



*Research Article*

## Phylogenetic Identification of Endemic *Lathyrus karsianum* and *Astragalus globosus* from Kars Province

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**Abstract:** Kars province has a very rich floral diversity representing 16% of Turkey's flora, with 1615 species, 71 of which are endemic. It exemplifies Turkey's Caucasian lands, being at the crossing point of Iran-Turan, Euro-Siberian and Mediterranean flora regions as well. The pressure of activities such as excessive and unconscious grazing and field clearing in the region adversely affects the continuity of species that contribute to biodiversity, especially endemic species. Giving molecular identity to endemic plants contributes to conservation and sustainable development of biological diversity. The current study distinguished two endemic species by using the 26S rDNA region. *Lathyrus karsianum*, *Astragalus globosus*, and six GenBank sequences of species in Fabaceae family were used to infer phylogenetic relationships among them. For the amplified and sequenced 26S rDNA region, three variable sites were observed for eight related species. 26S rDNA region for *Lathyrus karsianum* and *Astragalus sarykamychensis* were successfully amplified for the first time. The results of the current study could be used in future biodiversity studies to conserve these endemic species.

**Keywords:** Endemic, Biodiversity, Phylogenetic, *Lathyrus karsianum*, *Astragalus globosus*.

## Kars İli'ndeki Endemik *Lathyrus karsianum* ve *Astragalus globosus* Bitki Türlerinin Filogenetik Tanımlanması

**Özet:** Kars ili, 71'i endemik olmak üzere 1615 tür ile Türkiye florasının %16'sını temsil eden çok zengin bir çiçek çeşidine sahiptir. İran-Turan, Avrupa-Sibirya ve Akdeniz flora bölgelerinin geçiş noktasında yer aldığı gibi Türkiye'nin Kafkasya topraklarını da temsil etmektedir. Bölgede aşırı ve bilinçsiz otlatma, tarla açma gibi faaliyetlerin baskısı, endemik türler başta olmak üzere biyolojik çeşitliliğe katkı sağlayan türlerin devamlılığını olumsuz etkilemektedir. Endemik bitkilere moleküler kimlik verilmesi, biyoçeşitliliğin korunmasına ve sürdürülebilir gelişimine katkıda bulunmaktadır. Mevcut çalışma, 26S rDNA bölgesini kullanarak iki endemik türün ayırt edilmesini sağladı. *Lathyrus karsianum*, *Astragalus globosus* ve Fabaceae familyasındaki altı türün GenBank dizileri, türlerin arasındaki filogenetik ilişkileri ortaya çıkarmak için kullanıldı. Sekiz yakın tür için çoğaltılmış ve dizilenmiş 26S rDNA bölgesinde üç değişken bölge gözlemlenmiştir. *Lathyrus karsianum* ve *Astragalus globosus* için 26S rDNA bölgesi ilk kez başarıyla çoğaltılmıştır. Mevcut çalışmanın sonuçları, bu endemik türleri ve biyolojik çeşitliliği korumak için gelecekteki çalışmalarda kullanılabilir.

**Anahtar kelimeler:** Endemik, Biyoçeşitlilik, Filogenetik, *Lathyrus karsianum*, *Astragalus globosus*.

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## INTRODUCTION

The continuity of life on earth depends on protecting the populations of species that perform basic ecosystem services and shape biodiversity. The balanced functioning of ecosystems often depends on species richness, species composition, and functional relationships among species. Biodiversity, which keeps ecosystems in balance and makes them habitable, is decreasing daily due to many factors such as pollution, habitat loss, hunting, climate change and natural disasters brought about by the increasing world population. The genetic diversity provides evolutionary potential against changing environmental conditions of particular species that find themselves in vulnerable ecosystems due to the high environmental pressure accompanied by global warming. Thus, it needs to be uncovered and protected (Dobson et al., 1997; Khan et al., 2012).

Kars province has a very rich floral diversity representing 16% of Turkey's flora, with 1615 species, 71 of which are endemic. It demonstrates Turkey's Caucasian lands, in addition to being the crossing point of Iran-Turan, Euro-Siberian and Mediterranean flora regions (Güneş and Özba 2014). The pressure of activities such as excessive and unconscious grazing and field clearing in the region adversely affects the continuity of species, especially endemic ones, that further contribute to biodiversity, species (Ekim et al 2000). To protect biodiversity, it is necessary to reveal the genetic diversity that provides the evolutionary adaptation of species to changing environmental conditions. Out of 71 endemic species, 12 are distributed locally to only Lake Çıldır, Allahuekber Mountains and Sarıkamış forests, which are mentioned as Important Plant Areas in Turkey (Özhatay 2006; Güneş and Özba 2014). *Astragalus globosus* and *Lathyrus karsianum*, which are members of the Fabaceae family, are among the local endemic species. Giving molecular identity to endemic plants contributes to conservation and sustainable development of biological diversity in our country. In this study, phylogenetic identification of endemic *Lathyrus karsianum* and *Astragalus globosus* species was performed by using the 26SrDNA gene region to uncover intra- and inter-species phylogenetic relationships.

