RESEARCH PAPER

DNA barcoding based molecular identification and phylogenetic characterization of Vateria indica L. using rbcL marker

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ABSTRACT

Exact identification of red data plant species is essential for proper biodiversity management and conservation. In the current study, DNA barcoding was employed for the molecular identification of the vulnerable plant Vateria indica using rbcL barcode gene. Fresh leaf samples of Vateria indica plant species were collected from Bonacaud forest area, Agasthya hills, Trivananthapuram district, Kerala, India. Genomic DNA was isolated and the rbcL region was amplified through PCR, followed by the sequencing of the same. The obtained sequence was analysed using BLAST and phylogenetic techniques. The studied species identity was confirmed by BLAST analysis, and it revealed a high similarity (99%) with other reference sequences found in public databases. The present obtained sequence was further validated through phylogenetic analysis with other related reference taxa to show the closeness and differences among them.

Key words: BLAST, conservation genetics, DNA barcoding, IUCN, rbcL, red list, species authentication, Western Ghats.

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1. Introduction

Biodiversity conservation is heavily reliant on precise species identification, especially for vulnerable and rare plants. Misidentification can result in poor conservation initiatives, incorrect genetic resource use, and biodiversity loss. Conventional taxonomy based on morphological traits frequently has limitations due to phenotypic plasticity, environmental variation, and the absence of reproductive structures during field collection. In recent years, molecular techniques have gained importance for precise taxonomic authentication and biodiversity evaluation .[1-2]

DNA (Deoxyribonucleic Acid) barcoding technique has developed as a quick and standardized approach to identify floral and faunal species using small and conserved DNA sequences. Chloroplast markers like rbcL (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) and matK (maturase K) are widely acknowledged as core barcode regions in plants due to their universality and reproducibility across taxa.[3-4] Recent breakthroughs in DNA barcoding have emphasized its potential for conservation biology, medicinal plant authentication, ecological investigations and biodiversity monitoring.[5-6]

Though chloroplast barcoding genes rbcL and matK are used widely in plant DNA barcoding studies, the rbcL gene is preferred for its high amplification success, conserved primer regions and availability of extensive reference sequences in public databases which all lead to the base plant species identification. Further, due to its universality and consistent amplification over a wide range of plant taxa, rbcL is a useful marker for molecular identification and phylogenetic research.[7-8] Furthermore, recent research has shown that rbcL-based DNA barcoding can be valuable in conservation-oriented genetic studies as well as medicinal plants authentication.[9]

The India's Western Ghats is one of the world's important biodiversity hotspots because of its rich and diversity of living entity and ecological differences. The Agasthya hill region in Southern Western Ghats is harboured to different red data category of plant species with conservation significance. However, habitat fragmentation, deforestation, climate change and anthropogenic disturbances continue to endanger many native taxa, highlighting the importance of proper molecular documentation and conservation research.[10-11]

Vateria indica is a tree plant in the Dipterocarpaceae family, which is vulnerable and commercially important. The species is indigenous to India's Western Ghats, where it is found primarily in the evergreen forests of Kerala and surrounding areas. Economically, *Vateria indica* is valuable for

its wood, resin and medicinal properties. However, excessive exploitation, habitat degradation and forest ecosystem fragmentation have resulted in population decrease, classifying it as a Vulnerable species on the IUCN (International Union for Conservation of Nature) Red List. Hence, molecular characterization and correct species identification are necessary for its conservation and long-term management.[12]

For this purpose, the current work aims at the assessment of rbcL gene as effective barcode candidate in molecular identification and phylogenetic characterization of *Vateria indica* collected from the Bonacaud forest region of Agasthya hill in Kerala, India.

2. Materials and Methods

2.1. Sample collection

Fresh and healthy leaf samples of *Vateria indica* were collected from the Bonacaud forest region of the Agasthya hills in Thiruvananthapuram district, Kerala. The obtained plant material was authenticated by the Institute of Forest Genetics and Tree Breeding (IFGTB), with the authentication number 685/FRC/ID/FECC/IFGTB/2025.

Figure 1: *Vateria indica* L., Dipterocarpaceae

2.2. Systematic Position

Kingdom	Plantae
Phylum	Angiospermae
Class	Dicotyledons
Series	Thalamiflorae
Order	Malvales
Family	Dipterocarpaceae
Genus	*Vateria*
Species	*Indica*

Red List Category – Vulnerable (VU)

2.3. DNA isolation

Genomic DNA was isolated from fresh leaf tissues of *Vateria indica* using the CTAB method described by Doyle and Doyle (1987).[13] The approach was chosen due to its effectiveness in eliminating polysaccharides and secondary metabolites often found in plant tissues. The obtained DNA's purity and integrity were evaluated using standard molecular techniques like PCR amplification and 1.5% agarose gel electrophoresis.[14] In spectrophotometer, the absorbance ratio of A260/A280 was utilized to determine the DNA purity and concentration.

