Eurasian Journal of Applied Biotechnology. №.3, 2024
DOI: 10.11134/btp.3.2024.6

UDC: 575.174.015.3; 575.86 Original Article

DNA BARCODING OF THE GENUS *TULIPA* (LILIACEAE) IN KAZAKHSTAN

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ABSTRACT

DNA barcoding is a reliable and effective tool for analyzing genetic and species diversity. Three plastid genetic markers (rbcL, psbA-trnH, matK) and one nuclear marker (ITS) were amplified with universal primers for species diversity and phylogenetic analysis of eight species including *T. greigii, T. kaufmanniana, T. turkestanica, T. bifloriformis, T. patens, T. dubia, T. alberti, T. schrenkii* across subgenera *Tulipa* and *Eriostemones* in Kazakhstan. DNA samples were obtained from fresh leaves of plants collected from various protected areas of Kazakhstan, and subsequently subjected to PCR, sequencing and deep phylogenetic analysis using Bayesian models for calculating posterior probabilities. Phylogenetic trees generated based on data obtained from individual markers accurately divided the samples into clades representing the subgenera *Eriostemones* and *Tulipa*. The ITS marker gave the most reliable results, followed by matK and rbcL; psbA-trnH was the least informative. These finding highlight the importance of using DNA-barcoding markers for comprehensive phylogenetic analysis, and contribute to understanding the genetic diversity and conservation of the *Tulipa* genus in Kazakhstan.

Key words: *Tulipa* L., DNA-barcoding, ITS, matK, psbA-trnH, rbcL

INTRODUCTION

Tulipa genus

Tulipa (*Tulipa* L.) belongs to the Liliaceae family, Lilieae tribe, and *Tulipa* genus, and includes two subgenera – *Eriostemones* and *Orythia* [1] – with large genomes (1C = 25 GB for tulip) [2]. Most species have the same basic chromosome number (2n = 2x = 24) [3]. This plant grows and develops well at 22–30 °C, however under sufficient humidity; it can also survive at 37–42 °C. Moreover, it is characterized by high cold tolerance, and can survive at temperatures ranging from –15 °C to –30 °C. Tulipa is a spring-blooming perennial plant that produces flowers during March to May. Tulip flowers have a wide variety of colors with various components generally being pigmented red, pink, yellow, or white (usually in the warmest colors). It is bulbiferous, and contains five major parts such as basal plate, basal stem, fleshy scales, flower bud, and tunic [4].

Most wild tulips inhabit in the mountainous regions of Central Asia are considered the source of the world's most diverse tulip species. Currently, the species are distributed across Europe, North Africa, and Asia [5], and are even found in the western Himalayas, southern Siberia, and Inner Mongolia. In fact, the Tien Shan Mountain is one of the primary diversity centers of tulips. *Tulipa* genus is represented by 63 wild-type species in Central Asia [6], 37 of which are native to Kazakhstan and dispersed throughout the country. In total, there are 35 species of wild tulips in Kazakhstan, 18 of which are listed in the Red Book and are protected by the state (https://astana.citypass.kz/en/2021/03/10/v-kazakhstane-35-vidov-dikih-tyulpanov/). Ivaschenko A. described 34 species belonging to three subgenera (*Tulipa*, *Eriostemones*, and *Orythia*) [7].

Our study focused on eight species, including *T. greigii, T. kaufmanniana, T. turkestanica, T. bifloriformis, T. sylvestris, T. dubia, T. alberti,* and *T. suaveolens* across the subgenera *Tulipa* and *Eriostemones*. These species grow from perennial bulbs and generally reach between 10 and 50 cm tall. The seasons of interest for these species are early, mid, and late spring and sometimes extend to winter. These species are planted during the autumn in six main soil types, including chalky, loam, and sand with pH ranging from acid to alkaline. The flower characteristics are showy and fragrant, and flower color shades vary between red, yellow and white; some flowers have petals with two or more shades and colors. These species are endemic to Kazakhstan, and occur in Turkestan, Kyzylorda, the western extreme of the Zailiisky, Kungei Alatau, Kyrgyz Alatau, Chu-Ili Mountains, as well as Karatau and the south of the Betpakdala desert (https://silkadv.com/en/content/tulipa-greigii). Their core habitat areas are loamy steppes, slopes of foothills, and hills. They are listed in the Red Book of Kazakhstan as rare and endangered species [8]. Basic limiting factors to their distribution include construction of cities, plowing of land, and grazing.

