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Morphological and Molecular Identification of the Common Weeds *Alternanthera sessilis* and *Commelina benghalensis* using rbcL Gene-based Molecular Method

M. D. Kolte*[1], V. U. Pochhi[2] and D. L. Gavande[3]

[1-3] Department of Botany, Shri Shivaji Science and Arts College, Chikhli, District Buldana - 443 201, Maharashtra, India

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Abstract

Some plants that are documented as weeds in the literature have a high number of medicinal applications. *Alternanthera sessilis* and *Commenlina benghalensis* are herbaceous, weeds that are known for their medicinal applications in traditional medicine as well as in the modern pharmaceutical applications. In the present study, the selected plant species were collected from Chikhli tehsil, Buldhana district, Maharashtra. The collected healthy plant samples were utilized for the morphological identification of the plant species using the available literature sources. The molecular identification of the plant species was performed by DNA barcoding using the chloroplast rbcL gene. The observations noted during the study showed 99% similarity through BLAST analysis. We submitted these sequences to NCBI and received accession numbers for *Alternanthera sessilis* PX883717 and *Commelina benghalensis* PX892998.

***Key words*:** *Alternanthera sessilis*, *Commenlina benghalensis*, Chikhli, Morphological, Molecular, rbcL gene

Alternanthera sessilis is a perennial herb belonging to Amaranthaceae family. The medicinal plant *Alternanthera sessilis* is used for curing skin infections, diarrhea, stomach problems, dysentery, fever, snakebite, throat infections, asthma and bronchitis, headache and hepatitis [1]. The plant also shows several ethnomedicinal applications; the powder of dried leaves is used for stomach ailments like diarrhea and dysentery. Grounded leaf paste is applied on snake bite. The plant is also used for lung infections, asthma, bronchitis, skin infections and digestive problems traditionally. In the Ayurvedic and Siddha medicinal system the plant *Alternanthera sessilis* is mentioned as Ramayana drug [2].

Commenlina benghalensis is an herbaceous weed plant belonging to the family Commenlinaceae known for its medicinal applications [3]. The plant has been used traditionally since ages for ethnomedicinal uses. The leaves paste is used for curing burns, acne, warts and digestive abscesses and respiratory problems. The juice of root is used for curing acid reflux [4]. The plant is also used for the treatment of snakebite, insomnia, night blindness and cataract. The traditional texts suggest its use for curing female infertility. In modern medicine also the plants anti-inflammatory, anti-depressant, laxative and wound healing properties are utilized in pharmaceutical application [5].

Alternanthera sessilis (L.) R.Br. ex DC. and *Commelina benghalensis* L. are two widely distributed and economically important weed species commonly found in tropical and subtropical regions. *Alternanthera sessilis*, belonging to the family Amaranthaceae, is a perennial herb that frequently infests agricultural fields, wetlands, and roadside habitats. *Commenlina benghalensis*, a member of the family Commelinaceae, is a highly invasive species known for its prolific vegetative and sexual reproduction, making it particularly difficult to control in cropping systems [6]. Despite their distinct taxonomic positions, both species exhibit morphological variability that may lead to misidentification, especially at early growth stages. In recent years, molecular approaches have emerged as reliable tools for plant identification and taxonomic validation [7]. DNA barcoding, which uses short, standardized gene regions to identify species, has proven particularly effective in resolving taxonomic ambiguities. Among the chloroplast genes, the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene is one of the most widely used markers for plant DNA barcoding due to its universality, ease of amplification, and sufficient discriminatory power at the genus and species levels [8]. The integration of molecular data with classical morphological analysis enhances the accuracy and reliability of species identification.

The present study aims to perform a combined morphological and molecular characterization of *Alternanthera sessilis* and *Commelina benghalensis* using *rbcL* gene-based molecular methods. By correlating morphological traits with molecular sequence data, this research seeks to validate species identity and demonstrate the effectiveness of DNA barcoding in weed identification. The findings of this study are expected to contribute to improved taxonomic resolution, support weed management strategies, and strengthen the application of molecular tools in plant systematics and agricultural research. The present study deals with the plant collection, morphological

***Correspondence to:** Kolte M. D., E-mail: mukundakolte@gmail.com; Tel: +91 8888154009

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and molecular identification of the above two medicinal weed plants from the Chikhli tehsil, Buldhana district, Maharashtra. The molecular identification of the medicinal plants was performed with help of chloroplast rbcL gene.