2.4. PCR amplification

The chloroplast rbcL gene region was amplified with the universal primers rbcLa-F (5′ATG TCA CCA CAA ACA GAA AC-3′) and rbcLa-R (5′-TCG CAT GTA CCT GCA GTA GC-3′), which are widely used in plant DNA barcoding investigations.[4]

In the present study, PCR (Polymerase Chain Reaction) amplification was carried out in 30 µL of reaction mixture containing isolated genomic DNA, PCR master mixture, forward and reverse primers and also nuclease-free water. Amplification was performed using the following thermal conditions. In this way, initial denaturation at 95ºC for 5 minutes, followed by 34 cycles of final denaturation at 94° C for 45 seconds, annealing at 55° C for 1-minute, initial extension at 72ºC for 1 minute and final extension at 72ºC for 10 minutes.

Under UV light, in 1.5% agarose gel stained with safe stain, the amplified PCR products were visualized as bands.

2.5. Sequencing and data analysis

The PCR products of the present study were purified and sequenced using Sanger sequencing technique. The nucleotide sequences were edited using Bio Edit and aligned with MEGA11 software.[15] Low quality regions were removed to create a high-quality consensus sequence.

The NCBI (National Centre for Biotechnology Information) database's BLASTn (Basic Local Alignment Search Tool) program was used to identify the studied species by comparing sequences available in GenBank reference sequences.[16-17]

To analyse the evolutionary relationship between the obtained sequence and other related species, a phylogenetic analysis was performed with the Neighbor-Joining (NJ) method using the software MEGA11 with 1000 bootstrap repetitions.[18]

3. Results

3.1.DNA extraction

Using CTAB technique, from the fresh leaf samples of *Vateria indica*, high quality genomic DNA was extracted, and the agarose gel electrophoresis process revealed the intact and high-molecular weight DNA with no degradation. The DNA purity was in A260/A280 ratio = 1.81 and it was appropriate for all further downstream applications.

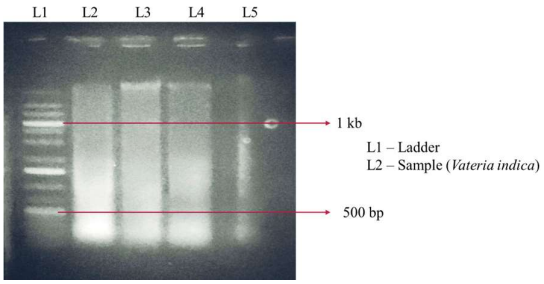

Figure 2: Agarose gel electrophoresis showing genomic DNA of *Vateria indica*

3.2. PCR amplification

The rbcL gene region of the studied plant sample was successfully amplified with 100% success rate. Agarose gel electrophoresis revealed a single band of approximately 455 bp, which is enough to the expected amplicon size with no non-specified bands.

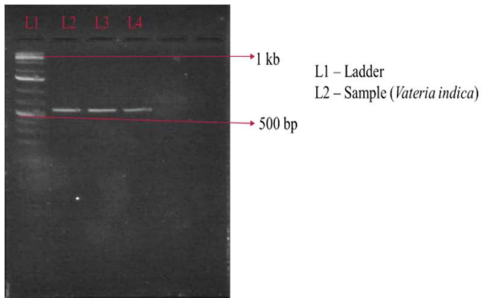

Figure 3: PCR amplification of rbcL gene in *Vateria indica*

3.3. Sequence quality and editing

With the present PCR products, high-quality nucleotide sequences were obtained by sanger sequencing method. The raw chromatograms showed unambiguous and well-defined peaks with low background noise, allowing for accurate base calling.

After trimming low-quality regions and deleting confusing bases, a consensus sequence of about 455 bp was produced. The sequence contained no stop codons, insertions or deletions indicating it as a functional chloroplast gene region suitable for further analysis.

3.4. BLAST examination and species authentication

The trimmed rbcL sequences were tested using the tool of BLAST based on the NCBI database to the exact identification of the present plant sample. The obtained rbcL sequence showed very close (99%) similarity with the other available reference sequence of *Vateria indica* in GenBank.

Job Title	Sample7R [organism=Vateria indica
RID	0JW65A6B014 Search expires on 05-18
Program	BLASTN Citation
Database	core_nt See details
Query ID	lcl|Query_3136377
Description	Sample7R [organism=Vateria indica]
Molecule type	dna
Query Length	445

Figure 4: Blast formatting of *Vateria indica*

3.5. Multiple Sequence Alignment

In the present study, the acquired rbcL sequence was examined through multiple sequence alignment pattern with other related available reference taxa and showed conserved nucleotide region(s) with negligble sequence variation.