DNA barcoding and its applications in plants

A standardized DNA barcode is a short (<1000 bp) and highly variable segment of DNA derived from specific regions of DNA [9], and can serve as an effective tool for exploring biological phenomena. Since 2003, it has been applied to identify species, infer ecological and evolutionary relationships between species, and accelerate taxonomic discovery. It is also used in germplasm conservation, community assembly, species interaction networks, and assessing priority areas for environmental protection [10, 11]. Collective progress is being made towards applying DNA barcodes for all groups of plants and making these data publicly available to assess, conserve, and adequately utilize the world's biodiversity.

Several reviews have highlighted recent barcoding studies on extensive research in the kingdom Plantae [12-15]. Specifically, 907 samples representing 445 angiosperm, 38 gymnosperm, and 67 cryptogam species have been sequenced and compared using the seven leading candidates DNA regions (atpF-atpH interval, matK gene, rbcL gene, rpoB gene, rpoC1 gene, psbK-psbI interval and trnH-psbA interval) by the CBOL Plant Working Group in 2009. Four primary gene regions (rbcL, matK, trnH-psbA, and ITS) have generally

DOI: 10.11134/btp.3.2024.6

been accepted as the standardized universal DNA barcodes for routine applications in plant species. This provides a platform for establishing a centralized plant barcode database. To date, 634 invasive plant species from China and around the world have been evaluated using five common markers (ITS, ITS2, matK, rbcL and trnH-psbA) [16]. In total, 1,130 specimens from 538 species, 323 genera, and 115 families of vascular plants from a highly diverse flora in the canga of the Serra dos Carajás in Eastern Amazon have been tested and barcoded using eight different DNA barcode markers (matK, rbcL, rpoB, rpoC1, atpF-atpH, psbK-psbI, trnH-psbA, and ITS2). This has led to the recommendation of rbcL and ITS2 as the most suitable markers for a broad application in the regional flora [17]. Moreover, 1,482 flowering plant species have been barcoded using rbcL, matK, and ITS2 markers, most of which (81%) were sourced from herbaria of the UK flora [18]. DNA barcoding was widely used on Orchidaceae, the second largest family of flowering plants. In the research of Li et al (2021), 4290 sequences including matK, rbcL, ndhF, and ycf1 genes of Orchids from the NCBI Nucleotide database were analyzed for genetic diversity, nucleotide pair frequencies, and phylogenetic analysis. In addition, a corresponding DNA QR code ID card was generated according to the SNP sites [11]. In total, 45 representative species from six sections of *Lilium* were evaluated using five barcode markers (ITS, rbcL, ycf1b, matK and psbA-trnH) to develop a reliable identification system according to DNA sequence polymorphisms [19]. DNA barcoding was also widely used on medicinal plants, and progress in the application of DNA barcodes in these plants was reviewed by Yu et al., 2021 [15]. A total of 31 individual plants represented by nine species and three genera of the Lamiaceae family from Indian Territory were evaluated using three barcode loci (matK, trnH-psbA and trnL) [20]. Moreover, nine rare and endangered endemic medicinal plant species collected from Saint Katherine Protectorate have been barcoded using rbcL, ITS and ycf1 with the aim of identifying biodiversity and phylogenetic relationships among them [21]. Nearly 7,000 sequences of 380 species (90%) of medicinal orchids in Asia were evaluated by five single barcodes (ITS, ITS2, matK, rbcL, trnH-psbA and their seven combinations) to analyze their genetic distance and phylogenetic relationships [22]. A total of 870 medicinal plants from herbal markets in Dar-es-Salaam and Tanga, Tanzania were identified using DNA barcoding markers such as rbcL, matK and nrITS [23]. Liu and colleagues assessed the identification efficiency of candidate DNA barcodes (ITS2, psbA-trnH, matK and rbcL) in 106 species across 27 families and 65 genera [24].

