all accessions having five leaves. This discrepancy may be attributed to varying environmental conditions. Additionally, it has been noted that *T. biebersteiniana* can readily undergo transformation to a triploid level under conditions that are unfavourable for sexual reproduction (Kutlunina *et al*., 2013). This adaptability may contribute to the observed variations in stem length and leaf number.

The establishment of a breeding program aimed at specific traits requires precise parent selection, thorough characterization of morphological attributes, assessment of genetic distances between individuals through molecular profiling, and an in-depth understanding of trait inheritance. Analysing genetic distances is essential for identifying genetic similarities and variations within the population, which informs the selection of optimal parent combinations for breeding. Additionally, comprehending the inheritance patterns of target traits is crucial for facilitating their efficient transmission to the next generations.

The aim of the present study was to assess genetic diversity using iPBS molecular markers. Genetic similarity among *Tulipa* species was found to range between 0.35 and 0.87. The results of this investigation were in line with those of other investigations. Using various molecular markers, the genetic diversity of *Tulipa* L. was examined.

In a study by Tarikahya-Hacıoğlu and Eker (2024), the genetic variation within the genus *Tulipa* was explored by examining 57 accessions from 19 taxa native to Türkiye. Using ISSR markers, 76 polymorphic bands were obtained. UPGMA clustering analysis revealed two major groups. The first group consisted of *T. sylvestris* subsp. a*ustralis, T. sprengeri, T. humilis, T. koyuncui, T. undulatifolia* var. *undulatifolia*, and *T. juliaaccessions*, with most of these taxa belonging to the subgenus *Eriostemones*, except *T. undulatifolia* var. *undulatifoliaand*, *T. julia*. In the second main group, taxa from both the subgenus *Tulipa* and *Eriostemones* were clustered according to their subgenera, reflecting their phylogenetic relationships. According to the findings of this study, *T. saxatilis* and *T. pulchella* were identified as sister species. It has been noted that *T. saxatilis* is a species growing at lower altitudes among Turkish tulips and is characterized by early flowering. Researchers have stated that although *T. saxatilis* is morphologically similar to *T. humilis* and *T. pulchella*, it is a distinctly different species due to its broad, glossy leaves and large petals (Eker *et al*., 2014). The results of our study corroborate these findings, as *T. saxatilis* was positioned in a distinct branch on the dendrogram. In previous studies, this species has been analysed in detail. For instance, in the study by Eker *et al*. (2014), *T. saxatilis* was associated with *T. cinnabarina* subsp. *cinnabarina*, *T. orphanidea*, *T. humilis*, and *T. pulchella*, which were grouped into two related clusters. Similarly, in the study by Eker and Tanış (2022), *T. saxatilis* was clustered with *T. humilis* and was linked to a group comprising *T. cinnabarina, T. orphanidea*, and *T. pulchella*. Moreover, *T. saxatilis*, along with *T. sylvestris*, *T. orphanidea, T. bifora,* and *T. humilis*, was placed in the same cluster in a study conducted by Turktas *et al*. (2013). In our study, however, *T. saxatilis* was clustered with *T. orphanidea*, *T. julia*, *T. agenensis*, and *T. sinensis* (Figure 3), further supporting the complex phylogenetic relationships within the genus *Tulipa*.

Asgari *et al*. (2020) conducted an AFLP-based analysis to evaluate the biodiversity and taxonomic relationships of 47 wild accessions from nine *Tulipa* species in Iran. The analysis uncovered substantial genetic diversity within the genus. A total of 342 fragments were generated using 12 AFLP primer sets, with 88.1% (304 fragments) identified as polymorphic. The study determined that *T. humilis* and *T. schrenkii* were the most genetically distinct species, while *T. montana* and *T. biflora* exhibited the closest genetic relationship.

In a separate study by Pourkhaloee *et al*. (2018), 70 genic microsatellites were screened, of which 15 highly polymorphic and reproducible markers were employed to assess genetic diversity, structure, and relationships among 280 individuals from 36 wild and cultivated tulip accessions originating from Iran and the Netherlands. The mean gene diversity and polymorphism information content (PIC) values were reported as 0.69 and 0.66, respectively, indicating the high discriminatory power of the markers. Wild *T. systole* stapf exhibited the highest genetic diversity. STRUCTURE analysis, based on a Bayesian model, identified five distinct gene pools within the 36 germplasms, aligning with both morphological observations and traditional classifications.

