

## PHYLOGENETIC ANALYSIS OF RARE AND ENDANGERED *TULIPA* SPECIES (*LILIACEAE*) OF KAZAKHSTAN BASED ON UNIVERSAL BARCODING MARKERS

M.Y. Sutula, A.K. Kakanay, D. Tussipkan, S.A. Manabayeva

National Center for Biotechnology

Kazakhstan, 010000, Astana, Qorgalzhyn Hwy. 13/5

e-mail: m.sutula@biocenter.kz

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In Kazakhstan, the genus *Tulipa* is represented by 35 species, 18 of which are listed in the Red Data Book of Kazakhstan and are protected by the state. Recent studies of tulip specimens from regions bordering Kazakhstan emphasize the significance of species inventory and report the discovery of several hybrids. In our study, 8 tulip species were identified based on morphological characteristics and using DNA barcoding methods. All plant material was collected in Aksu-Zhabagly, Karatau Nature State Reserves and Kostanay region under the guidance of State Reserve botanists. The plant material was identified by the State Reserve botanists using a special identification key of the botanical database. Permission to collect of endangered species was obtained from the Forestry and Wildlife Committee of the Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan. The corresponding voucher specimens are deposited in a herbarium of the National Center for Biotechnology (Astana, Kazakhstan). To expand the representation of and genetic variation of Kazakh tulip species, data of 154 accessions were downloaded from NCBI GenBank. Molecular genetic markers, including nrDNA (ITS) and cpDNA markers (*rbcL*, *matK*), of the studied species were sequenced and analyzed using Bayesian inference and Maximum Likelihood phylogenetic analysis methods. The basic indicators of genetic diversity were examined, including nucleotide divergence ( $P_i$ ), the proportion of conservative (C), polymorphic, and segregating (S) regions. The use of the BLAST tool to search for identical sequences in the NCBI database revealed limited effectiveness at the species level for the chloroplast DNA markers *rbcL*

and *matK*. All investigated DNA barcodes successfully identified species at the genus level with 100% accuracy. This study presents a molecular analysis of the genus *Tulipa*, covering a wide range of species, including all available variants from the border regions of Central Asia (such as China, Russia, Uzbekistan). These results complement the existing knowledge of the phylogenetic relationships among the species and allow for a more in-depth analysis of their classification. However, some discrepancies between nrDNA and cpDNA-based phylogenies, especially regarding the placement of certain taxa (*T. patens*, *T. kaufmanniana*), require special attention and discussion. Our work highlights the effectiveness of using single genetic markers as species-specific barcodes in molecular genetics. The selection of a molecular marker with significant variability is crucial for accurate phylogenetic analysis. We demonstrated the reproducibility of the sequencing results. Analysis of individual markers revealed that nuclear ITS sequences provided better support than plastid markers. The variability of the ITS region allows accurate identification at both intragenic and intraspecific levels. To improve the accuracy of identifying evolutionary relationships among closely related species, we combined nuclear and chloroplast DNA data sets. This approach significantly improved the resolution and power of the phylogenetic analysis, revealing a previously undescribed hybrid *T. patens* × *T. alberti* and identifying a previously characterized hybrid *T. kaufmanniana* × *T. greigii*. Our results suggest the need for further population studies to validate these observations.