## MATERIAL and METHODS

Leaf samples for two endemic species were collected from Sarıkamış Yağmurlu Dağı in Kars province. Nuclear DNA was extracted from leaf tissues by using a modified CTAB DNA isolation method of Kistler (2012). DNA concentrations were determined with a (Biodrop ILite 7141 V.1.0.4 spectrophotometer. The sequences of 26S forward and reverse primers are 5'-ttcccaacaaccgactc-3' and 5'- gccgtccgaattgtagtctg - 3', respectively (Alvarez and Vandel, 2003). PCR reaction was carried out in a 20 µl total volume, consisting of 4 µl HOT FIREPol Blend Master Mix (Solis BioDyne, Tartu, Estonia), 0.5 µl of 200 nM forward and reverse primers, 5 µl template DNA diluted as 10 ng, and 10 µl water. The PCR protocol was one cycle at 95 °C for 5 min followed by 30 cycles of 30 s at 94 °C, 58 °C (Ta) for 30 s and at 72 °C for 45 s, following a final extension at 72 °C for 10 min. PCR products were run on 3% agarose gels at 90 V for 30 min in electrophoresis. PCR products with the desired amplification were purified and sequenced by the BM Labosis (Cankaya, Ankara). The chromatogram data visualization, BLAST search (Altschul et al. 1990), and CLUSTAL alignment (Thompson et al. 1994) were performed with MEGA 10 Software (Kumar et al. 2018). The 26SrDNA sequences of *Astragalus globosus* and *Lathyrus karsianum* were compared with the sequences of close species from the NCBI database by BLAST analysis. The 26SrDNA sequences of six relative species were used for the construction of phylogenetic tree.

## RESULTS and DISCUSSION

A large amount and good quality of genomic DNA was obtained from endemic plant species with the help of the modified CTAB method from Kistler and Saphiro 2011. 26S rDNA region for *Lathyrus karsianum* and *Astragalus globosus* were successfully amplified for the first time. The length of the 26S rDNA region was found to be about 151 base pairs for both endemic species. There were not any aligned sequences belonging to *L. karsianum* and *A. globosus* in BLAST search. The obtained sequences for these two endemic plants were aligned to their relatives in the same family (Table 1). Three variable sites between sequences of endemic species and their relatives were at base positions 52, 144 and 148 (Table 5). No variation was seen between *L. karsianum* sequence and *Vicia ervilia*, *Cicer arietinum* sequences retrieved from NCBI. Two variations between *L. karsianum* and *L. decaphyllus* sequences from NCBI were observed at 144 and 148 bp locations. One variation between *A. globosus* sequence and *A. canadensis*, *A. Crassicaarpus* sequences from NCBI was at 144 bp location.

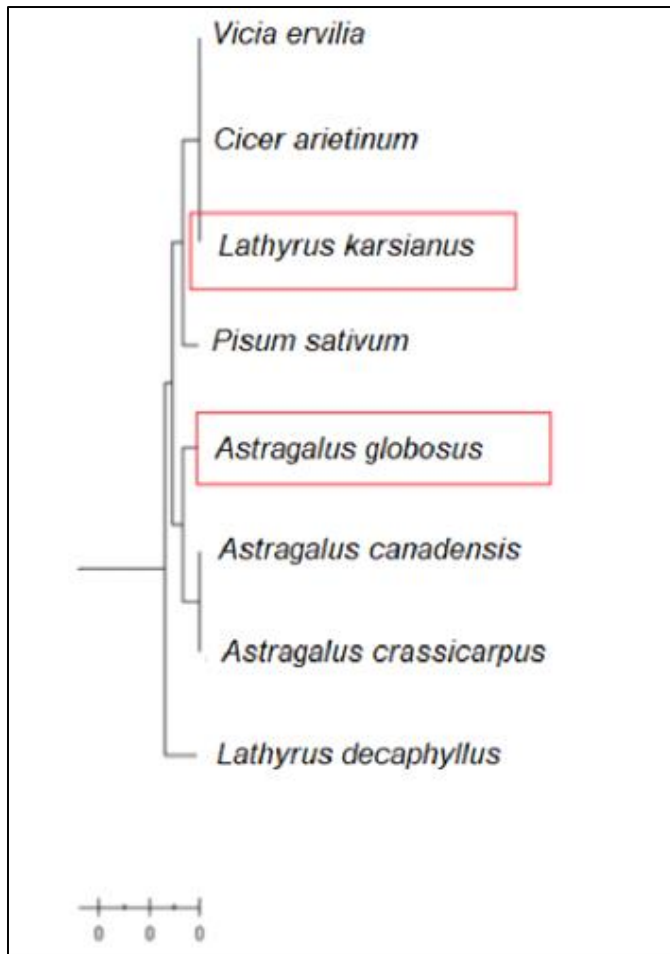
**Table 1.** Alignment results of 26SrDNA sequences for *L. karsianus* and *A. globosus*.