Application of DNA barcode in the genus *Tulipa*

Many studies over the last decade have investigated the genetic diversity of the Liliaceae family assessed by genetic barcoding markers [25]. Generation and analysis of expressed sequence tags in the extremely large genomes of Tulipa was published in 2012. In this study, the first set of 81,791 contigs with an average length of 514 bp for the tulip was developed, thus providing a platform for genetic research improvement [2]. Christenhusz et al. investigated the phylogenetic relationships of 25 accessions, representing 23 species in the genus *Tulipa* using DNA sequences from five plastid regions (trnL intron and trnL–trnF spacer, rpl16 intron, rps12–rpl20 intergenic spacer and matK) and the internal transcribed spacer (ITS) region of nuclear ribosomal DNA [25]. The genetic diversity of *T. edulis* collected from eight different regions in China were studied using four plastid (rbcL, psbA-trnH, matK, trnL-F) and ITS markers [26]. The genetic diversity and population structure of 65 natural populations of T. suaveolens from the Astrakhan, Volgograd, Orenburg, Rostov, Samara and Saratov provinces, the Krasnodar Region, the Republic of Kalmykia, the Republic of Dagestan, the Crimea and western Kazakhstan were evaluated by amplifying the psbE–petL region of chloroplast DNA and the complete ITS of nuclear ribosomal DNA. That study also revealed *T. suaveolens* to be the most likely wild ancestor of early *T. gesneriana* [27]. Altogether, 15 species of Tulipa from Uzbekistan were sequenced and characterized for their phylogenetic relationship using four plastid (rbcL, psbA-trnH, matK, trnL-F) and ITS markers [5]. Eight taxa including six species and two subspecies of the genus Tulipa from Kosovo were explored using trnL-trnF, rbcL and psbA-trnH plastid and ITS markers [28].

MATERIALS AND METHODS

All plant materials were gathered at the collection sites in Aksu-Zhabagly and Karatau Nature State Reserves with the guidance of state reserve botanists. Permission for the collection of endangered species was obtained from the Forestry and Wildlife Committee Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan. The detailed list of accessions is described in Appendix 1.

Young leaves of accessions were stored at –80 °C until DNA was extracted. DNA was extracted from fresh tulip leaves by the CTAB method [29] with slight modifications. The extracted DNA was checked for intactness, homogeneity, and purity by 1% agarose gel electrophoresis, run at 120V for 30 minutes. The DNA was stored at –20 °C until use in the next step of the experiment.

The universal barcode primers were selected based on published papers; their detailed information is given in Table 1. All primers were synthesized by the Laboratory of Organic Synthesis of the National Center for Biotechnology (Astana, Kazakhstan). PCR was performed in a 40 µl total reaction volume consisting of 2 µL of genomic DNA (50ng), 0.4 µL of 10x Taq polymerase (Gen Lab), 4 µl of 25mM $MgCl_2$ (Thermo Scientific), 4 µL of 10x Taq buffer (Thermo Scientific), 1 µL of 10mM dNTP (Thermo Scientific), 1 µL of forward and reverse primers (10 µmol L stock) and 27.6 µl of ddH2O. Amplification of the PCR reaction was performed in a T100 Thermal Cycler (Bio-Rad) with the following program: 5 minutes at 95 °C for initial denaturation (one cycle), 30 cycles of 1 minute at 95°C for denaturation, 1-minute optimal annealing temperature for each primer (50 °C–56 °C; Table 1), and 1 minute at 72 °C for elongation; finally one cycle of 10 minutes at 72 °C for extension temperature and held at 4 °C thereafter. The obtained PCR products were checked in 2% agarose gel electrophoresis, run for 30 minutes under 120V and cleaned using PureLink Quick Gel Extraction kit by Invitrogen. The purified PCR products were Sanger sequenced using a 3730xl DNA analyzer (Applied Biosystems). The obtained sequences of both forward and reverse primers of each accession were analyzed with Invitrogen ContigExpress (Vector NTI Advance® 11.5) software, and contigs were assembled to minimize possible reading errors. The assembled se-

DOI: 10.11134/btp.3.2024.6

Table 1. Nucleotide sequences of PCR primers used for DNA barcoding.

Primer Name	Nucleotide sequence of primer (5'-3')	Barcoding locus	Tm (°C)
3F_KIMf [31]	CGTACAGTACTTTTGTGTTTACGAG	matK	50
1R_KIMr [31]	ACCCCATTCATCTGGAAATCTTGGTTC	matK	50
rbcLa_F [32]	ATGTCACCAACAAACAGAGACTAAAGC	rbcL	58
rbcLa_R [32]	GTAAAATCAAGTCCACCRCG	rbcL	58
psbA3f [33]	GTTATGCATGGTGGATTCACAATCC	trnH-psbA	53
trnHf_05 [33]	CGCGCATGGTGGATTCACAATCC	trnH-psbA	53
ITS4 [34]	TCCTCCGCTTATTGATATGC	ITS1 and ITS2	55
ITS5 [34]	GGAAGTAAAAGTCGTAACAAG	ITS1 and ITS2	55

quences were compared with existing DNA sequences using BLAST on the National Center for Biotechnology GenBank (NCBI). The sequences of Kazakhstan Tulip species were uploaded to the NCBI database with accession numbers reported in Appendix 1. Reference and outgroup sequences (*L. lancifolium, E. oregonum*) taken from the GenBank are presented in Appendix 1.

