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IDENTIFICATION OF THREATENED AND ENDEMIC ANGELICA GLAUCA: INSIGHT FROM DNA BARCODING AND PHYLOGENETIC ANALYSIS

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ABSTRACT

Angelica glauca Edgew is a perennial non-vascular herb belonging to the family Apiaceae, having a high medicinal and economic value. However, *A. glauca* faces threats from climate change, habitat loss, and overharvesting, which reduce its natural populations. To address these issues, the DNA barcoding technique was employed as a taxonomic tool to confirm the accurate identification and support the conservation of this species. 2% CTAB was used to extract total genomic DNA, and DNA barcode markers such as (ITS2, *matK, rbcL,* and *rpoB*) were amplified and sequenced. Nucleotide sequence analysis of Angelica species revealed that the conserved, parsimony-informative, and singleton sites vary from primer to primer. The highest number of variable sites was recorded at 192 in the *matK* marker, followed by 170 in the *rpoB* marker. A phylogenetic tree was generated through the neighbour-joining method. It showed that *A. glauca,* based on the ITS2 sequence (LC860493), was closely clustered with *A. archangelica*-MT735413.1, based on *matK* (LC860494), with *A. archangelica*-GQ248079.1 based on *rbcL* (LC860492), with *A. archangelica*-GQ248549.1, and based on *rpoB* (LC593680) with *A. megaphylla*-NC057128.1, with bootstrap support of 63, 70, 100, and 43, respectively. The present study concluded that *A. glauca* is closely related to *A. archangelica* within the genus *Angelica*, and the markers used in this study are recommended for improved identification and conservation of other threatened or endangered plant species.

Keywords: *Angelica glauca,* Endemic, Threatened, Himalayan, DNA barcoding, and Phylogeny.

INTRODUCTION

For centuries, medicinal plants have been an essential component of the human healthcare system, especially in traditional medicine (Jamshidi-Kia *et al.,* 2017; Islam *et al.,* 2021). A variety of phytochemicals, including terpenoids, alkaloids, flavonoids, and phenolics, contribute to their therapeutic potential, which makes them essential for use in pharmaceuticals, nutraceuticals, and herbal remedies (Riar and Panesar, 2024). The Himalayan Mountain range, home to a wide range of medicinal plants, is also known as the pharmacy of the world. Due to the region's unique climate, elevation changes, and diverse ecosystems, a variety of economically viable plants native to the area have emerged (Wambulwa *et al.,* 2021). Himalayan Angelica, or *A. glauca*, is very important for medicine and economics (Singh *et al.,* 2020). This species comes from the temperate and sub-alpine areas of the western Himalayas (Gautam *et al.,* 2023).

A. glauca has been utilized in diverse herbal remedies for the treatment of respiratory, digestive, and inflammatory diseases (Arya *et al.,* 2021). People have known about the roots of this plant for a long time because they are full of essential oils, coumarin, and flavonoids, which make them suitable for digestion, killing bacteria, and fighting fungi (Rizvi *et al.,* 2022). It is also used as a spice and flavoring in local foods, which illustrates the relevance of spices to the area (Khoja *et al.,* 2024). Recent efforts to highlight it as a promising drug discovery have focused on its pharmacological capability to mitigate oxidative stress and its natural ability to treat a wide range of medical conditions (Dmytriv *et al.,* 2024). The threats to this species include habitat loss, overexploitation, and climate change, which are all harmful for the species' natural population. Based on these issues, conservation policies should be based on molecular data to help people use them in ways that are good for the environment. (Frankham *et al.,* 2019; Shinwari *et al.,* 2024).

DNA barcoding is a cost effective, modern, advanced, and reliable way to accurately identify a person down to the genus and species level. It is defined as a reference assembly by a short piece of DNA (400–

800 bp) (Antil *et al.,* 2023). In this approach, ITS2 and *matK* are clearly presented while the *rbcL, rpoB, trnH-psbA* are lineup for plant identification (Chen *et al.,* 2024). The main aim of this approach is to characterize the species in as short a time as possible. Furthermore, it can also be applied to study both intraspecies and interspecies variations in unidentified species (Letsiou *et al.,* 2024; Van Elst *et al.,* 2025).

However, *A. glauca* has not previously been studied and examined through advanced genomic and bioinformatics tools and techniques. The current study tested the use of DNA barcoding markers to determine the genetic variation in collected *A. glauca* and other database sequences of the genus *Angelica.* The finding of this work will not only contributes to the conservation of this species and improve its sustainable use as a medicinal resource, but also provide a framework for the identification and classification of new plant species using a similar method from Pakistan.

MATERIALS AND METHODS

STUDY AREA AND SAMPLES COLLECTION: This study was carried out in the Himalayan region of Pakistan. The western Himalayan region, including Azad Jammu and Kashmir, Gilgit-Baltistan, and parts of Khyber Pakhtunkhwa, is an ecologically significant area known for its diverse habitats, ranging from subtropical forests to alpine meadows, with elevations from 1,000 to 8,000 m (Sher *et al.,* 2021). *A. glauca* samples were collected from the Chorr valley of the district Battagram, the Himalayan region, Pakistan (Figure 1). Samples were identified and dried properly. For molecular analysis, the fresh leaves and dried roots were stored in a deep freezer at -20°C, and then three replicates were used for further experiments. The basic information of the collected species was recorded in Table. 1 and (Fig. 1).

