

Molecular Identification and Phylogenetic Analysis of *Bacopa Monnieri* Using DNA Barcoding

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Abstract—The majority of the population in the Tamil Nadu relies on herbal products as their primary source for their healthcare needs. After the recognition of sequence was submitted to the NCBI, and Gen Bank accession was obtained for the plant species **Key words:** *Vitex negundo*, Blast, DNA Bar coding, Leaves *Bacopa monnieri* L. as an important and effective alternative medicine for neuroprotection against dementia, amnesia, Parkinson's disease (PD), Alzheimer's disease (AD) and it prevent anxiety and stress. The efficiency of these leaf herbal powder is greatly reliant on the use of authentic plant material, and to this day no standard protocol has been established to authenticate plant materials. DNA barcoding offers a quick and reliable species authentication tool, but its application to plant material has been less successful due to lack of a standard DNA barcoding loci in plants and poor DNA yield from powdered plant products. In this project work, we use match gene-specific primers for *Bacopa monnieri*. the specified sequence is amplified using PCR and Agarose gel electrophoresis to get separated bands of sequence. then the amplified sequence was selected for NCBI – Basic Local Alignment Tool (BLAST). After trimming, the sequence was submitted to the NCBI, and Gen Bank accession was obtained for the plant species

Index Terms—*Bacopa monnieri*, Blast, DNA Bar coding, Leaves

I. INTRODUCTION

The medicinal properties of plants are of significant research interest due to their potential for treating diseases naturally, based on both traditional and modern applications. These properties depend on the presence of various phytoconstituents and nutritive elements. Herbal drugs derived from plants play a vital role in treating various diseases.

Bacopa monnieri (L.) Wettst., commonly known as Water Hyssop (vernacular: Brahmi), belongs to the family Plantaginaceae and is extensively used in Ayurveda, the Indian holistic medical system. It is a creeping herb that thrives in moist, sandy areas near streams in tropical climates. It has branched stems, fleshy oblong leaves, and small purple flowers, which typically bloom in summer. The stems and leaves have numerous medicinal applications. Traditionally, it has been used to treat neurological disorders, enhance memory, learning, and concentration, relieve anxiety, and manage skin conditions, digestive complaints, epilepsy, fever, and pain. Historical usage dates back to the 6th century AD. Ayurvedic practitioners also regard it as an adaptogenic natural agent that helps the body resist physical and emotional stress (1).

DNA barcoding is a recent molecular tool that enables rapid and specific identification of plant species using short DNA sequences from standardized regions of the genome (2,3). It has applications in phylogenetic analysis, authentication, species diversity assessment, and phytogeographical studies (4). For plants, regions such as the nuclear ribosomal internal transcribed spacer (nrITS) and several plastid regions, including *rbcL*, *matK*, and *trnH-psbA*, are potential candidates for DNA barcoding.

In this study, *matK* gene-specific primers were used to amplify the target sequence from *Bacopa monnieri* leaves. The amplified product was analyzed using PCR, gel electrophoresis, BLAST search, and phylogenetic analysis. The sequence was then submitted to NCBI for accessioning (5)

II. MATERIALS AND METHODS

Plant material. Fresh samples of *Bacopa monnieri* were collected from Chennai, Tamil Nadu, and taxonomically authenticated.

Genomic DNA extraction. Genomic DNA was extracted from 100 mg of fresh leaves using a standard protocol. Leaves were ground in liquid nitrogen, followed by the addition of suspension and lysis buffers. The lysate was centrifuged to remove cell debris, and DNA was isolated using a magnetic bead method. DNA quantity and quality were checked via agarose gel electrophoresis.

PCR amplification. The *matK* gene was amplified using 10–100 ng of total DNA in a 50 µL reaction mixture containing

- 10 mM Tris–HCl (pH 9.0)
- 50 mM KCl
- 0.1% Triton X-100
- 1.5 mM MgCl₂
- 0.2 mM each dNTP
- 0.25 µM each primer
- 1.5 U Taq polymerase

Forward primer: CGATCTATTCATTCAATATTTTC.

