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Research Article

Genetic Authentication of *Syzygium mundagam* Leaves using rbcL DNA Barcoding

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ABSTRACT

Syzygium mundagam belongs to Myrtaceae family. DNA barcoding is an effective molecular method used for the identification and authentication of medicinal plants. Syzygium mundagam, a medicinal plant species belonging to the family Myrtaceae and native to the Western Ghats, is widely known for its therapeutic importance. Due to similarities with other species, correct identification based only on morphology can be difficult. This study aimed to authenticate Syzygium mundagam leaves using DNA barcoding techniques. Fresh leaf samples were collected, and genomic DNA was isolated and amplified using standard barcode markers such as rbcL and ITS through PCR analysis. The amplified sequences were compared with reference sequences available in genetic databases for species confirmation. The results showed accurate identification of Syzygium mundagam, proving that DNA barcoding is a reliable tool for medicinal plant authentication. The study highlights the importance of molecular identification in ensuring quality control and preventing adulteration in herbal medicines.

INTRODUCTION

Reliable identification of plant species is fundamental to taxonomic research, biodiversity conservation, and the effective management of biological resources. Although morphological characters remain the primary basis for species identification, they may vary with developmental stage, environmental conditions, and natural phenotypic variation, making the discrimination of closely related taxa difficult. Molecular approaches have therefore become valuable tools for confirming species identity and supporting traditional taxonomic methods.

DNA barcoding has become an established method for plant identification by analyzing short, standardized DNA regions that can distinguish

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species based on genetic variation. Among the recommended barcode loci for land plants, the chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene is one of the most widely used because it is easily amplified across a broad range of taxa and produces high-quality sequence data. Its extensive representation in global sequence repositories also makes *rbcL* suitable for comparative analyses and species authentication.

The genus *Syzygium* (Myrtaceae) is a diverse group of woody plants distributed throughout tropical and subtropical regions, with many species possessing ecological, medicinal, and economic importance. However, accurate identification within the genus is often complicated by similarities in vegetative and reproductive characters. *Syzygium mundagam*, an endemic species of the Western Ghats, has received limited molecular attention despite its restricted distribution and conservation significance. Generating DNA barcode data for this species will strengthen its molecular documentation and facilitate future taxonomic and conservation studies.

Therefore, the present study employs the chloroplast *rbcL* gene as a DNA barcode to genetically identify *Syzygium mundagam* using leaf samples. The obtained sequence was compared with authenticated reference sequences available in public databases to verify species identity and contribute to the expanding molecular database of the genus *Syzygium*.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Syzygium mundagam* were collected from healthy plants and used for molecular analysis. Approximately 100 mg of leaf tissue was used for genomic DNA extraction.

Genomic DNA Isolation

Genomic DNA was extracted using the NucleoSpin® Plant II Kit (Macherey-Nagel, Germany) following the manufacturer's protocol. Leaf tissue was ground to a fine powder in liquid nitrogen before lysis. After RNase treatment, the lysate was purified through a silica membrane column to remove proteins, polysaccharides, and other contaminants. Purified DNA was eluted in 50 µL of the supplied elution buffer and stored at 4°C until further use.

DNA Quality Assessment

The quality of the extracted DNA was assessed by electrophoresis on a 0.8% agarose gel prepared with 0.5× Tris–Borate–EDTA (TBE) buffer containing ethidium bromide (0.5 µg mL^{-1}). DNA samples were mixed with 6× loading dye before loading onto the gel. Electrophoresis was carried out at 75 V, and DNA bands were visualized under ultraviolet illumination using a Bio-Rad Gel Documentation System.

PCR Amplification of the *rbcL* Barcode Region

The chloroplast *rbcL* gene was amplified using the universal primer pair rbcL-AF (5′-ATGTCACCACAAACAGAGACTAAAGC-3′) and rbcL-724R (5′-TCGCATGTACCTGCAGTAGC-3′). PCR amplification was performed in a total reaction volume of 10 µL containing 5 µL of 2× Phire Master Mix, 1 µL of genomic DNA, 0.25 µL each of forward and reverse primers, and nuclease-free water to the final volume. Amplification was carried out in a GeneAmp PCR System 9700 (Applied Biosystems, USA) under the following conditions: an initial denaturation at 98°C for 1

min; 40 cycles of denaturation at 98°C for 5 s, annealing at 58°C for 10 s, and extension at 72°C for 15 s; followed by a final extension at 72°C for 2 min.