Figure: 1. Morphology of *A. glauca*. (A) Young plant (B) flowers (C) freshly extracted root (D) dried roots

Table 1. Coordinates of *A. glauca* collected from the study area

Species name	Sample Collection	Location	Latitude N	Longitude E	Collection year
A. glauca	Flower/Leaves/Root	Chorr valley, Battagram, Himalayan Pakistan.	34.682346	73.017548	2019

DNA isolation and gel documentation: Genomic DNA was isolated with some modifications in the CTAB (Cetyl trimethyl ammonium bromide) method (Verma and Biswas, 2020). Fresh tissue was ground into a fine paste with the help of a mortar and pestle. About 900 µl of pre-warmed CTAB extraction buffer was added to 100 mg of ground tissue, and vortexed. The mixture was then incubated at 56°C for an overnight. After incubation, phenol chloroform-isoamyl alcohol (PCI) 600 µl was added, and the homogenate was centrifuged at 12,000 rpm for 20 minutes. After centrifugation, the supernatant was kept in the deep freezer for 1 hour. The chilled mixture was then centrifuged for 15 minutes at 12,000 rpm, the isopropanol was removed, and the pellet remained at the bottom of the Eppendorf tube. Then, 500 µl of 70% ethanol was added to wash the DNA and centrifuged for 5 minutes at 10,000 rpm (Abbasi Kejani *et al.,* 2010). The tubes were inverted to dry the pellet and to remove ethanol. For DNA dilution, 60 µL of ddH20 was added, and 5 µL of diluted DNA, along with 2 µL of loading dye, was loaded into the 1% agarose gel to confirm the extracted DNA **(Fig. 2)** (Cseke *et al.,* 2011).

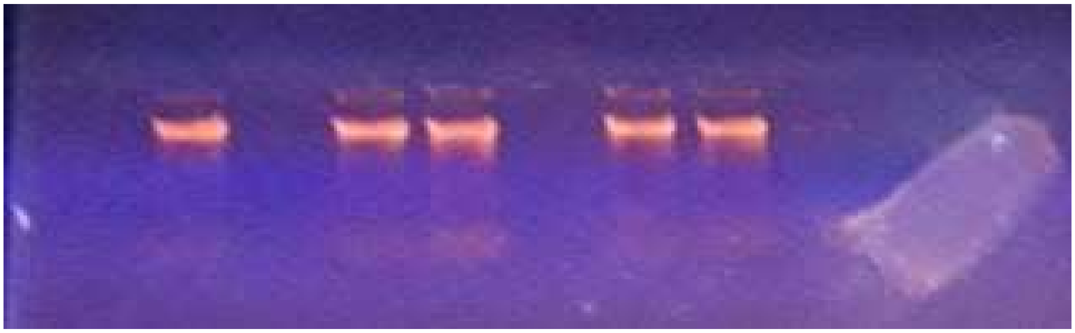

Figure: 2. A representative photograph showing the total genomic DNA isolated from the fresh leaves of the collected samples.

Primers selection and pcr amplification: Four DNA markers (Table 2) were selected, of which ITS2 is nuclear (Chen *et al*., 2010), whereas *matK, rbcL,* and *rpoB* are located on the chloroplast (Gao *et al.,* 2011; Singh *et al.,* 2012; Liu *et al.,* 2013). Amplification was carried out in thin-walled PCR tubes with a total volume of 25 µL of master mix. The master mix comprises 10x buffer 2.5 µL, MgCl2 2 µL, dNTPs 2 µL, DNA 2 µL, Taq polymerase 0.5 units, 2 µL primers (forward and reverse), and 14 µL distilled water. DNA marker amplification was carried out using a thermocycler (PCR). The thermocycler cycling protocol begins with an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 40 seconds, and extension at 72°C for 35 seconds. A final extension step was performed at 72°C for 10 minutes. The amplified PCR products were then analysed on a 2% agarose gel **(Fig. 3).**

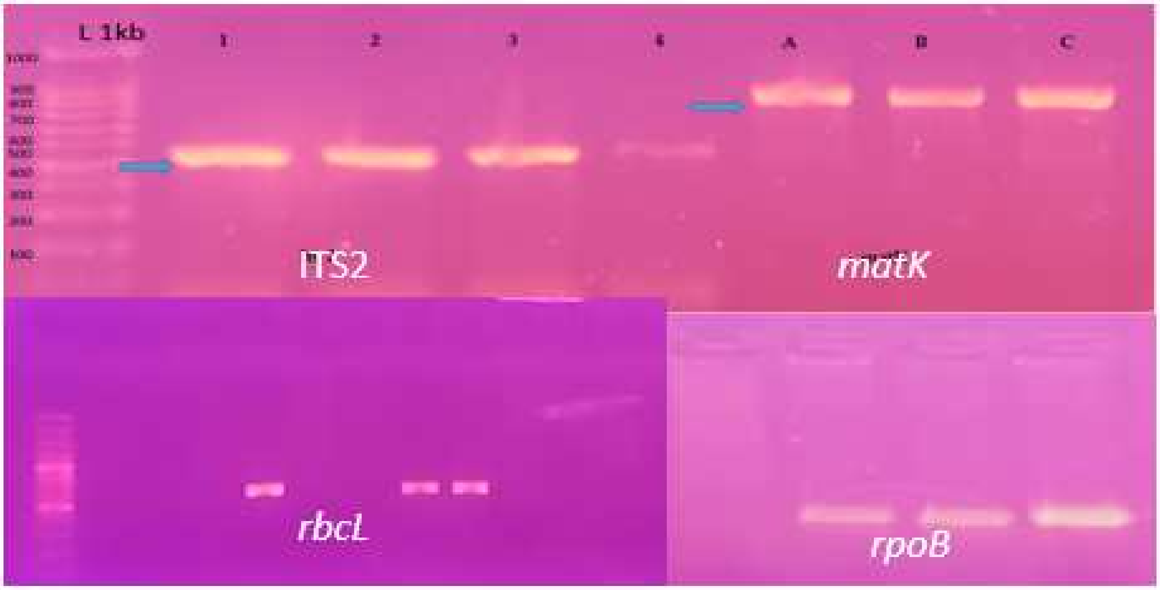

Figure: 3. A representative photograph showing the desired PCR amplified regions of the experimental organisms

