Eco. Env. & Cons. 30 (October Suppl. Issue) : 2024; pp. (S53-S57)
Copyright@ EM International
ISSN 0971–765X

DOI No. http://doi.org/10.53550/EEC.2024.v30i06s.008

Comparison of Identification Efficiency of RBCL and Mat K Primers using Bold and Blast Databases in *Anisomeles malabarica*

Bhuvaneshwari R.[1] and R. Anandhan[2]*

[1]Plant Molecular Biology and Biotechnology, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar 608 002, T.N., India
[2]Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar 608 002, T.N., India

(Received 1 March, 2024; Accepted 7 May, 2024)

ABSTRACT

Anisomeles malabarica also known as Indian Malabar catamint is a medicinal plant classified under the Lamiaceae family, is found in the southern tropical and subtropical regions of Asia. In the present study comparison of identification efficiency of rbcl and mat K markers was done using BOLD and BLAST data bases. The sequences identified from BLAST and BOLD databases were different from each other. The sequence identified from BLAST using matk and rbcl are same which are as follows, *Anisomeles indica* - 99.6%, *Microtoenaurticifolia* - 97.99%, *Microtoenarobusta* - 97.99%, *Microtoenadelavayi* - 97.99% and *Colebrookiaoppositifolia* - 97.35%. Similarly, the sequence identified from BOLD using matk and rbcl are same which are as follows, *Anisomeles indica* - 99.6%, *Anisomeles malabarica* - 98.38%, *Rostrinuculas inensis* - 97.08%, *Anisomeles indica* - 99.3 % and *Cymaria acuminata* - 96.82 %.

Key words: *Barcode, Barcode of Life Database, Basic Local Alignment Search Tool, Gel electrophoresis. Polymerase chain reaction*

Introduction

Identification of a plant species is important when it comes to preserving the plant and using it further for any commercial or medicinal purpose. Morphological identification of plant is difficult as it needs specialised taxonomists (Chase and Fay, 2009). Hence alternate system of plant identification is being suggested by Consortium of Barcode of Life (CBOL) in 2009 that is the use of DNA barcodes. This method is able to identify the plant using the small region from any geographical region. A DNA barcode consists of a standardized short sequence of DNA (400–800 bp) that, in principle, can be easily generated and would be unique for every species on the planet (Maloukh *et al.,* 2017). The most commonly used barcode for plant species identification is a small region in plastid gene viz., ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL), and maturase K (matK). These two sequences have been adopted as standard plant DNA barcodes by the international body of the Plant Working Group (PWG) of the Consortium for the Barcoding of Life (CBOL, 2009). Universal primer sequences designed by CBOL were used successfully for identification and authentication of some international as well as regional plant species. *Anisomeles malabarica* also known as Indian Malabar

([1]Ph.D. Scholar, [2]*Assistant Professor)

catamint is a medicinal plant classified under the Lamiaceae family, is found in the southern tropical and subtropical regions of Asia (Tandonand Gupta 2004). The whole plant, especially the leaves and the roots are used as astringent, carminative, febrifuge and tonic. The plant possesses many phytochemical properties and is used in the treatment of various diseases in traditional medicine. Only a very few studies have been done in DNA barcoding in *Anisomeles malabarica*. No study is about the comparison of matK and rbcl primers in identification of the sequences of *A. malabarica*. Hence the present study has been focussed on the comparison of identification efficiency of rbcl and matK markers using BOLD and BLAST data bases. The program compares nucleotide or protein sequences and calculates the statistical significance of matches. BLAST is an acronym for Basic Local Alignment Search Tool which can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families. The Barcode of Life Data System (bold) is an informatics workbench aiding the acquisition, storage, analysis and publication of DNA barcode records (Ratnasingham and Hebert, 2007). In the present study DNA barcoding work for *Anisomeles malabarica* was done and the primer efficiency was compared from BLAST and BOLD databases.

Materials and Methods

The study was done at the Plant molecular biology laboratory, Department of Genetics and Plant Breeding, Annamalai University. DNA barcoding was first proceeded by the isolation of DNA from the plant. Young leaves were collected and removed entirely and washed completely with sterilized water for a few times and cut off to small pieces with a sterile scalpel under sterile conditions and genomic DNA was isolated using method of Doyle and Doyle, 1987 with slight modification without using liquid nitrogen. Genomic DNA yield was expressed as µg (micro gram) DNA per mg (milli gram) of leaves tissue. Using a spectrophotometer, the yield of DNA per gram of freshly isolated leaf tissue was calculated at 260 nm. The purity of DNA was decided by calculating the ratio of absorbance at 260 nm to that of 280 nm. Then the isolated DNA was amplified by doing PCR using matK and rbcl primers. Then the purity of the PCR products was analysed by using Gel electrophoresis. The PCR product was sequenced by outsourcing.The Sanger sequencing method was carried out by outsource (Eurofins Genomics India Pvt Ltd, Bangalore). The resulted sequences after sequencing were quality checked and some of the ambiguous nucleotides on both the ends were trimmed carefully using the chromas software. The sequences were analysied using the Chromas (version 2.6) and Molecular Evolutionary Genetics Analysis software (MEGA version 6.0). The sequences were blasted using NCBI website. http://blast.ncbi.nlm.nih.gov/Blast.cgi and BOLD systems.