PCR Analysis

2X Phire Master Mix	5µL
D/W	4µL
Forward Primer	0.25µL
Reverse Primer	0.25µL
DNA	1µL

Primers used

Target	Primer Name	Direction	Sequence (5' → 3')
RBCL	RBCL-AF	Forward	ATGTCACCACAAACAGAGACTAAAGC
	RBCL-724R	Reverse	TCGCATGTACCTGCAGTAGC

PCR amplification profile

RBCL

98 ºC - 1 min

98 ºC - 5 sec

58 ºC - 10 sec } 40 cycles

72 ºC - 15 sec

72 ºC - 2 min

4 ºC - ∞

Analysis of PCR Products

Amplification products were separated on a 1.2% agarose gel prepared in 0.5× TBE buffer containing ethidium bromide. Electrophoresis was performed at 75 V using a 2-Log DNA Ladder (New England Biolabs, USA) as the molecular size marker. The amplified fragments were visualized under UV light and photographed using a gel documentation system.

Purification and DNA Sequencing

PCR products that produced clear single bands were purified using ExoSAP-IT™ (GE Healthcare, USA) to eliminate excess primers and unincorporated nucleotides. Purified amplicons were sequenced by the Sanger dideoxy chain termination method using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequencing reactions were purified by ethanol precipitation before capillary electrophoresis on an ABI 3500 Genetic Analyzer (Applied Biosystems, USA).

The Sequencing PCR mix consisted of the following components:

D/W	6.6µL
5X Sequencing Buffer	1.9µL
Forward Primer	0.3µL
Reverse Primer	0.3µL
Sequencing Mix	0.2µL
Exosap treated PCR product	1µL

Sequencing PCR amplification profile

96ºC -2min

96ºC -30sec

50ºC -40sec } 30 cycles

60 ºC -4min

4 ºC -∞

Sequence Editing and Identification

Raw sequence chromatograms were inspected for quality using Sequence Scanner Software v1.0 (Applied Biosystems, USA). Forward and reverse sequences were assembled, edited, and aligned in

Geneious Pro v5.1 to generate a consensus sequence. The resulting *rbcL* sequence was compared with reference sequences available in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST) to confirm the genetic identity of *Syzygium mundagam*. The validated sequence was subsequently used for downstream analyses.

RESULTS AND DISCUSSION

DNA Isolation and Quality Assessment

High-quality genomic DNA was successfully isolated from fresh leaves of *Syzygium mundagam* using the NucleoSpin® Plant II Kit. Agarose gel electrophoresis revealed intact, high-molecular-weight DNA with minimal smearing, indicating good DNA integrity and the absence of significant degradation .The extracted DNA was of sufficient quality for subsequent PCR amplification and sequencing.

PCR Amplification of the *rbcL* Gene

The chloroplast *rbcL* region was successfully amplified using the universal primer pair rbcL-AF and rbcL-724R. Agarose gel electrophoresis of the PCR products showed a distinct single band of approximately 550 bp ,indicating specific amplification without detectable non-specific products .The successful amplification demonstrates the suitability of the extracted DNA for DNA barcoding studies.

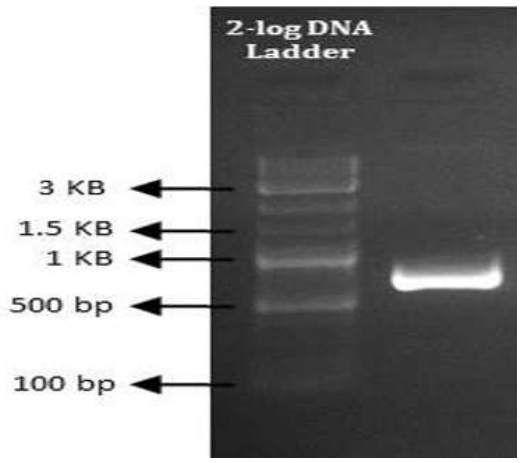

Fig 1: Agarose gel electrophoresis of the PCR-amplified rbcL gene from *Syzygium mundagam* leaves

DNA Sequencing and Species Identification

The purified PCR product yielded high-quality bidirectional sequences. After sequence editing and assembly, a consensus *rbcL* sequence of approximately 550 bp was obtained. BLAST analysis against the NCBI GenBank database showed the highest similarity 98.73 % with *Syzygium mundagam* confirming the taxonomic identity of the collected specimen. The sequence quality was sufficient for reliable molecular identification and may serve as a reference barcode for this species.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc Len	Accession
Syzygium caryophyllatum ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; chloroplast	1114	1114	86%	0.0	98.73%	1255	KU381786.1
Syzygium cumini voucher CIMAP:SC1 E702 Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial c...	1107	1107	88%	0.0	97.82%	647	OR923588.1
Syzygium cumini voucher CIMAP:SC2 E703 Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial c...	1105	1105	87%	0.0	97.82%	646	OR923589.1
Syzygium kanneliyense chloroplast, complete genome	1122	1122	89%	0.0	97.78%	158630	PX138333.1
Syzygium cinereum chloroplast, complete genome	1122	1122	89%	0.0	97.78%	158399	NC_086715.1
Syzygium glomeratum chloroplast, complete genome	1122	1122	89%	0.0	97.78%	158469	PP734815.1
Syzygium alubo chloroplast, complete genome	1122	1122	89%	0.0	97.78%	158786	PX138396.1
Syzygium latifolium chloroplast, complete genome	1122	1122	89%	0.0	97.78%	158503	PX125982.1
Syzygium cumini chloroplast, complete genome	1116	1116	89%	0.0	97.55%	158996	NC_053327.1
Syzygium polyestalobeum chloroplast, complete genome	1116	1116	89%	0.0	97.55%	158420	PX138356.1
Syzygium fluviatile chloroplast, complete genome	1116	1116	89%	0.0	97.55%	158550	NC_082026.1
Syzygium formosii chloroplast, complete genome	1116	1116	89%	0.0	97.55%	158996	NC_044106.1