Table: 2. Description of selected primers with the desired sequence

S. no	DNA Markers	5' to 3' sequence	References
1	ITS2	*5'ATGCGATACTTGGTGTGAAT3'* *5'GACGCTTCTCCAGACTACAAT3'*	(Chen *et al*., 2010)
2	*matK*	*5'CCTATCCATCTGGAAATCTTAG3'* *5'CGTAGTATATATCTCAATATACGC3'*	(Gao *et al*., 2011)
3	*rbcL*	*5' ATGTCACCACAAACAGAGACTAAAG 3'* *5'GCAGCAGCTAGTTCCGGGCTCCA3'*	(Liu *et al.,* 2013)
4	*rpoB*	*5'ATGCAACGTCAAGCAGTTCC3'* *5'GATCCCAGCATCACAATTCC3'*	(Sudmoon *et al.,* 2014)

Sequencing and data analysis: The desired amplified fragments were sequenced from Macrogn Inc., South Korea. The sequence quality was checked on Geneious software version 7 (Awomukwu *et al.,* 2015). The sequences were blasted in the online NCBI database to retrieve the most similar sequences for further analysis (Lindahl *et al*., 2013). BioEdit software was used to delete the rough sequences from both ends, start, and end of the sequences and arrange the sequence data for phylogenetic analysis (Sajjad *et al*., 2024). The phylogenetic tree was generated by the NJ method through Mega software (version 11) with 1000 replicates (Umer *et al.,* 2020).

RESULTS

In the current research, the collected specimens of *A. glauca* were evaluated using 4 different DNA markers, such as *matK, rbcL*, *rpoB*, and ITS2, which were further used for evaluation, identification, and genetic diversity. All the markers showed a 100% success rate in amplification and sequencing, while the identity success rate was greater than 90% **(Fig. 4),** and the percent identity, query cover, and E value of desired markers are presented in **Table 3**.

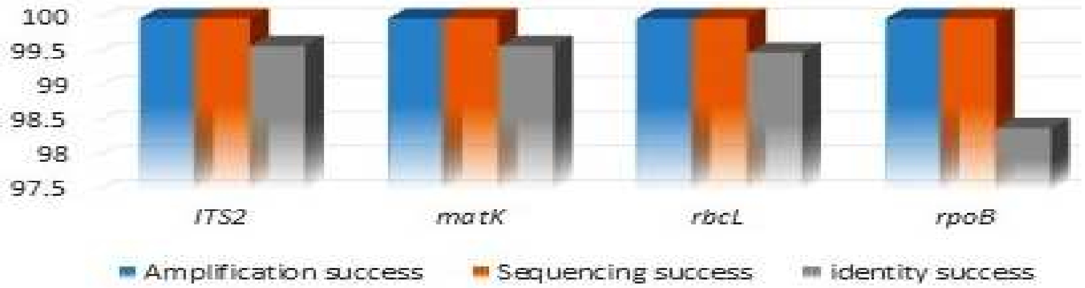

Figure: 4. A detailed description of the desired markers amplification, sequencing, and species identification

Table: 3. A detailed description of desired markers in Blast analysis.

DNA markers	Align seq (bp)	Query cover	Identity success	E-value
ITS2	599	100	99.6	0.0
matK	562	100	99.6	0.0
rbcL	564	100	99.5	0.0
rpoB	600	100	98.4	0.0

Sequences Analysis and Characterizations: The nucleotide sequence analysis was conducted between the query genotype and available reference sequences to determine the evolutionary lineage. The NJ method, which used 1000 replicates, was used to construct the phylogeny. The branch's value of less than 50% collapsed. To compute the pairwise distance and number of substitution sites, the Tamura-Nei model was used. In sequence analysis, various characteristics, conserved, variable, parsimony-informative, and singleton sites were recorded and presented in **Table. 4,** and these nucleotide sequences were submitted to the online NCBI database to obtain accession IDs **(Table 5).**

Table: 4. A detailed description of the molecular characterisation of DNA markers

DNA Markers	Total	Conserved	Variable	Parsimony	Singleton
ITS2	599	575	24	09	15
matK	562	369	192	190	02
rbcL	564	552	01	01	00
rpoB	600	333	170	02	168

Table: 5. The NCBI submitted sequences along with Accession IDs.