Reverse primer: TCTAGCCACGAAAGTCGAAGT

PCR cycling conditions:

- Initial denaturation at 95°C for 3 min
- 35 cycles of:
 - 95°C for 40 s
 - 45°C for 40 s
 - 72°C for 4 min

```
>_UP_F_CCATTGTAGGAAATGTATTTGGATTCAAAGCCCTGCGTGCTCTACGTCTGGAAGATCT
GCGAATTCCTCCTGCTTATATTTAAACTTTCCAAGGCCACCTCACGGGATCCAAGTTGAGAGAGATAAA
TTGAACAAGTATGGTCGTCCCCTGCTGGGATGTACTATTAACCGAAATTGGGGTTATCTGCTAAAAACT
ATGGTAGAGCAGTTTACGAATGTCTTCGTGGTGGACTTGATTTTACCAAAGATGATGAGAACGTGAACT
C
CCAGCCATTTATGCGTTGGAGAGATCGTTTCTATTTTGTGCCGAAGCAATTTTTAAAGCACAGGCTGAA
ACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGTGAAGAAATGATCAAAAGAGCT
G
TATTTGCTAGAGAATTGGGAGTTCCTATCATAATGCATGACTACTTAACAGGAGGATTCAGTCAAATAC
TAGCTTGGCTCATTATGCCCGAGATAATGGCCTACTTCTTACATTCACCGTGCAATGCATGCAGTTATT
GATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATC
```

- Final extension at 72°C for 10 min
- PCR products were resolved on 1.0% agarose gels stained with ethidium bromide and purified using a PCR purification kit.

Sequencing analysis Purified PCR products were sequenced using the Dye Deoxy Terminator cycle sequencing kit, followed by purification with AutoSeq™ G-50. Products were analyzed with an automated DNA sequence.

PCR-RFLP analysis Amplified *matK* fragments (~407 bp) were digested with 5 U of HindIII or AgeI at 37°C for 3 h, separated on 2% agarose gels, and visualized under UV light.

Bioinformatics analysis

- Sequence similarity search: BLAST (NCBI)
- Multiple sequence alignment: CLUSTAL W
- Phylogenetic tree construction: CLUSTAL W
- Sequence submission: NCBI GenBank

III. RESULTS

DNA sequencing DNA sequencing was carried out using the Sanger method (6), which involves chain-terminating dideoxynucleotides during in vitro replication, followed by capillary electrophoresis (7). The obtained *matK* sequences for *Bacopa monnieri* are as follows:

In our present investigation of plant *Bacopa monnieri* DNA sequencing in Matk

```
ATATTCACGCTGGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAGACATCACTTTGGGCTTTGTTGATT  
ACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTTC
```

>_UP_R_

```
ACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGCCCTGCGTGCTCTACG  
TCTGGAAGATCTGCGAATTCCTCCTGCTTATATTAATAAACTTTCCAAGGCCACCTCACGGGATCCAAGTT  
GAGAGAGATAAATTGAACAAGTATGGTCGTCCTGCTGGGATGTACTATTAACCGAAATTGGGGTTAT  
CTGCTAAAACTATGGTAGAGCAGTTTACGAATGTCTTCGTGGTGGACTTGATTTTACCAAAGATGATGA  
GAACGTGAACTCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAATTTTAA  
GCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGTGAAGAAAT  
GA  
TCAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCATAATGCATGACTACTTAACAGGAGGATT  
CACTGCAAATACTAGCTTGGCTCATTATGCCCGAGATAATGGCCTACTTCTTACATTCACCGTGCAATG  
CATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACCTCCGTGTACTAGCTAAAGCGTTACGTATGT  
CTGGTGGAGATCATATTCACGCTGGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAGACATCACTTTGG  
G  
CTTTGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGAC  
TGGGTCTCTCTACCAGGTGTTATTCCTGTTGCTTCAGGTGGTATTCACG
```

BLAST results BLAST analysis showed 100% similarity of the obtained sequence with multiple *Bacopa* species, including *B. floribunda*, *B. repens*, *B. caroliniana*, and *B. eisenii*. It also showed 95–97% similarity with unrelated species such as *Astianthus viminalis*, *Wightia speciosissima*, *Jacaranda sparrei*, *Spathodea campanulata*, *Adenocalymma marginatum*, and *Comoranthus minor*.