Fig 2 : NCBI BLAST results of the rbcL gene sequence of Syzygium mundagam

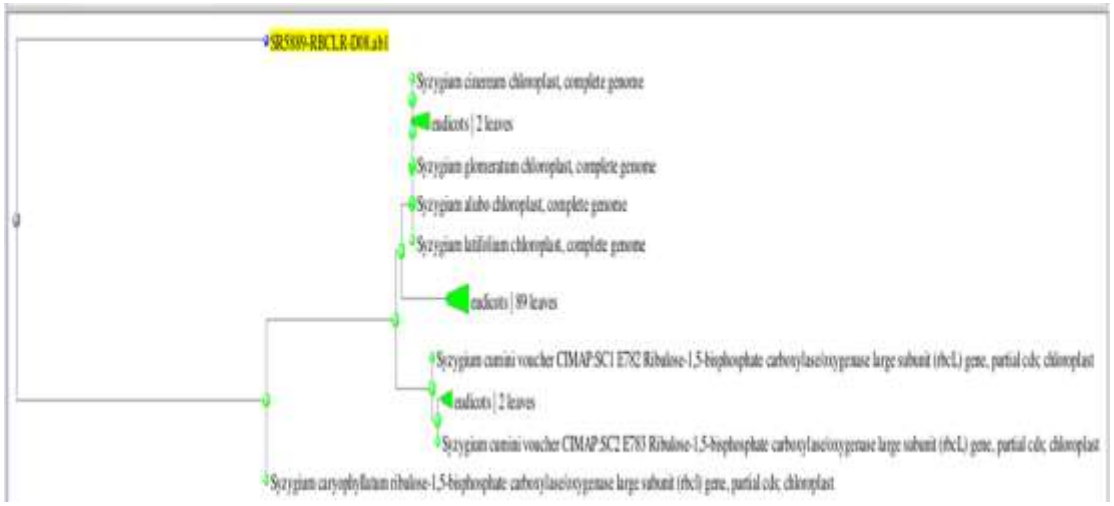

Fig 3 : Phylogenetic tree based on rbcL gene sequences illustrating the evolutionary relationship of *Syzygium mundagam* with closely related Syzygium retrieved from NCBI BLAST

DISCUSSION

DNA barcoding has become an effective molecular approach for the identification and authentication of plant species, particularly those exhibiting high morphological similarity. In the present study, the chloroplast *rbcL* marker was successfully amplified and sequenced from *Syzygium mundagam*. The production of a single, distinct PCR amplicon and the acquisition of high-quality sequence data demonstrate the effectiveness of the *rbcL* primers for this species.

The high sequence similarity observed during BLAST analysis confirms the identity of *S. mundagam* and supports the reliability of the *rbcL* gene as a barcode marker for species authentication. The conserved nature of the *rbcL* locus enables consistent amplification across a broad range of angiosperms while providing

sufficient sequence variation for species-level identification in many plant groups. These characteristics have led to its widespread use in plant taxonomy, biodiversity assessment, and molecular authentication.

The barcode sequence generated in this study contributes valuable molecular information for *S. mundagam*, an endemic species of the Western Ghats. Such reference sequences are important for supporting future taxonomic studies, conservation planning, phylogenetic analyses, and the development of authenticated DNA barcode databases. The integration of molecular barcoding with conventional morphological identification enhances confidence in species identification and provides a robust framework for documenting endemic plant diversity.

CONCLUSION

The present study demonstrated the effectiveness of chloroplast *rbcL*-based DNA barcoding for the genetic identification of *Syzygium mundagam*. The study successfully isolated good-quality genomic DNA, amplified the target barcode region, and generated sequence information suitable for molecular analysis. The obtained *rbcL* sequence provided reliable evidence for species authentication and highlights the usefulness of this marker in supporting taxonomic identification of *Syzygium* species. The generated molecular data serve as a valuable reference resource for future studies involving species diversity, phylogenetic relationships, and conservation management of this endemic plant.

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