Kutlunina *et al*. (2013) investigated the genetic diversity of four *Tulipa* species (*T. biebersteiniana, T. patens, T. scytica, and T. riparia*) using AFLP analysis. A total of 81 individuals from 13 populations were genotyped with 87 loci using three selective EcoRI/MseI primer pairs. Low genetic variability was observed across all species, with *T. biebersteiniana* (P = 20.41%, UHe = 0.075), *T. patens* (26.97%, UHe = 0.082), *T. scytica* (27.53%, UHe = 0.086), and *T. riparia* (27.72%, UHe = 0.096). *T. patens* was found to be well-differentiated based on Nei's distances and STRUCTURE analysis, which identified four genetic groups, though they did not fully align with geographic populations.

In another study, 236 polymorphic SNPs were identified from the tulip cultivars 'Kees Nelis' and 'Cantata'. For genetic analysis, 121 SNPs with a minor allele frequency (MAF) above 0.1 were selected. The total observed heterozygosity (Ho) among the 72 accessions was 0.35, with Ho values ranging from 0.22 in *T. fosteriana* to 0.43 in *T. gesneriana* × *T. fosteriana* hybrids. Genetic distances among *T. gesneriana* cultivars were relatively small, with differentiation based on flowering time and morphology. Both principal coordinate analysis and STRUCTURE analysis revealed three genetic clusters among the accessions (FST = 0.208, P < 0.0001) (Tang *et al*., 2013).

When considering all these studies collectively, it becomes evident that there is a high degree of variation within *Tulipa* species. Studies conducted in Türkiye have revealed the presence of species with remarkably diverse characteristics across different regions and altitudes. The naturally distributed tulip species exhibit significant genetic diversity, highlighting the adaptive capacity of these species to various environmental conditions. The results of population analysis further support this diversity, as tulip individuals assigned to four or five subpopulations often represent highly pure genotypes and/or species. This finding underscores the distinct genetic integrity and diversity maintained within natural tulip populations.

In this study, different *Tulipa* species originating from Kazakhstan and Türkiye, along with their genotypes, were evaluated in comparison to commercial varieties in terms of morphological and genetic traits, revealing a high level of genetic diversity. The findings are consistent with previous research and provide valuable insights for guiding future studies.

Conclusions

In the present study, 47 tulip genotypes belonging to 14 different species were comprehensively evaluated in terms of their morphological and molecular characteristics. The findings revealed significant morphological diversity among the tulip species, including variations in plant growth habits, leaf and flower traits, and bulb characteristics. iPBS molecular markers were used to assess the extent of genetic diversity. The results demonstrated clear genetic differentiation among the tulip species, with genotypes sampled from Türkiye and Kazakhstan exhibiting a rich genetic pool compared to commercial varieties. For tulips, which have been valuable not only as wild ornamental bulbs but also as cut flowers in recent years, developing varieties with favourable agronomic traits, such as resistance to drought and various abiotic and biotic stress conditions, is of great importance. Therefore, it is essential to identify naturally occurring species and evaluate them in comparison with commercial cultivars. The findings of this study provide a foundational framework for future

breeding efforts aimed at cultivating tulip varieties that are aligned with sustainable agricultural objectives. The practical significance of this research lies in the obtained values of genetic diversity between *Tulipa* species of Kazakhstan and Türkiye, which are expected to be useful for tulip breeders who could improve their productivity with these new data. Investigating the genetic diversity in plant populations is vital for various scientific, ecological, and practical purposes. This study has certain limitations, such as was the lack of comparison between iPBS efficiency and the previously used genetic analysis methods. Future studies could add such a comparison and verify the rationale for using the iPBS approach with this study.

Authors' Contributions

The contributions of authors to the manuscript; Data curation: NS, ADS, KG, MT; Formal analysis: NS, ADS, MT, FBT; Funding acquisition: NS, AA, BY, GT, KG; Investigation: NS, ADS, MT, OS, FGC; Resources: NS, OS, MT, FGC; Writing - original draft and editing: ADS, KG.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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