MATERIALS AND METHODS

Sample collection

Plant samples for the study were collected from Chikhli Tehsil, Buldhana district, Maharashtra at 20° 20' 56.7528" N and 76° 15' 55.0404" E during the study period of August 2022 to August 2023. Healthy plant samples of *Alternanthera sessilis* and *Commelina benghalensis* was collected from the study site and used for morphological investigation

Plant DNA extraction procedure

The whole genomic DNA was extracted from the plant samples using a standard plant DNA isolation protocol. Moreover, the purity of the extracted DNA was assessed on a 1.0% agarose gel, and a single high-molecular-weight band confirmed suitability for downstream applications. The barcode marker chloroplast rbcL gene is a selected gene for species identification. The universal rbcL primers were used for PCR amplification under optimized cycling conditions. The amplification was confirmed by agarose gel electrophoresis, and the PCR products were purified to remove residual primers, nucleotides, and enzymes. The amplicons were added to Sanger sequencing using the BDT v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer. Furthermore, both forward and reverse reads were generated. Forward and reverse sequences were aligned to generate a high-confidence consensus sequence. The final consensus rbcL sequence was queried against the NCBI GenBank 'nr' database and the BOLD (Barcode of Life Data System) reference library using BLASTn. Species identification was founded on percentage similarity, alignment score, and top-hit consistency across databases.

RESULTS AND DISCUSSION

In the present study morphological identification of the selected medicinal plants *Alternanthera sessilis* and *Commenlina benghalensis* was performed with the help of the book Flora of Kolhapur District [9] and Handbook of Weed Identification [10].

Classification

Kingdom	:	Plantae
Phylum	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Caryophyllales
Family	:	Amaranthaceae
Genus	:	*Alternanthera*
Species	:	*sessilis* (L.) R. Br. ex DC.

Plant description

It is a procumbent/prostrate annual/perennial herb. Stem is prostrate; 0.2 to 1m in height; strong creeping roots; roots emerge at nodes; creeping sometimes floating and ascending at the tips. Roots are cylindrical, slightly hairy with erect branches. Leaves simple, opposite, slightly petiolate/sessile; generally lanceolate/spatulate to almost linear [11]. Leaves are around 0.6 to 5cm long and 0.3 to1 cm wide. Leaves are attenuated at the base; apex acute to blunt; margin glabrous/pilose. Flowers are inconspicuous; white in colour; axillary, sessile, borne in small, dense, silver-white clusters of compressed spikes. Fruits are Urticle cordiform; highly compressed. Seeds are dark brown to black in colour; 0.8 to mm in diameter; disc shaped and shiny [12] (Fig 1).

Fig 1 Morphological Identification of *Alternanthera sessilis*

Classification

Kingdom	:	Plantae
Phylum	:	Tracheophyta
Class	:	Liliopsida
Order	:	Commenlinales
Family	:	Commenlinaceae
Genus	:	*Commenlina*
Species	:	*benghalensis* L.

Plant description

Perennial herb creeping or decumbent; tuberous roots; dichotomous stems and diffused branches; rooting at nodes. Leaves ovate to lanceolate or ovate to elliptic; apex, obtuse, leaf base unequal, rounded, hairy on the margins, petiole sessile or short [13]. Flowers are few in axillary spathes; funnel shaped. Flowers blue in colour; branched cyme; clawed at base. Fruit is a capsule, pyriform membranous. Seeds are oblong and closely pitted (Fig 2).