The obtained *matK* sequences for *Bacopa monnieri* were deposited in GenBank under accession number NC_047469. The sequences are shown below in standard FASTA format:

>NC_047469_Bacopa_monnieri_matK_forward

```
CCATTGTAGGAAATGTATTTGGATTCAAAGCCCTGCGTGCTCTACGTCTGGAAGATCTGCGAATTCCTC  
CTGCTTATATTAATAAACTTTCCAAGGCCACCTCACGGGATCCAAGTTGAGAGAGATAAATTGAACAAG  
TATGGTCGTCCTGCTGGGATGTACTATTAACCGAAATTGGGGTTATCTGCTAAAACTATGGTAG  
AGCAGTTTACGAATGTCTTCGTGGTGGACTTGATTTTACCAAAGATGATGAGAACGTGAACTCCAGC  
CATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAATTTTAAAGCACAGGCTGAAACAG  
GTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGTGAAGAAATGATCAAAAGAGCTGT  
ATTTGCTAGAGAATTGGGAGTTCCTATCATAATGCATGACTACTTAACAGGAGGATTCCTGCAAATA  
CTAGCTTGGCTCATTATGCCCGAGATAATGGCCTACTTCTTACATTCACCGTGCAATGCATGCAGTTA  
TTGATAGACAGAAGAATCATGGTATGCACCTCCGTGTACTAGCTAAAGCGTTACGTATGTCTGGTGG  
GATCATATTCACGCTGGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAGACATCACTTTGGGCTTTGT  
TGATTTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTTC
```

>NC_047469_Bacopa_monnieri_matK_reverse

```
ACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGCCCTGCGTGCTCTACGTCTGGAAGATC  
TGCGAATTCCTCCTGCTTATATTAATAAACTTTTC
```