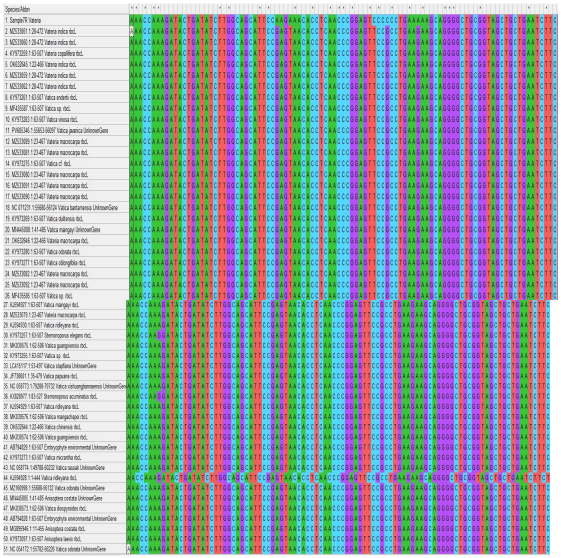

Figure 5: Multiple sequence alignment of *Vateria indica* using rbcL sequence with other related taxaPhylogenetic tree construction

In the present study, with the obtained rbcL sequence, a phylogenetic tree was constructed utilizing MEGA11's Neighbor-Joining (NJ) method with other available related reference sequences in GenBank. The phylogenetic analysis confirmed the present species identity with the matching of other related species in cluster. The resulting tree showed a clear evolutionary relationship between the tested sample and the related taxa. Bootstrap analysis with 1000 replicates supported the clustering pattern of phylogenetic tree. The present phylogenetic tree construction was compatible with BLAST study and confirmed the rbcL's efficacy for the molecular identification of *Vateria indica* through DNA barcoding technique.

Figure 6: Neighbor-Joining phylogenetic tree construction of *Vateria indica*

4. Discussion

The present study demonstrated the usage of chloroplast gene rbcL for the molecular based species identification and its phylogenetic detection of present *Vateria indica*. In general, the CTAB protocol is used to isolate genomic DNA from medicinal, forest tree species and also plants with strong primary and secondary metabolites in high success rates. In this study also, it is proved.[19]

The rbcL gene was successfully amplified, resulting in a unique amplicon of around 455 bp. Although the acquired sequence length was lower than that of full-length barcode regions, earlier studies have demonstrated that partial rbcL sequences are sufficient for reliable species identification and phylogenetic placement in many angiosperms. The current study's excellent amplification efficiency supports the rbcL marker's universality and reproducibility in plant DNA barcoding research.[5,7,20-21]

The acquired nucleotide sequence shared 99% similarity with *Vateria indica* reference sequence from the GenBank database, validating the sample's taxonomic identity. In molecular taxonomy investigations, high BLAST sequence similarity values are typically regarded as valid indications for species-level identification. The availability of extensive rbcL reference sequences in public databases enhances the usefulness of the marker for reliable species authentication.[17,22]

The phylogenetic tree construction using Neighbor-Joining method is proved and showed the evolutionary relationship and taxonomic position of *Vateria indica*. In the present study, the bootstrap worked with 1000 replicates of rbcL nucleotides showed the high reliability in the obtained phylogenetic cluster. Recent molecular systematic studies of tropical and medicinal plant taxa have found similar phylogenetic relationships based on chloroplast DNA sequences.[23-25]

The Western Ghats are a worldwide recognized biodiversity hotspot, home to a number of endemic and vulnerable plant species. *Vateria indica* is an ecologically and economically important tree species that is under threat from habitat degradation, overexploitation and environmental disturbances. Molecular characterization via DNA barcoding thus plays a significant role in species conservation, biodiversity assessment and sustainable management of vulnerable taxa.[26-27]

The present findings showed the high efficacy of chloroplast rbcL barcode gene in the study of molecular based identification and phylogenetic position of *Vateria indica*. Through this study, the generated genomic DNA data of *Vateria indica* may helpful to species conservation, identification and for future genetic studies of red data plant species of Southern Western Ghats.

5. Conclusion

It is concluded that the present DNA barcoding work established the high efficacy of the chloroplast rbcL gene for the molecular based species identification and phylogenetic construction of *Vateria indica*. High quality genomic DNA was extracted and effectively amplified, yielding a valid rbcL sequence of approximately 455 bp. In the present study, the BLAST and phylogenetic construction also used and confirmed the taxonomic position and species identification of the present study plant and also with the other closely related plant species. The present findings also demonstrated the high effectiveness of DNA barcoding in proper species identification, biodiversity conservation and the generation of genetic information which all lead to the future utilisation in the way of retrieving molecular information of a particular species or at a whole.

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