Sequences were aligned in MEGA 11 using automatic algorithm selection and default settings. Aligned sequences were reviewed in BioEdit and manually realigned. Bayesian inference was undertaken in MrBayes v. 3.2. For the nuclear DNA alignment, Markov chain Monte Carlo analyses with four chains were run for 10 M generations, sampling every 1,000 steps, with a burnin of 25% and Dirichlet distribution unlinked. For the chloroplast DNA alignment, the analysis was run for 2.5 M generations and sampled every 500 steps. Output files were viewed in Tracer to check for convergence. The average standard deviation of split frequencies was also reviewed and confirmed to be below 0.01 upon completion of analyses. Branches with values of < 0.95 PP were considered unsupported.

RESULTS

The length of the rbcL sequences after alignment was 486 bp. The intragroup alignment includes 12 variable sites, four potentially informative sites, eight single nucleotide polymorphisms (SNPs), 471 conserved sites, and a G + C content of 44.5% (Table 2). The psbA-trnH sequences in the data set were 451 bp long. The intragroup alignment had 138 codons, 360 conserved sites, 52 variable sites, 21 potentially informative sites, 31 SNPs, and 31.7% G + C content. The matK sequences after alignment were 597 bp long. The analyzed sequences showed eight potentially informative sites, 34 single nucleotide polymorphisms, 42 variant sites, 555 conserved sites, and a G + C content of 30%. The length of the aligned ITS sequences (ITS1, complete 5.8S rDNA gene, ITS2 and a small part of the 26S rDNA gene) of Tulipa species was 558 bp. The intragroup alignment showed 367 conserved sites, 184 variable sites, 81 potentially informative sites, 103 single nucleotide polymorphisms, and 59.5% G + C content (Table 2).

Phylogenetic analysis

A total of 75 sequences were used in the phylogenetic analysis. Phylogenetic trees were created based on individual marker genes (rbcL, psbA-trnH, matK, and ITS). All phylogenetic trees were generated using Bayesian analysis (Likelihood algorithm with determination of the posterior distribution probability).

rbcL region

A total of 21 sequences were used to build a phylogenetic tree based on the rbcL marker. As a result, we observe a clear distribution of sequences across subgenera (Figure 1). Control specimens of *T. clusiana* (subgenus *Clusianae*) and *T. uniflora* (subgenus *Orithyia*) taken from GenBank were identified as representatives of separate subgenera (BPP = 1). This is followed by a division into two clades, also with a high de-

Table 2. Aligned sequence characteristics for rbcL, psbA-trnH, matK, and ITS analyses.

Parameters	rbcL	psbA-trnH	matK	ITS
No. of taxa	21	15	19	18
Alignment length (bp)	486	451	597	558
Conserved sites	474	360	555	367
Variable sites	12	52	42	184
Parsimony informative sites	4	21	8	81
Singleton sites	8	31	34	103
Average codons	162	138	199	175
G + C contents	44,5	31,7	30	59,5

DOI: 10.11134/btp.3.2024.6

gree of support (BPP = 1). The first strongly supported clade (BPP = 1) consists of members of the subgenus *Eriostemones*, with *T. turkestanica* and *T. bifloriformis* showing a close relationship, as well as *T. patens* and *T. sylvestris*. The second strongly supported clade (BPP = 0.97) consists of members of the subgenus *Tulipa*. Samples of the subgenus *Tulipa* are divided into three groups: the first consists of sequences of the *scardica* complex (*T. albanica, T. serbica, T. scardica*) taken from GenBank (BPP = 0.9); the second group included *T. thianschanica, T. ferganica, T. intermedia*, and *T. schrenkii*; and the third group consisted of sequences of *T. affinis* and *T. mogoltavica* species taken from GenBank, which were expectedly separated from the Kazakhstan species (*T. greigii, T. kaufmanniana, T. dubia, T. alberti*) despite the relatively low posterior probability (BPP = 0.84).