Fig 2 Morphological Identification of *Commenlia benghalenis*

In this study, the high-quality genomic DNA was successfully isolated, as shown by a single intact band on the agarose gel (Fig 3). This PCR amplicon of the rbcL gene produced a clear, specific amplicon of the expected size. These results demonstrated successful target amplification. Sanger sequencing produced high-quality forward and reverse reads. After trimming and alignment, a clean consensus sequence of the rbcL region was obtained:

For *Alternanthera sessilis*
Consensus data

ATGTCACCACAAACAGAGACTAAAGCAAGTGTTGGA
TTTAAAGCTGGTGTTAAAGATTACAAATTGACTTATT
ATACTCCGGAGTATGAAACCCTAGATACTGATATCTT
GGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCA
CCTGAAGAAGCAGGGGCTGCAGTAGCTGCCGAATCT
TCTACTGGTACATGGACAACTGTATGGACTGACGGG
CTTACCAGTCTTGATCGTTACAAAGGACGATGCTACC
ATATCGAGCCTGTTGCTGGTGAAGAAAACCAATATA
TTTGTTATGTAGCGTATCCTTTAGACCTTTTTGAAGA
AGGTTCTGTTACTAATATGTTTACTTCCATTGTAGGT
AACGTATTTGGGTTCAAAGCCCTGCGTGCTCTACGTT
TGGAGGATTTGCGAATCCCTGTTGCTTATATAAAAAC
TTTCCAAGGCCCGCCTCACGGTATCCAAGTTGAAAG
AGATAAATTGAACAAGTATGGTCGTCCCCTATTGTGA
TGCACTATTAAACCT

For sample *Commelina benghalensis*
Consensus data

ATAAATTGACTTATTATACTCCTGAGTATCAAACCAA
GGATACTGATATATTGGCAGCATTCCGAGTAACTCCT
CAACCAGGAGTTCCACCTGAGGAAGCAGGGGCTGCG
GTAGCTGCCGAATCTTCTACTGGTACATGGACAACCG
TGTGGACTGATGGGCTTACCAGTCTTGATCGTTACAA
AGGACGATGCTACCGCATCGAGCCCGTTACTGGGGA
GGATAATCAATATATTGCTTATGTAGCTTATCCTTTA
GACCTTTTTGAAGAAGGTTCTGTTACTAATATGTTTA
CTTCCATTGTAGGTAATGTATTTGGTTTCAAAGCCTT
GCGAGCTCTACGTTTGGAGGATTTGCGAATTCCCACT
TCTTATACCAAAACTTTTCAAGGCCCGCCTCACGGTA
TCCAAGTTGAAAGAGATAAGTTGAACAAGTATGGTC
GTCCTCTATTGGGATGTACTATTAAACCAAAATTGGG
ATTATCCGCAAAGAACTATGGTAGAGCAGTTTACGA
ATGTCTGCG

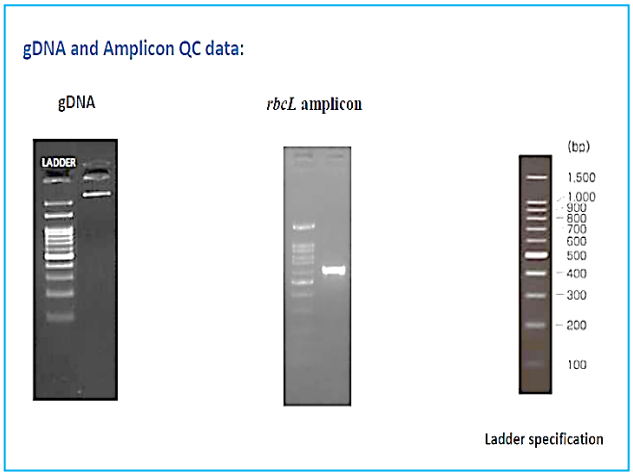

Fig 3 Single intact band on Agarose gel

We compared these sequences with available sequences in the NCBI Database. We carried out BLAST analysis. These sequences showed of the consensus sequence showed greater than 99% similarity to sequences belonging to the genus *Alternanthera* and *Commelina*. The highest-scoring matches corresponded specifically to *Alternanthera sessilis* in both NCBI GenBank and BOLD databases. No conflicting species-level matches were observed. The genus of the sample is *Commelina*. The *rbcL* gene used for the analysis showed similarity of more than 99% to *benghalensis* species. Thus, we can conclude that the given sample might *Commelina benghalensis* (Table 1). We submitted these sequences to NCBI and received accession numbers for *Alternanthera sessilis* PX883717 and *Commelina benghalensis* PX892998.