S. No	Markers	Locus	Accessions
1	ITS2	Nuclear	LC860493
2	*matK*	Chloroplast	LC860494
3	*rbcL*	Chloroplast	LC860492
4	*rpoB*	Chloroplast	LC593680

Molecular characterizations and phylogeny based on ITS2 marker: The *A. glauca* sequence was subjected to BLAST in the online NCBI database to verify the alignment score, homology, and retrieve the closely related reference sequences. The total sequence length of 625 nucleotides was blasted, resulting in 100% identity and 99% query coverage, and having an E-value of 0.0. The query sequences, along with retrieved database sequences, were trimmed from both ends, and the final dataset of 599 base pairs was obtained and utilised for molecular characterisation and phylogeny. As a result, 575 conserved, 24 variables, 09 parsimony, and 15 singletons were recorded. Based on sequence data, a phylogenetic tree was constructed using the neighbour-joining method by MEGA software version 11. In the phylogenetic tree, 15 sequences, including the query sequence, were used; as a result, two major clades, i.e., clades A and B, and three sub-clades in AI, AII, and AIII were formed. Clade A comprises 10 genotypes, of which 4 are in clade AI (*A. glauca*, *A. decurrens*-MT735414.1, *A. archangelica*-MT735411, and *A. archangelica*-MT735413.1), 3 in AII (*A. brevcaulis*-MT735423.1, *A. ampla*-MT735409.1, and *A. atropurpurea*-MT735421.1), and 3 in AIII (*A. sylvestris*-MT922576.1, *A. tschimganica*-MT735418.1, and *A. brevcaulis*-MT735417.1) (highlighted with green and light green color). While clade B comprises 5 reference sequences (*A. acutiloba*-GU3951447.1, *A. stenoloba*-AB697608.1, *A. razulii*-OQ064660.1 *A. yakusimensis*-MT735472.1, and *A. pubescens*-MT735475.1) (highlighted red). The query sequence *A. glauca* makes a close tie with (*A. archangelica*-MT735413.1) with strong bootstrap support of 63 in sub-clade AI (highlighted with a dark green) (**Fig. 5**). DNA barcodes and QR (quick response codes) of query sequences were generated through online Bio-Rad and QR code generator software and presented in **Fig. 6.**

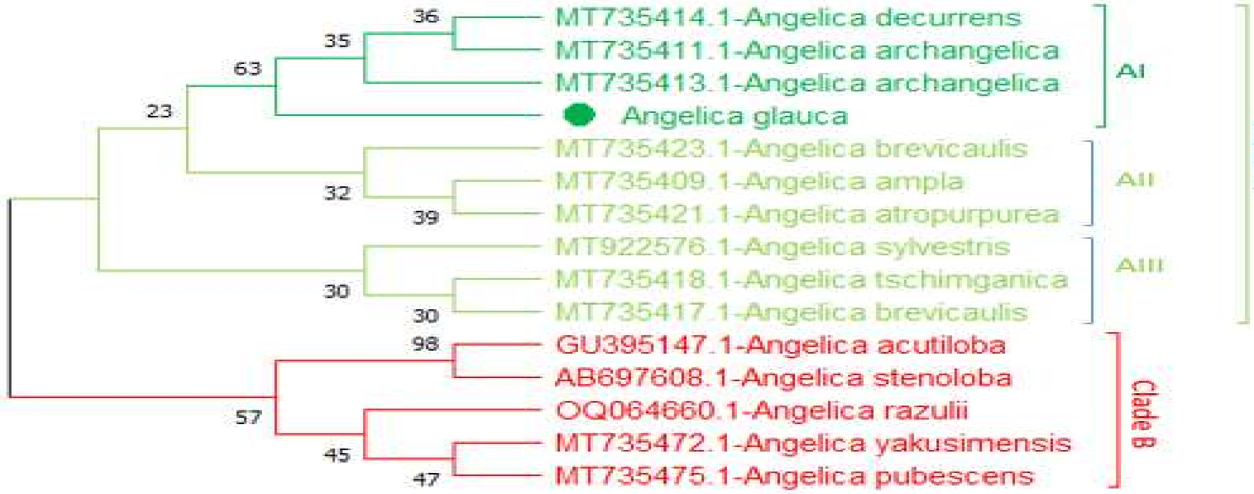

Figure 5. A phylogenetic tree based on an ITS2 marker shows an evolutionary relationship between *A. glauca* and reference species through the neighbour joining method.

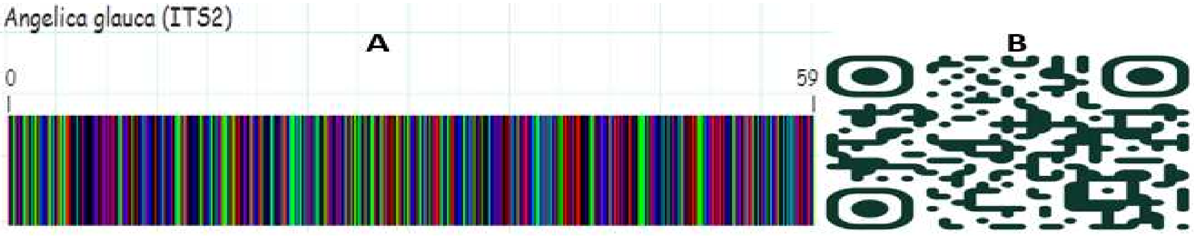

Figure: 6. DNA barcode (A) and QR code (B) of the *A. glauca* based on *ITS2 marker.*

Molecular characterizations and phylogeny based on *matK* marker: The total sequence length of 580 nucleotides was blasted, resulting in 100% identity, a query cover of 99%, and an E-value of 0.0. After trimming in BioEdit software, both the query and retrieved database sequences from both ends, the final dataset of 562 base pairs was obtained. In which 369 conserved sites, 192 variable sites, 190 parsimonies, and only 02 singleton sites were recorded. In the phylogenetic tree, a total of 15 sequences, including query sequences, were used; as a result, two major clades, i.e., clades A and B, and two sub-clades, AI and AII, were formed. Clade A comprises 9 accessions, 5 in clade AI (*A. glauca*, *A. archangelica*-GQ248079.1, *A. decurrens*-