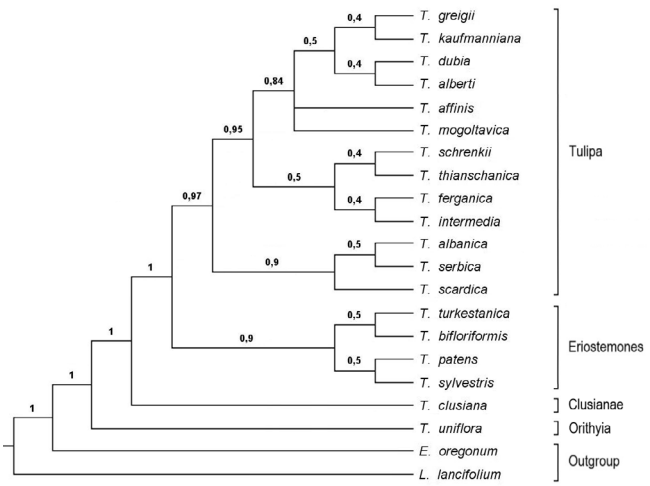

Figure 1. Phylogenetic tree based on rbcL sequences, including posterior probabilities (BPP > 0.4) provided above each branch.

psbA-trnH region

The phylogenetic tree generated from 15 psbA-trnH sequences was the least informative for a single marker (Figure 2), with consistently low posteriors. The marker was able to distinguish representatives of the subgenera *Eriostemones* and *Tulipa* with very limited resolution (BPP = 0.9). However, the specimen *T. clusiana* (Subgenus *Clusianae*) showed a closer relationship to the subgenus *Eriostemones* and was not identified as a separate taxon of *Clusianae*, indicating a low resolution of the marker. In the *Eriostemones* clade, specimens of *T. turkestanica* and *T. bifloriformis* show a close relationship. Within the *Tulipa* clade, Bayesian analyses showed low support for taxa separation (BPP = 0.6), especially taxa of the Kazakhstan species (*T. greigii, T. kaufmanniana, T. dubia, T. alberti*, and *T. schrenkii*). However, Kazakhstan taxa were separated from *T. affinis* and *T. lehmanniana* (BPP = 0.86). Moreover, a representative of the *scardica* complex, *T. albanica*, was identified as a separate taxon with a fairly high level of support (BPP = 0.9).

matK region

The phylogenetic tree generated from 19 matK sequences was divided into two clades representing two subgenera: *Eriostemones* and *Tulipa*, as well as an offshoot of the control sample *T. clusiana* representing the subgenus *Clusianae* (Figure 3). In the first clade, *Eriostemones* (*T. turkestanica, T. bifloriformis, T. patens, T. sylvestris*), and specimens of *T. turkestanica* and *T. bifloriformis* show a close relationship that is largely supported by Bayesian analysis (BPP = 0.9), although the structure of the phylogenetic tree was slightly different from that generated using the rbcL sequences. The structure of the second clade is represented by specimens of the subgenus *Tulipa*, which have formed several distinct subclades. In the first subclade, samples taken from GenBank (*T. thianschanica* and *T. intermedia*) were separated and showed a close relationship (BPP = 0.9). The closely related *T. lehmanniana* and *T. ferganica* are grouped together with *T. affinis*. In addition, *T. schrenkii* was identified as a separate taxonomic entity with a relatively high probability (BPP = 0.87). In the last major subclade, Kazakhstani closely related *T. kaufmanniana* and *T. dubia* (BPP = 0.9) were grouped separately from *T. greigii* and *T. alberti*, which showed a closer relationship to *T. mogoltavica* than to *T. vvedenskyi*. However, the low posterior probability does not allow us to confidently conclude about such a close relationship between the last four species (BPP = 0.5).

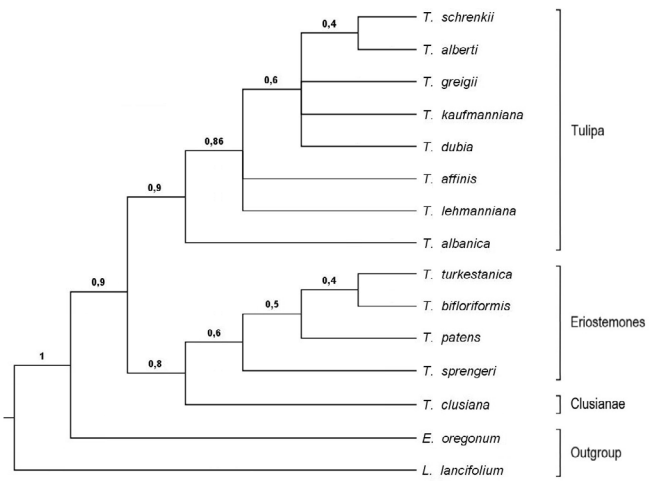

Figure 2. Phylogenetic tree based on trnH-psbA sequences, including posterior probabilities (BPP > 0.4) provided above each branch.