26SrDNA	GenBank Accession Number	Aligned species	Query Cover %	Identity %
<i>Lathyrus karsianus</i>	KT459234.1	<i>Lathyrus decaphyllus</i>	100	98.68
	MK413193.1	<i>Cicer arietinum</i>	99	100
	MK413190.1	<i>Vicia ervilia</i>	99	100
	XR_007796967.1	<i>Pisum sativum</i>	97	99.32
<i>Astragalus globosus</i>	MT610924.1	<i>Astragalus canadensis</i>	96	99.32
	KT459220.1	<i>Astragalus crassicaarpus</i>	96	99.32

The obtained sequences were merged with GenBank sequences of six relatives in genus *Astragalus* and *Lathyrus* to generate a phylogenetic tree. Even though, studied species were constituted two branches of the pylogenetic tree, low level of genetic differentiation was seen among them (Figure 2). *L. decaphyllus* is in one branch. *L. karsianum* and *A. globosus* were clustered in the same branch but different subgroups. *L. karsianum* was clustered with *Cicer arietinum* and *Vicia ervilia* which are agricultural crop plants in the same group instead of *L. decaphyllus*. *A. globosus* was found to be genetically close to other *Astragalus* species in the same group. It is seen that the 26S rDNA region is highly polymorphic for two endemic species and their six relatives. The obtained preliminary results from the current study will contribute to future genetic studies to conserve these endemic species and biodiversity. The genetic data to be added to the databases will be a source for national and international scientific studies on endemic species and their close relatives. In addition to preliminary genetic data DNA barcoding for endemic species could be performed for DNA-based identification system which has the potential to be a resource for the studies to be carried out to protect our biodiversity in the national and international arena.

Comparison of 26SrDNA sequences	
	52
<i>Lathyrus karsianus</i>	T T C C C A A C A A C C C G A C T C G C C G A C A G G C C T C G T G G T G C G A C A G G G T C C G A G C A C A A C G G G G C T C T C A C C C T C T C C
<i>Lathyrus decaphyllus</i> voucher Jones 1001 26S ribosomal RNA gene	.
<i>Vicia ervilia</i> isolate Contemporary C 26S large subunit ribosomal RNA gene	.
<i>Cicer arietinum</i> isolate Contemporary D 26S large subunit ribosomal RNA gene	.
<i>Pisum sativum</i> uncharacterized LOC127114519	.
<i>Astragalus globosus</i>	.
<i>Astragalus canadensis</i> isolate DPP8 small subunit ribosomal RNA gene	G
<i>Astragalus crassicaarpus</i> voucher Jones 1003 26S ribosomal RNA gene	G
	144      148
<i>Lathyrus karsianus</i>	G C G C C C C C T T C C A G G G G A C T T G G G C C C G G T C C G C C G C T G A G G A C G C T T C T C C A G A C T A C A A T T C G G A C G G C A A
<i>Lathyrus decaphyllus</i> voucher Jones 1001 26S ribosomal RNA gene	.
<i>Vicia ervilia</i> isolate Contemporary C 26S large subunit ribosomal RNA gene	.
<i>Cicer arietinum</i> isolate Contemporary D 26S large subunit ribosomal RNA gene	.
<i>Pisum sativum</i> uncharacterized LOC127114519	.
<i>Astragalus globosus</i>	.
<i>Astragalus canadensis</i> isolate DPP8 small subunit ribosomal RNA gene	A
<i>Astragalus crassicaarpus</i> voucher Jones 1003 26S ribosomal RNA gene	A

Figure 1. 26SrDNA BLAST result for endemic *L. karsianum* and *A. globosus* species.



**Figure 2.** Phylogenetic tree for two endemic species distributed in Kars (*L. karsianum* and *A. globosus*) with their relatives in Fabaceae family.

**Conflict of Interest:** There is no conflict of interest among the authors.

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