KX526484.1. *A. archangelica*-EF590395.1, and *A. sylvestris*-MT917208.1) (highlighted with a dark green colour) and 4 in AII (*A. shikokiana*-AB697588.1, *A. polymorpha*-MH714151.1, *A. amurensis*-MT119236.1, and *A. anomala*-MT119239.1) (highlighted light green). While clade B comprises 6 accessions (*A. tsinlingensis*-OM281944.1, *A. porphyrocaulis*-NC057136.1, *A. acutiloba*-KX352468.1, *A. apaensis*-NC057132.1, *A. porphyrocaulis*-OR209147.1, and *A. gigas*-MW900177.1) (highlighted in red). The query *A. glauca* sequence closely ties with (*A. archangelica*-GQ248079.1) with bootstrap support of 70 **(Fig. 7).** DNA barcodes and QR (quick response codes) of the query sequence were generated through online Bio-Rad and QR code generator software and presented in **Fig. 8.**

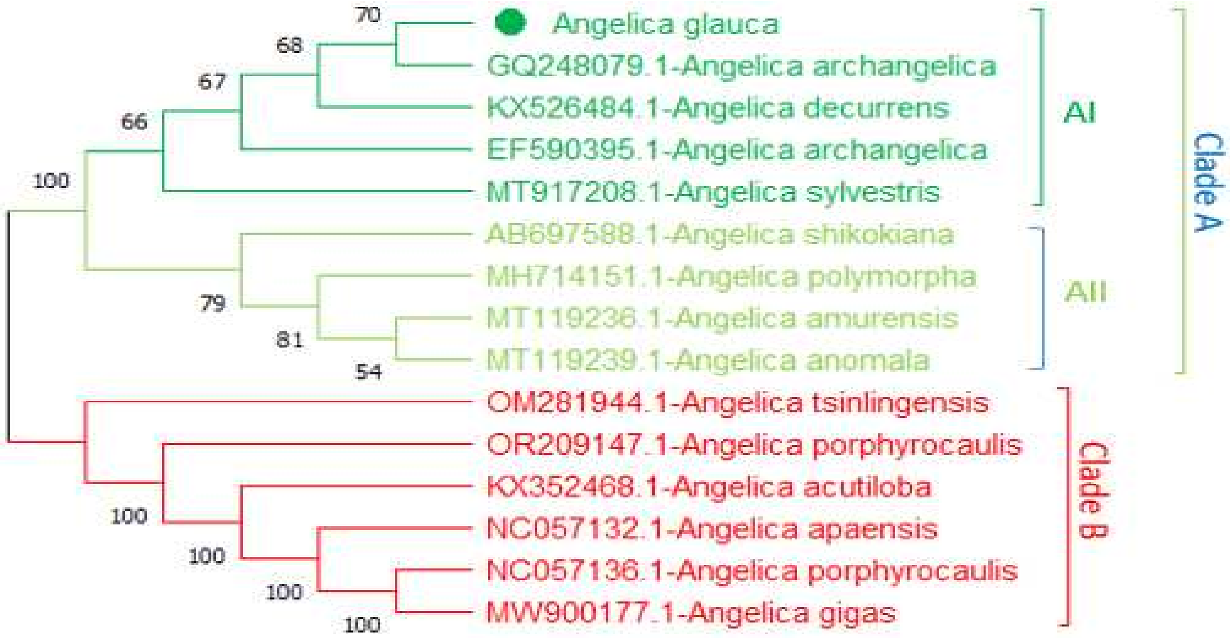

Figure: 7. Phylogenetic tree based on *matK* marker showing evolutionary relationship of *A. glauca* with reference species through neighbour joining method.

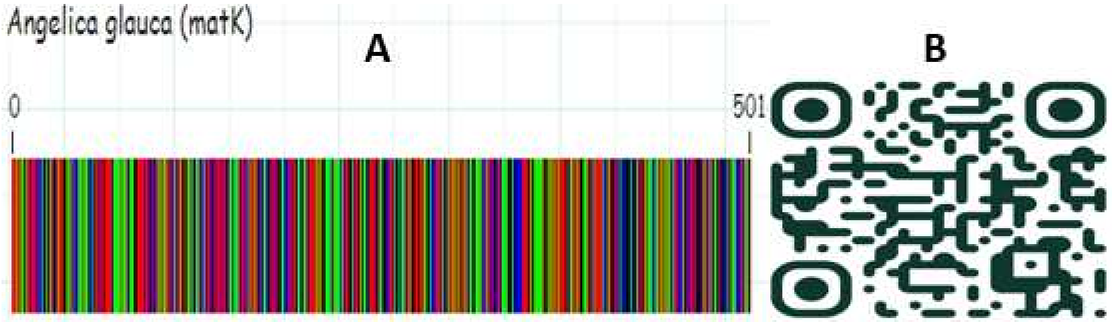

Figure: 8. DNA barcode (A) and QR code (B) of the *A. glauca* based on *matK* marker.