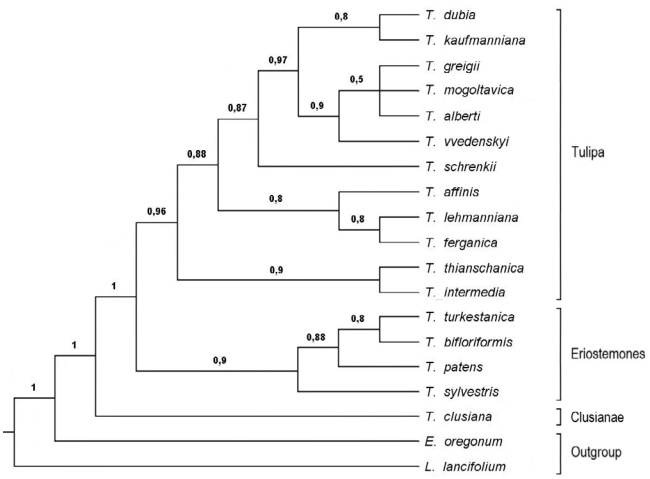

Figure 3. Phylogenetic tree based on matK sequences, including posterior probabilities (BPP > 0.4) provided above each branch.

ITS region

A phylogenetic analysis of 18 ITS sequences is shown in Figure 4. The generated tree shows that *Tulipa* taxa are di-

DOI: 10.11134/btp.3.2024.6

vided into two main clades with strong support (BPP = 1). The first clade includes representatives of the subgenus *Eriostemones* (*T. turkestanica, T. bifloriformis, T. patens, T. sylvestris, T. sprengeri, T. humilis, T. orthopoda, T. saxatilis*), and the second clade includes representatives of the subgenus *Tulipa* (*T greigii, T. kaufmanniana, T. dubia, T. alberti, T. schrenkii, T. thianschanica, T. ferganica, T. intermedia*). In the first clade, the closely related *T. patens* and *T. sylvestris* are separated from the *T. turkestanica* complex and *T. bifloriformis* (which are also closely related to each other) and show a close relationship to the GenBank-derived *T. orthopoda* (BPP = 0.9). The control group of Turkish and Iranian specimens taken from GenBank (*T. sprengeri, T. saxatilis, T. humilis*) are grouped in a separate clade (BPP = 0.9), due to the geographical remoteness of these species from other groups. In the second clade, all species of *T. greigii, T. alberti, T. kaufmanniana, T. dubia, T. schrenkii* form a single clade with a more distant relationship with specimens taken from GenBank (*T. thianschanica, T. intermedia, T. ferganica*). The species *T. ferganica* was identified as a separate taxonomic entity (BPP = 0.97).

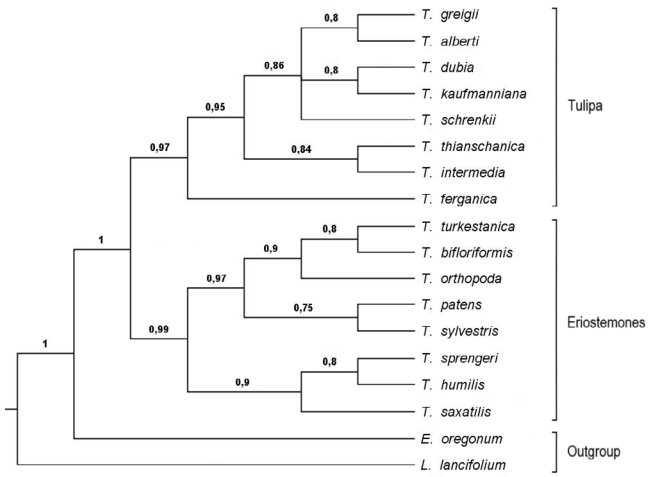

Figure 4. Phylogenetic tree based on ITS sequences, including posterior probabilities (BPP > 0.4) provided above each branch.

DISCUSSION

In order to conduct a phylogenetic analysis on rare and protected species of Kazakhstan tulips, genetic markers rbcL, psbA-trnH, matK and ITS were used. Our data supplement the information on the possibilities of using chloroplast (rbcL, psbA-trnH, matK) and nuclear markers (ITS) [25-28] to study evolutionary relationships between wild tulip species. In general, we found that the use of individual genetic markers is not enough to distinguish and structure evolutionary relationships between closely related species, which supports previous reports [30]. We can also confirm that the use of the matK marker can serve as a very good tool for phylogenetic analysis. Since information on the matK gene in the public domain is extremely limited, our research will be especially useful in studying the biological and genetic diversity of tulips with a wide geographic coverage.