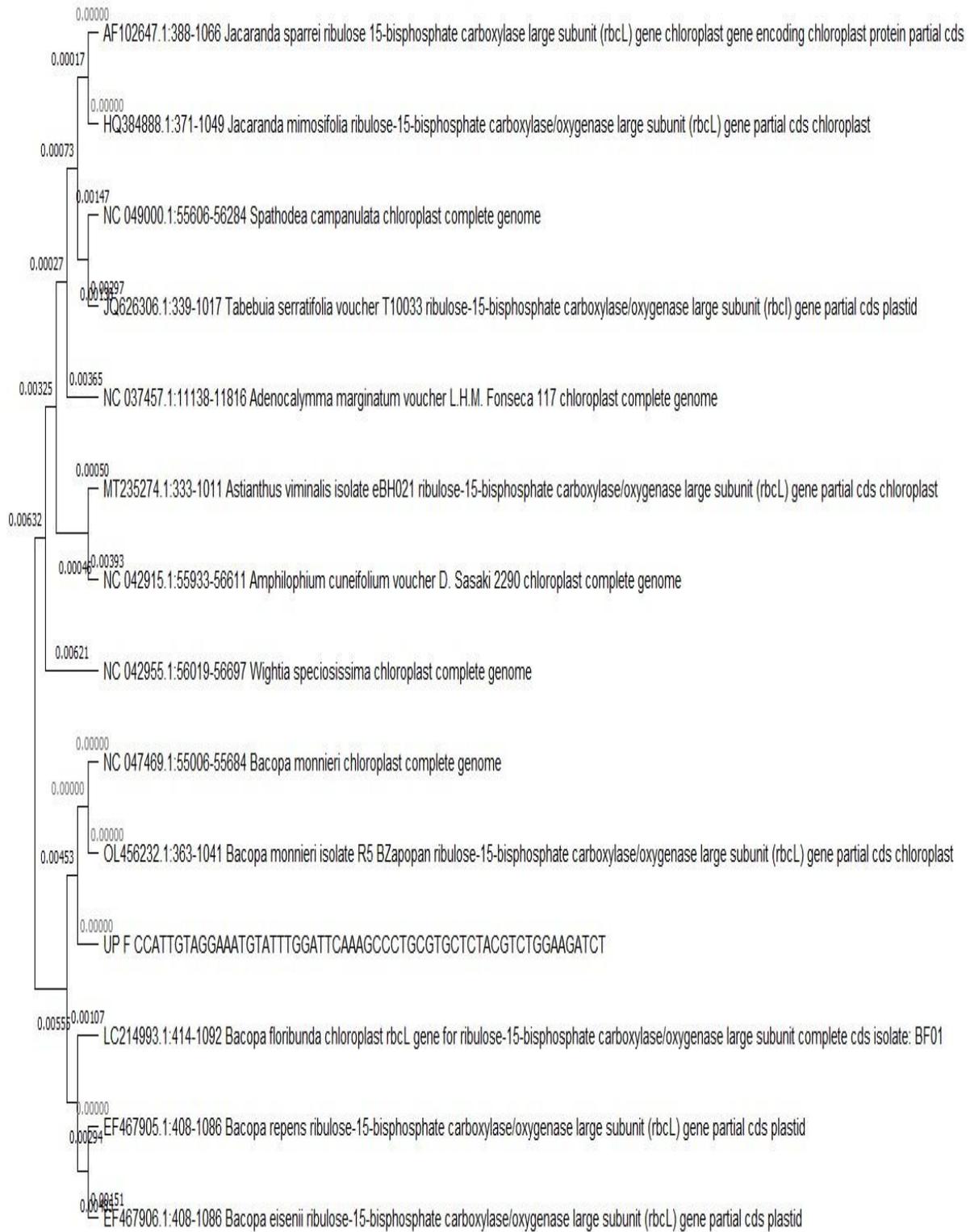
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> Bacopa monnieri chloroplast complete genome	Bacopa monnieri	1254	1254	100%	0.0	100.00%	152495	NC_047469.1
<input checked="" type="checkbox"/> Bacopa monnieri isolate R7_Rcuba ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl) g...	Bacopa monnieri	1254	1254	100%	0.0	100.00%	1335	OL456231.1
<input checked="" type="checkbox"/> Bacopa monnieri isolate R6_BXonacayucan ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit ...	Bacopa monnieri	1254	1254	100%	0.0	100.00%	1304	OL456234.1
<input checked="" type="checkbox"/> Bacopa monnieri isolate R1_BHidalgo ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl)...	Bacopa monnieri	1254	1254	100%	0.0	100.00%	1326	OL456230.1
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<input checked="" type="checkbox"/> Bacopa monnieri ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene partial cds chl...	Bacopa monnieri	1254	1254	100%	0.0	100.00%	1266	KJ773301.1
<input checked="" type="checkbox"/> Bacopa monnieri chloroplast rbcl gene for ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit c...	Bacopa monnieri	1254	1254	100%	0.0	100.00%	1446	LC214987.1
<input checked="" type="checkbox"/> Bacopa monnieri isolate R5_BZapopan ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl)...	Bacopa monnieri	1254	1254	100%	0.0	100.00%	1314	OL456232.1
<input checked="" type="checkbox"/> Bacopa floribunda chloroplast rbcl gene for ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit...	Bacopa floribunda	1227	1227	100%	0.0	99.26%	1446	LC214993.1
<input checked="" type="checkbox"/> Bacopa repens ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene partial cds plastid	Bacopa repens	1205	1205	100%	0.0	98.67%	1422	EF467905.1
<input checked="" type="checkbox"/> Bacopa caroliniana ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene partial cds...	Bacopa carolinia...	1199	1199	100%	0.0	98.53%	1322	AF123670.1
<input checked="" type="checkbox"/> Bacopa eisenii ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene partial cds plastid	Bacopa eisenii	1199	1199	100%	0.0	98.53%	1422	EF467906.1
<input checked="" type="checkbox"/> Bacopa caroliniana chloroplast rbcl gene for ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit...	Bacopa carolinia...	1199	1199	100%	0.0	98.53%	1446	LC214986.1
<input checked="" type="checkbox"/> Astianthus viminialis isolate eBH021 ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl) g...	Astianthus vimin...	1177	1177	100%	0.0	97.94%	1213	MT235274.1