Molecular characterizations and phylogeny based on *rbcL* marker: The total sequence length of 589 nucleotides was blasted, resulting in 100% identity and 99.5% query coverage, and having an E-value of 0.0. After trimming in BioEdit, the query and retrieved database sequences were trimmed from both ends, yielding a final dataset of 564 base pairs. In which 552 conserved sites, 01 variable site, 01 parsimony, and no singleton sites were recorded. Based on the observed characterisations, a phylogenetic tree was constructed using 15 sequences, including the query sequence; as a result, two major clades, i.e., clade A and B, and two subclades in clade A (AI and AII) were formed. Clade A comprises 12 accessions, 9 of which are in sub-clade AI, such as *A. stenoloba*-LC632480.1, *A. lucida*-MG224582.1, *A. saxatilis*-MW436381.1, *A. hirsutiflora*-NC080210.1, *A. acutiloba*-KX352468.1, and *A. atropurpurea* KF613025.1) (highlighted light green) and 3 in sub-clade AII (*A. glauca*, *A. sylvestris*-MT930258.1, and *A. archangelica*-GQ248549.1) (highlighted with a dark green). While clade B comprises only 3 sequences, such as (*A. valida*-NC057129.1, *A. polymorpha*-

MN192452.1, and *A. dahurica*-OR209160.1) (highlighted in red). The query *A. glauca* sequence closely ties with (*A. archangelica*-GQ248549.1) in sub-clade AII with a strong bootstrap support of 100 **(Fig. 9).** DNA barcode and QR (quick response codes) of query sequences were generated through online Bio-Rad and QR code generator software and presented in **Fig. 10.**

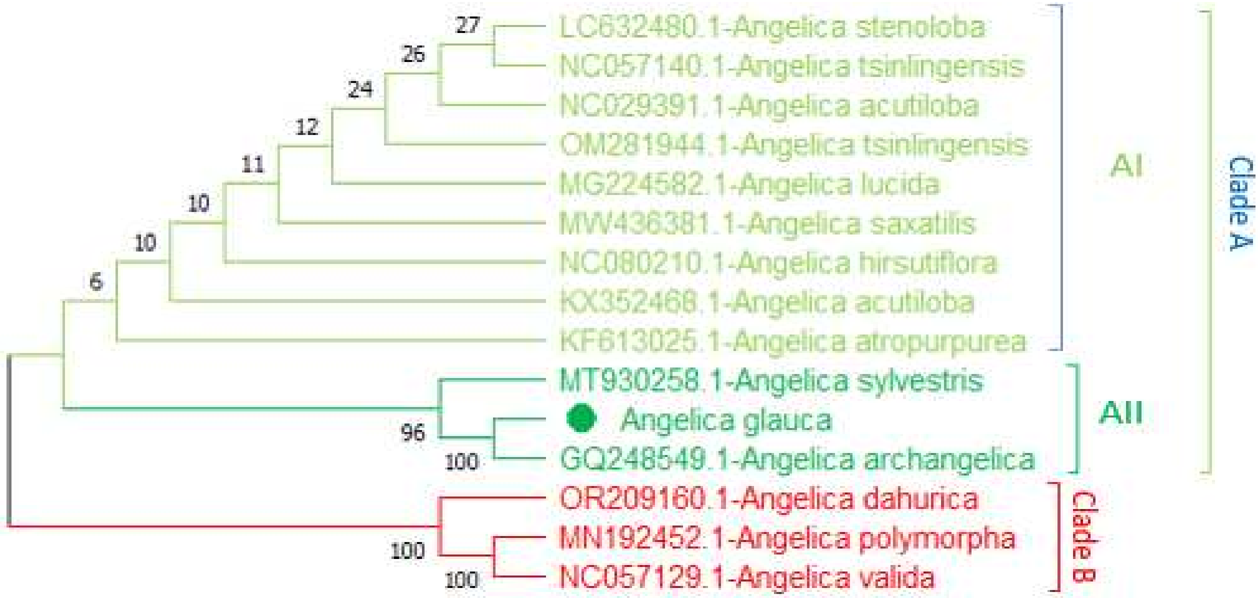

Figure: 9. Phylogenetic tree based on *rbcL* marker showing the evolutionary relationship of *A. glauca* with reference species through neighbour joining method.

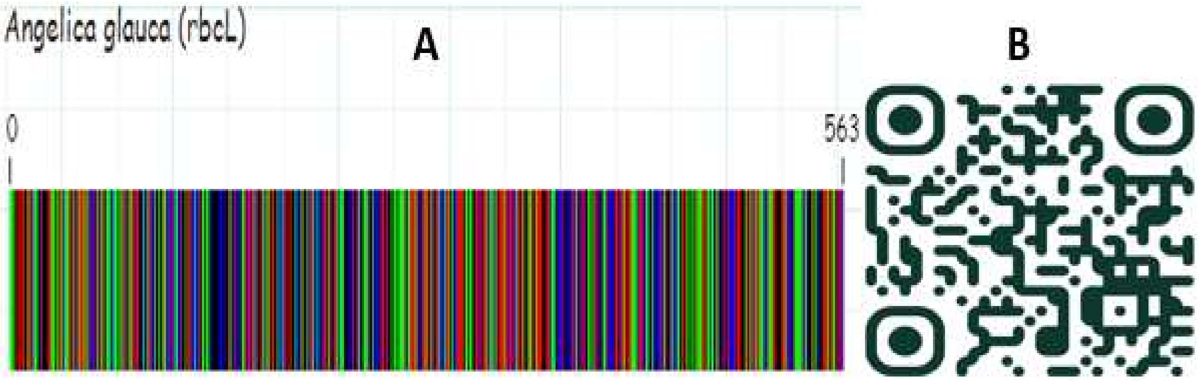

Figure: 10. DNA barcode (A) and QR code (B) of the *A. glauca* based on *rbcL* marker.