When considering data on individual markers, the phylogenetic tree generated using the nuclear ITS sequences was more reliable than the trees generated from the sequences of individual plastid markers (rbcL, psbA-trnH, matK). This is because the nuclear genome accumulates nucleotide substitutions at approximately the same rate, in contrast to the chloroplast and mitochondrial genomes. In this regard, the ITS region has sufficient genetic diversity, which makes it possible to detect not only intergeneric differentiation, but also differentiation between closely related species in some cases. The phylogenetic tree based on the ITS marker had monophyletic groups into which specimens of the subgenus *Eriostemones* (*Biflores, Sylvestres*) and *Tulipa* (*Kolpakowskianae, Vinistriatae, Spiranthera*) were distributed with a high posterior probability (BPP = 0.8). However, the generated tree shows that *Saxatiles* in the subgenus *Eriostemones* does not form a monophyletic group, while the *T. sprengeri* specimen (section *Sylvestres*) turned out to be more closely related to the species of *Saxatiles* than to *Sylvestres*. Representatives of *Vinistriata* of the subgenus *Tulipa* (*T. greigii, T. alberti, T. vvedenskyi*, and *T. mogoltavica*) are grouped into one subclade, although no clear structure is observed within this subclade.

In our case, the psbA-trnH phylogenetic tree was the least informative, due to the low resolution of the studied taxa and the inability to identify a specimen of the subgenus *Clusianae* as a separate taxonomic entity that was erroneously placed in the *Eriostemones* clade. The rbcL marker demonstrated a higher resolution than psbA-trnH, although it does not have enough informative sites to separate taxa within one section, mixing species within subgenera boundaries. However, when using matK sequences, the Bayesian analysis gave a rather high level of posterior probability in the distribution of the studied samples on the phylogenetic tree, dividing individuals into clades within subgenera with 100% accuracy and sections with an accuracy of about 80% in comparison with the taxonomy of tulip species proposed by Zonneveld in 2009 [3]. Thus, representatives of *Vinistriata* of the subgenus *Tulipa*, accessions *T. greigii, T. alberti, T. vvedenskyi*, and *T. mogoltavica* are distributed in one subclade, although within this subclade (similarly to the ITS tree) no clear structure is observed. On the other hand, *T. affinis* (section *Lanatae*) has been identified as being closely related to *T. lehmanniana* and *T. ferganica* (section *Kolpakowskianae*) [25, 28].

From the aforementioned information, it becomes clear that each individual marker gene has its disadvantages and advantages, which manifest themselves differently in the distribution of taxa and identification of species. Incorrect distribution of closely related species with one marker can be resolved by a more accurate analysis with another genetic marker [26].

Based on the results of our studies and the works of other authors, we can conclude that the use of individual genetic markers does not guarantee the exact identification of closely related species, but provides a high resolution in the distribution by subgenera. A detailed analysis of the distribution of sections within the genus *Tulipa* revealed significant limitations in the use of individual genetic markers (rbcL, psbA-trnH, matK, and ITS) due to the low representation of species and the limited number of nucleotide sequences of marker genes in the public domain [27]. Therefore, we cannot accurately state the distribution of *Tulipa* sections, and note that not all of them may be correct. Hence, it is necessary to continue updating the sequence database and expanding the geographic coverage of phylogenetic studies of rare and protected

DOI: 10.11134/btp.3.2024.6

species of wild *Tulipa*.

DATA AVAILABILITY STATEMENT

The DNA barcoding sequences of 8 *Tulipa* species referred to in this work were uploaded into the GenBank of NCBI (https://www.ncbi.nlm.nih.gov/). All the DNA barcoding sequences' raw data are available at NCBI, and accession numbers were listed in Appendix 1.

FUNDING

This research was funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan, Program ID BR18574125 and OR11465422.

ACKNOWLEDGEMENT

We are grateful to the curator of Aksu-Zhabagly State Nature Reserve Samatulla Dzhumanov for providing access to the samples for DNA barcoding. Thanks also to acknowledge Dilnur Tussipkan for assistance in obtaining NCBI accession numbers.

CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix 1. Species and GenBank/NCBI accession numbers used in this study