Table 1 Sequences producing significant alignments for *rbcL* gene

Description	Max score	Total score	Query cover	E value	Per. ident	Accession
Alternanthera sessilis bio-material CBT05-25	953	953	100%	0.00%	99.24%	PX138770.1
Alternanthera philoxeroides	953	953	100%	0.00%	99.24%	MK450441.1
Alternanthera denticulata	953	953	100%	0.00%	99.24%	PP869626.1
Alternanthera philoxeroides	953	953	100%	0.00%	99.24%	NC_042798.1
Alternanthera philoxeroides	953	953	100%	0.00%	99.24%	OQ354385.1
Alternanthera sessilis	953	953	100%	0.00%	99.24%	PP239384.1
Alternanthera philoxeroides isolate S10	929	929	98%	0.00%	99.22%	MH070608.1
Alternanthera philoxeroides voucher RQHN00944	929	929	98%	0.00%	99.22%	MH049882.1
Alternanthera philoxeroides voucher J.R	928	928	97%	0.00%	99.22%	GU135193.1
Alternanthera philoxeroides voucher Q575	928	928	97%	0.00%	99.22%	MH658507.1
Commelina benghalensis	966	966	100%	0.00%	100.00%	MH092643.1
Commelina benghalensis voucher Z467	966	966	100%	0.00%	100.00%	JF941286.1
Commelina benghalensis voucher JH200806001	966	966	100%	0.00%	100.00%	OR165418.1
Commelina diffusa	966	966	100%	0.00%	100.00%	MW186251.1
Commelina benghalensis	966	966	100%	0.00%	100.00%	NC_072999.1
Commelina benghalensis voucher LSC75	966	966	100%	0.00%	100.00%	MH767485.1
Commelina benghalensis voucher TuTY1395	965	965	100%	0.00%	100.00%	MH767486.1
Commelina communis voucher Z468	961	961	100%	0.00%	99.81%	JF941292.1
Commelina benghalensis voucher DWQGZ612	957	957	99%	0.00%	100.00%	PQ332054.1
Commelina benghalensis voucher DWQJM213	955	955	99%	0.00%	100.00%	PQ332247.1

The morphological identification of the plant samples was performed on the basis of morphological characters like habitat, root, stem, shape and length of leaf, inflorescence and fruit by Kashyap *et al*. [14]. The authors identified the plant sample to be of *Alternanthera sessilis*. Gupta *et al*. [15] also performed the morphological and anatomical identification of *A. sessilis* collected from Nilgris district, Tamil Nadu. Orni *et al*. [16] showed the brief description of *Commelina benghalensis* based on morphological characteristics along with its medicinal properties. Dolaabh *et al*. [17] also briefed regarding the morphological characterization of *Commelina benghalensis*. These studies collectively highlight the importance of morphological traits as a primary and reliable approach for preliminary identification of *Alternanthera sessilis* and *Commelina benghalensis*.

The identification of plant samples at both the genus and species levels DNA barcoding, using the rbcL gene, is one of the most effective methods [18]. The DNA band confirmed that it yielded genomic DNA suitable for amplification. The amplified amplicon of the rbcL region further demonstrated that universal chloroplast markers are robust tools for plant identification. Previous studies showed similar results, even when working with unknown or unclassified samples; chloroplast markers are robust tools for plant identification [19-20]. In plant barcoding, the rbcL gene is widely used because of its conserved nature and reliable amplification across diverse taxa [21]. In this study, the >99% sequence similarity to *Alternanthera sessilis* and *Commelina benghalensis* in both GenBank and BOLD databases indicates a high degree of confidence in the identification. The consistency of results across two independent reference databases further validates the conclusion.

CONCLUSION

In this study, we used DNA (Deoxyribonucleic acid) barcoding using the chloroplast rbcL gene to identify the plant sample at the genus and species levels. The plants were identified by using a molecular marker in the rbcL region. This marker is the robustness of this marker for plant identification. Our results demonstrated that 99% similarity by using BLAST analysis. These results confirmed that the plant is in the genus *Alternanthera sessilis* and *Commelina benghalensis*. Hence, the study proved that the rbcL-based DNA (Deoxyribonucleic acid) barcoding as a powerful tool for accurate species identification. Therefore, the integration of rbcL-based DNA barcoding with traditional morphological observations enhances the reliability of weed identification and supports its application in taxonomic and biodiversity studies.

Conflict of interest

The authors declare that there is no conflict of interest.

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