Molecular characterization and phylogeny based on *rpoB* marker: The total sequence length of 623 nucleotides was blasted, resulting in 100% identity and 98% query coverage, and having an E-value of 0.0. After trimming in BioEdit software, both the query and retrieved database sequences from both ends, the final dataset of 600 base pairs was obtained. In which 333 conserved sites, 170 variable sites were, 02 parsimony, and 168 singleton sites were recorded. Based on the observed characterisations, a phylogenetic tree was established in which 13 accessions, including the query sequence, were used; as a result, two major clades formed, i.e., clade A and B. Clade A comprises a total of 8 sequences, such as (*A. glauca, A. megaphylla*-NC057128.1, *A. laxifoliata*-MT921969.1, *A. acutiloba*-NC029391.1, *A. tsinlingensis*-OM281944.1, *A. saxatilis*-MW436381.1, *A. laevigata*-MW696157.1 and *A. valida*-NC057129.1) (highlighted with a dark green colour), and clade B comprises only 5 sequences (*A. dahurica*-OR209160.1, *A. ternate*-MT561043.1, *A. multicaulis*-MT561027.1, *A. paeoniifolia*-MT561026.1 and *A. sinensis*-MW820164.1) (highlighted in red colour). The query *A. glauca* sequence closely ties with (*A. megaphylla*-NC057128.1) with a bootstrap value of 43 **(Fig. 11).** DNA barcode and QR (quick response code) of query sequence were generated through online Bio-Rad and QR code generator software and presented in **Fig. 12.**

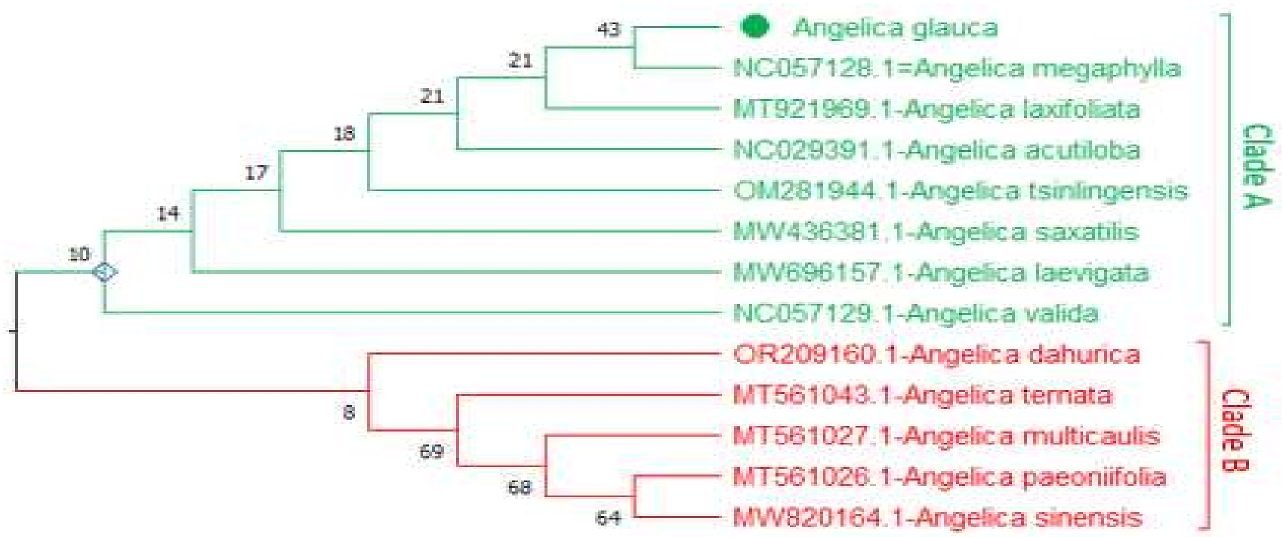

Figure: 11. Phylogenetic tree based on *rpoB* marker showing evolutionary relationship of *A. glauca* with reference species through neighbour joining method.

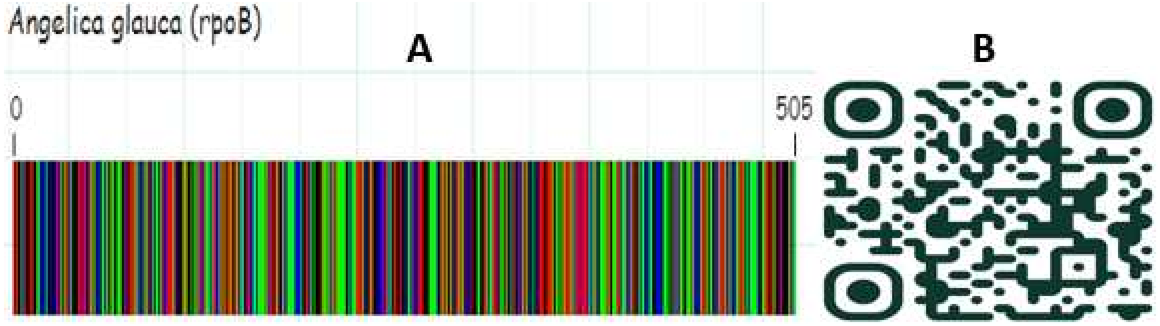

Figure: 12. DNA barcode (A) and QR code (B) of the *A. glauca* based on *rpoB* marker.