T. greigii: ON010708, ON423208, ON423211, OP279724; *T. kaufmanniana*: ON186589, ON423207, ON952472, OP279725; *T. turkestanica*: ON186590, ON423209, ON952473, OP279723; *T. bifloriformis*: ON186591, ON423210, ON952474, OQ733258; *T. patens*: OP261551, OQ718219, OP261547, OP279727; *T. dubia*: OP261549, ON983982, OQ718220, OQ733267; *T. alberti*: OP261548, ON983980, OQ718218, OP279728; *T. schrenkii*: OP261550, ON983981, OP261546, OP279726; *T. sylvestris*: MF572249, MF543700, MF543559, MF543811; *T. affinis*: MW847262, MW847269, MW847256, MW854641; *T. sprengeri*: AM085141, MH555233; *T. clusiana*: KM085528, AM085140, KM085657; *T. lehmanniana*: EU939291, EU912163; *T. uniflora*: KM085540; *T. albanica*: MZ147068, MZ147043; *T. serbica*: MZ147085; *T. scardica*: MZ147082; *T. thianschanica*: MT930330, MT917276, MT923872; *T. ferganica*: MW828759, MW828755, MW826216; *T. intermedia*: MW828758, MW828754, MW826215; *T. mogoltavica*: MW847265, MW847259; *T. vvedenskyi*: MW847258; *T. humilis*: MH555263; *T. orthopoda*: MH555217; *T. saxatilis*: MH555181; *E. oregonum*: KX679106, EU311867, KX677389, EU311823; *L. lancifolium*: MZ969911, KX346970, MZ970142, MZ960538.

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DOI: 10.11134/btp.3.2024.6

УДК: 575.174.015.3; 575.86

ДНК-БАРКОДИРОВАНИЕ РОДА *TULIPA* (LILIACEAE) В КАЗАХСТАНЕ

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АННОТАЦИЯ

Баркодирование ДНК – надежный и эффективный инструмент анализа генетического и видового разнообразия. Три пластидных генетических маркера (rbcL, psbA-trnH, matK) и один ядерный маркер (ITS) были амплифицированы с помощью универсальных праймеров для видового разнообразия и филогенетического анализа восьми видов, включая *T. greigii, T. kaufmanniana, T. turkestanica, T. biflоriformis, T. patens, T. dubia, T. alberti, T. schrenkii* в подродах *Tulipa* и *Eriostemones* в Казахстане. Образцы ДНК были получены из свежих листьев растений, собранных на различных охраняемых территориях Казахстана, и в дальнейшем подвергнуты ПЦР, секвенированию и глубокому филогенетическому анализу с использованием Баесовского анализа. Филогенетические деревья, построенные на основе данных, полученных по отдельным маркерам, точно разделили выборки на клады, представляющие подроды *Eriostemones* и *Tulipa*. Маркер ITS дал наиболее надежные результаты, за ним следовали matK и rbcL; psbA-trnH оказался наименее информативным. Эти результаты подчеркивают важность использования маркеров ДНК-баркодирования для комплексного филогенетического анализа и способствуют пониманию генетического разнообразия и сохранения рода *Tulipa* в Казахстане.

Ключевые слова: *Tulipa* L., ДНК-баркодирование, ITS, matK, psbA-trnH, rbcL

ӘОК: 575.174.015.3; 575.86

ҚАЗАҚСТАНДАҒЫ *TULIPA* (LILIACEAE) ТУЫСЫНЫҢ ДНҚ-БАРКОДЫ

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ТҮЙІН

ДНҚ баркодинг – генетикалық және түрлік әралуандылықты талдаудың тиімді құралы болып табылады. Үш пластидті генетикалық маркер (rbcL, psbA-trnH, matK) және бір ядролық маркер (ITS) түрлердің әртүрлілігі мен сегіз түрдің соның ішінде *Tulipa* және *Eriostemones* кіші туыстастарына жататын *T. greigii, T. kaufmanniana, T. turkestanica, T. biflоriformis, T. patens, T. dubia, T. alberti, T. schrenkii* филогенетикалық талдауы үшін әмбебап праймерлермен амплификацияланды. ДНҚ үлгілері Қазақстанның әртүрлі қорғалатын аумақтарында жиналған өсімдіктердің балғын жапырақтарынан алынды және одан әрі ПТР, секвенирлеу және Баестік талдауды пайдалана отырып, терең филогенетикалық талдаудан өтті. Жеке маркерлерден алынған мәліметтер негізінде салынған филогенетикалық ағаштар үлгілерді *Eriostemones* және *Tulipa* субгенустарын білдіретін кладаларға дәл бөлді. ITS маркері ең сенімді нәтиже берді, содан кейін matK және rbcL; psbA-trnH ең аз ақпараттылықты дәлелдеді. Бұл нәтижелер кешенді филогенетикалық талдау үшін ДНҚ-баркодинг маркерлерін пайдаланудың маңыздылығын көрсетеді және Қазақстандағы *Tulipa* тұқымының генетикалық әртүрлілігін және сақталуын түсінуге ықпал етеді.

Түйінді сөздер: *Tulipa* L., ДНҚ-баркодинг, ITS, matK, psbA-trnH, rbcL