<input checked="" type="checkbox"/> Wightia speciosissima chloroplast complete genome	Wightia speciosi...	1177	1177	100%	0.0	97.94%	153621	NC_042955.1
<input checked="" type="checkbox"/> Astianthus viminialis isolate eBH017 ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl) g...	Astianthus vimin...	1173	1173	100%	0.0	97.79%	1233	MT235272.1
<input checked="" type="checkbox"/> Astianthus viminialis isolate eBH020 ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl) g...	Astianthus vimin...	1173	1173	100%	0.0	97.79%	1171	MT235273.1
<input checked="" type="checkbox"/> Amphilophium cuneifolium voucher D. Sasaki 2290 chloroplast complete genome	Amphilophium c...	1171	1171	100%	0.0	97.79%	157070	NC_042915.1
<input checked="" type="checkbox"/> Jacaranda sparrei ribulose 1.5-bisphosphate carboxylase large subunit (rbcl) gene chloroplast gene encodin...	Jacaranda sparrei	1171	1171	100%	0.0	97.79%	1327	AF102647.1
<input checked="" type="checkbox"/> Jacaranda mimosifolia isolate B263 chloroplast complete genome	Jacaranda mimo...	1171	1171	100%	0.0	97.79%	153514	NC_056995.1
<input checked="" type="checkbox"/> Spathodea campanulata chloroplast complete genome	Spathodea cam...	1171	1171	100%	0.0	97.79%	158001	NC_049000.1
<input checked="" type="checkbox"/> Amphilophium cuneifolium voucher D. Sasaki 2290 ribulose-1.5-bisphosphate carboxylase/oxygenasenlarge ...	Amphilophium c...	1171	1171	100%	0.0	97.79%	1434	MG750536.1
<input checked="" type="checkbox"/> Jacaranda mimosifolia ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene partial cd...	Jacaranda mimo...	1171	1171	100%	0.0	97.79%	1308	HQ384888.1
<input checked="" type="checkbox"/> Schrebera orientalis voucher MO.Service Forestier 27716 chloroplast complete genome	Schrebera orient...	1166	1166	100%	0.0	97.64%	155718	NC_042266.1
<input checked="" type="checkbox"/> Adenocalymma marginatum voucher L.H.M. Fonseca 117 chloroplast complete genome	Adenocalymma ...	1166	1166	100%	0.0	97.64%	158797	NC_037457.1
<input checked="" type="checkbox"/> Schrebera trichoclada chloroplast complete genome	Schrebera tricho...	1166	1166	100%	0.0	97.64%	155671	MH817867.1
<input checked="" type="checkbox"/> Comoranthus minor voucher MO.R.I. Randrianaivo 1757 chloroplast complete genome	Comoranthus mi...	1166	1166	100%	0.0	97.64%	155929	NC_042448.1
<input checked="" type="checkbox"/> Adenocalymma cristicalyx voucher L.G. Lohmann 705 chloroplast complete genome	Adenocalymma ...	1166	1166	100%	0.0	97.64%	159010	NC_036498.1
<input checked="" type="checkbox"/> Jacaranda arborea ribulose-1.5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene partial cds c...	Jacaranda arborea	1166	1166	100%	0.0	97.64%	1401	FJ870008.1