DISCUSSION

Current research work is aimed at evaluating the potential DNA barcoding markers, such as ITS2, *matK, rbcL,* and *rpoB;* their amplification success; sequencing success; and identification success rate for the *A. glauca* in the western Himalayan region, and establishing their phylogenetic relationships with other members of the genus *Angelica*. The current study results of the desired markers in amplification and sequencing were 100%, while they were greater than 90% in *Angelica* species identification. In DNA barcoding and characterisation of barcode regions, there were 575 conserved sites in ITS2, 369 in *matK,* 552 in *rbcL,* and 333 in *rpoB.*

The variable sites were 24 in ITS2, 192 in *matK,* 01 in *rbcL,* and 170 in *rpoB*. The parsimony informative and singleton sites in ITS2 were 09 and 15, in *matK* were 190 and 02, in *rbcL* were 01 & 00, and in *rpoB* were recorded. In the phylogenetic analysis, the trees were generated through the neighbour-joining method, and the resultant query species *A. glauca* makes a close tie with (*A. archangelica*-MT735413.1) based on ITS2 with strong bootstrap support of 63. The internal transcribed spacer (ITS) region, especially ITS2, has demonstrated high species discrimination power due to its high variability and has been recommended as a universal DNA barcode for medicinal plants (Chen *et al.,* 2010; Yao *et al.,* 2010). (*A. archangelica*-MT735413.1) is previously reported in the USA (Yang *et al.,* 2012). The ITS2 region was previously used and studied to identify *A. sinensis* within the genus *Angelica* (Nazar *et al.*, 2024). In a previous study, Hollingsworth *et al.,* (2011) used the ITS2 region as a DNA barcode to differentiate the *A. dahurica* and *A. sinensis.* Based on chloroplast marker *matK A. glauca* make a close relationship with (A. archangelica-GQ248079.1) and having a strong bootstrap support of 70. (A. archangelica-GQ248079.1) was previously reported from Canada by Liao *et al.,* (2022). In the previous study of He *et al.,* (2011), *matK* was used as a core barcode to differentiate Angelica species such as A. dahurica, A. sinensis, and A. pubescens.

Based on rbcL-phylogeny, *A. glauca* closely shares a clade with 100 bootstrap support with *A. archagelica* (GQ248549.1). (*A. archagelica-*

GQ248549.1) was previously documented from Canada by Liu *et al.* (2021). A previous work on the comparative analysis of the chloroplast genome within the genus *Angelica* confirmed the efficacy of both *matK* and *rbcL* in clarifying the phylogenetic linkage of *A. sinensis* (Hollingsworth *et al.,* 2009). Both the chloroplast regions *matK* and *rbcL* are the most predominant DNA barcoding markers in plants. In the genus *Angelica, matK* shows higher variability as compared to *rbcL*, rendering it more effective in differentiating the closely related species (Noh *et al.,* 2021). However, *rbcL* has greater rates of amplification and sequencing success because it has conserved region but may not have enough resolution power to identify the species within the genus *Angelica* (Hollingsworth *et al.,* 2009).

The chloroplast marker *matK* was effective in evolutionary and phylogenetic relationships, and both *matK* and *rbcL* were previously used to discriminate the closely related Angelica species such as *A. gigas* and *A. archangelica* (Huang *et al.,* 2021). The efficacy of the *matK* marker in describing the evolutionary relationship differentiation of many Angelica species, such as *A. pubescens* and *A. glauca* (Plunkett *et al.,* 1997). Similarly, in the previous studies, the DNA barcoding method, including the *matK* and their authentication in medicinal *Angelica* species, and the results effectively distinguish between *A. sinensis* (Yuan *et al.,* 2015; Zhu *et al.,* 2022). *A. biserrata* and *A. dahurica* with good identification achievement (Lyu *et al.,* 2022).

Based on *rpoB*, the current sequence of *A. glauca* makes a close tie with *A. megaphylla* (NC057128.1) with a bootstrap value of 43. (*A. megaphylla*-NC057128.1) was previously reported from the USA. Similar results were found that the *rpoB* marker alone is less discriminatory than *rbcL* and *matK* but can complement other markers in specific cases (Chase *et al*., 2007). On the other hand, *rpoB,* among other plastid markers, identifies the genetic diversity in medicinal plants from the family Apiaceae, emphasising its usage in species differentiation (Haider, 2011). Similarly, in a previous study, the *rpoB* marker was combined with other markers to genetically characterise *O. arabicus*, a medicinal plant, distinguishing it from closely related species (Al-Juhani, 2019).

Conservation through conventional methods mainly focuses on protecting plants in their natural habitats (in situ) or in controlled settings (ex situ) (Zegeye, 2017). This technique needs the establishment of protected areas, reserves, national parks, or cultivation in botanical gardens. The strength of this method is that it preserves the whole ecosystem, not just species, maintains ecological interaction, and is easy for local communities to adopt (Sadhana, 2024). However, these techniques have some limitations, as this method is time-consuming and resource-intensive, morphological identification may be inaccurate, especially in closely related species, which cannot always detect cryptic diversity, and seed viability may decline in storage (Krishnamoorthi, 2024).

In comparison with the conventional conservation method, DNA barcoding uses a short standardized DNA region for species identification, authentication, and genetic assessment. In this technique, small tissues, powders, or seedlings can be used for identification, detection of adulteration and substitution in herbal trade, phylogenetic analysis for evolutionary relationships, and identification of cryptic, endangered, or misidentified species (Antil *et al.,* 2023). This technique is rapid and accurate, helps authenticate medicinal plants in trade, reducing overharvesting of rare species, detects genetic variation useful for selecting genotypes for conservation and cultivation, and complements conventional taxonomy when morphology is insufficient (Abdi *et al.,* 2024).

Conclusion: The current study found that all primers yielded satisfactory results in both amplification sequencing and species identification. Nucleotide sequence analysis revealed conserved, variable, parsimony-informative, and singleton sites in *Angelica* species. Phylogenetic analysis revealed the highest genetic relationships of *A. glauca* with *A. archangelica*. DNA barcodes generated during this study for *A. glauca* Edgew, which is an economically important threatened/endangered medicinal species, can be saved in the online database for future use.

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