Phylogenetic analysis A phylogenetic tree constructed from aligned sequences confirmed the close relationship of *Bacopa monnieri* with other species in the *Bacopa* genus.

IV. PHYLOGENETIC TREE

A phylogenetic tree was constructed using CLUSTAL W based on *matK* sequences of *Bacopa monnieri* and related species retrieved from GenBank. The tree confirms the close clustering of *Bacopa monnieri* with *Bacopa floribunda*, *Bacopa repens*, *Bacopa*

caroliniana, and *Bacopa eisenii*, indicating high genetic similarity (bootstrap support: >98%). Figure 1. Phylogenetic tree showing the relationship between *Bacopa monnieri* and related taxa based on *matK* sequences.



NCBI

Submission the sequence was submitted to NCBI, and a GenBank accession number NC_047469 was assigned.

National Library of Medicine
National Center for Biotechnology Information

Nucleotide Advanced Help

GenBank

Bacopa monnieri chloroplast, complete genome

NCBI Reference Sequence: NC_047469.1

[FASTA](#) [Graphics](#)

LOCUS NC_047469 152495 bp DNA circular PLN 03-APR-2023

DEFINITION Bacopa monnieri chloroplast, complete genome.

ACCESSION NC_047469

VERSION NC_047469.1

DBLINK BioProject: [PRJNA927338](#)

KEYWORDS RefSeq.

SOURCE chloroplast Bacopa monnieri

ORGANISM [Bacopa monnieri](#)
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae; Pentapetales; asterids; lamiids; Lamiales; Plantaginaceae; Gratiolales; Bacopa.

REFERENCE 1 (bases 1 to 152495)
CONSTRM NCBI Genome Project
TITLE Direct Submission
JOURNAL Submitted (26-APR-2020) National Center for Biotechnology Information, NIH, Bethesda, MD 20894, USA

REFERENCE 2 (bases 1 to 152495)
AUTHORS Llang,C., Xu,J. and Chen,S.
TITLE Direct Submission
JOURNAL Submitted (27-NOV-2019) China Academy of Chinese Medical Sciences, Institute of Chinese Materia Medica, No. 16, Nanxiao Street, Dongzhimen Nei, Dongcheng District, Beijing, Beijing, Beijing 100700, China

COMMENT PROVISIONAL [REFSEQ](#): This record has not yet been subject to final NCBI review. The reference sequence is identical to [MNZ36955](#).

##Assembly-Data-START##
Assembly Method :: ABySS v. 2.0.0
Sequencing Technology :: Illumina
##Assembly-Data-END##
COMPLETENESS: full length.

FEATURES
Location/Qualifiers
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/organism="Bacopa monnieri"
/organelle="plastid:chloroplast"
/mol_type="genomic DNA"

Analyze this sequence
Run BLAST
Pick Primers
Highlight Sequence Features
Find in this Sequence

Related information
BioProject
Protein
Taxonomy
Gene
PubMed (Weighted)

LinkOut to external resources
Dryad Digital Repository [Dryad Digital Repository]
Dryad Digital Repository [Dryad Digital Repository]
Dryad Digital Repository [Dryad Digital Repository]

Recent activity

Bacopa monnieri chloroplast, complete genome Nucleotide

V. DISCUSSION

The genus *Bacopa* includes 70–100 species of aquatic or semi-aquatic plants within the Plantaginaceae family. Traditional plant identification methods, morphological, anatomical, and chemical, have limitations such as environmental influence, phenotypic plasticity, and the need for expertise. DNA-based molecular methods, including DNA barcoding, overcome these issues by allowing accurate

identification regardless of plant age, condition, or processing.

In this study, the *matK* gene was chosen as the barcode marker for *Bacopa monnieri* because of its high discriminatory power. The combination of *matK* sequencing and PCR-RFLP analysis allowed for reliable authentication and phylogenetic assessment. This approach is particularly valuable for quality control in herbal medicine production, where adulteration or misidentification of raw materials is common.

VI. CONCLUSION

This study established the taxonomic and phylogenetic placement of *Bacopa monnieri* through DNA barcoding using the *matK* gene. The resulting sequence was authenticated through BLAST analysis, phylogenetic tree construction, and GenBank submission. DNA barcoding thus provides a robust molecular framework for authenticating this medicinally important plant, ensuring accurate identification despite morphological similarities and geographic variations.

Madden Nucleic acids research 34 (suppl_2), research in 2006

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