Results and Discussion

Following the Doyle and Doyle, 1990methodology, the genomic DNA was isolated from the fresh tender leaves of *Anisomelesmalabarica* using the Cetyl Trimethyl Ammonium Bromide (CTAB) method. Extracted DNA samples were amplified using DNA barcode markers *Rbcl and MatK,* in a thermo cycler (96 well-Applied Biosystem). The PCR assay using the standardized thermal settings gave satisfactory results. The amplified PCR product size was validated using 1.0% of agarose gel. The image obtained in Gel electrophoresis of PCR product is given in the Fig.1. The PCR product, confirmed to yield only single band (250-350 bp) on electrophoresis. The sequences generated after outsourcing are furnished here under:

matK

ATCTTGGTTCAAATCCTTCGCTATTGGGTA
AAAGATGCTTCCTCCTTGCATTTATTACGAGT
TTTTCTCAACGAATATTGTAATTGGAATAG
TCTTATTATTCCAAAGAAAGCGAGTTCCTCTT
TTTCAAAAAAAAAATCAAAGATTATTCTTATTC
TTATATAATTCTCATGTATGTGAA TATGAATC
CATTTTCGTCTTTCTACGTAACCAATCTTTT
CATT TACGATCAACATCTTCTAGAGTTCTTC
TTGAACGAATCTATTTCTATATAAAAATAG
AACGTCTTGTGAACGTCTTTG TCAAG ATTAA
GGATTTTCGGGCAAACCCGTGGTTGGTCA
AGGAACCTTTCATGCATTATATTAGGTA
TCAAAGAAAATCCATTCTGGCTTCAAAAGG
GACATCTATTTTCATGAATAAATGGAAA
TTTTACCTTGTCACTTTTTGGCAATGGCAT
TTTTCCTTGTGGTTTCATCCAACAAGGATTT
ATATAAACCAATTATCCAAGCATTCCCTT
GAGTTTTTGGGCTATCTTTTAAGCGTGCGAAT
GAACCCTTCTGTAGTACGGAGTCAAATTC

TAGAAAATTCATTTCTAATCAATAATCCTA
TTAAGAAGTTTGATACTCTTGTTCCAATTAT
TCCTCTGATTGCGTCATTGGCTAAAGCCAA
ATTTTGTAACGTATTGGGGCATCCCATTAGTA
AGCCGATTCGGGGCTGATTTATCAGATTCTAA
TATTATTTACCGATCTGGGCGT

Rbcl

CCCAGTGTTGGATTCAAAGCGGGTGTTAAAG
AGTACAAATTGACTTATTATACCCCTGAA
ATACCAAACCAAAGATACTGATATCTTGGCA
GCATTCCGAGTAACTCCTCAACCTGGAGT
TCCGCCCGAAGAAGCAGGGGCCGCAGTAG C
TGCCGAATCTTCGACTGGTACATGGACAA
CTGTGTGGACCGATGGACTTACCAGCCTT
GATCGTTACAAAGGGCGATGCTACGACAT
TGAGCCCGTTCTTGGAGAAAAAGATCAA
TATATCTGTTATGTAGCTTACCCTTTAGACC
TTTTTGAAGAAGGTTCTGTTACTAACATGT
TTACTTCCATTGTAGGAAATGTATTTGGAT
TCAAAGCCCTACGTGCTCTACGTCTGGAAGA
TCTGCGAATCCCTCCTGCTTATACTAA AACTT
TCCAAGGCCCGCCTCATGGGATCCAA GTTGA
GAGAGATAAATTGAACAAGTATGGTCGTCCT
CTGTTGGGATGTACTATTAAACCGAAATT
GGGGTTATCTGCTAAAAACTATGGTAGAG
CGGTTTATGAATGTCTTCGCGGTGGA

Sequence analysis

For homologous identification, only sequences of minimum 95% percentage identity were considered. The first five species of BLAST and BOLD databases were compared for identification percentage. The identification percentage of BLAST and BOLD for matk and rbcl was given in the Table 1.

The sequences identified from BLAST and BOLD databases were different from each other. The sequence identified from BLAST using matk and rbcl are same which are as follows, *Anisomelesindica* - 99.6%, *Microtoenaurticifolia* - 97.99%, *Microtoenarobusta* - 97.99%, *Microtoenadelavayi* - 97.99% and *Colebrookiaoppositifolia* - 97.35%. Similarly, the sequence identified from BOLD using matk and rbcl are same which are as follows, *Anisomelesindica* - 99.6%, *Anisomelesmalabarica* - 98.38%, *Rostrinuculasinensis* - 97.08%, *Anisomelesindica* - 99.3 % and *Cymariaacuminata* - 96.82 %.

Plants are the major source of therapeutic ingredients in complementary and alternative medicine (CAM). However, species adulteration in traded medicinal plant raw drugs threatens the reliability and safety of CAM. Since morphological features of medicinal plants are often not intact in the raw drugs, DNA barcoding was employed for species identification. Species authentication is a challenging task as most of the raw drugs are traded in the form of dried, fragmented, or powdered leaves, flowers, seeds, stem bark, roots, and other plant parts, which do not possess intact diagnostic characters required for morphological identification by Linnaean taxonomy. Alternative methods such as chemotaxonomy, chromatography, and microscopy, which were adopted for this purpose, showed only limited success in species identification. Major limitations of these methods include involvement of complex chemistries, lack of unique compounds, influence of environmental factors, plant's age, and geographical variations Techen *et al*. (2014). All these challenges can be largely overcome by using DNA barcoding, which does not rely on the morphology of plants, and therefore, is not affected by the above-mentioned factors. Moreover, DNA barcoding can be done using live or dead tissue from any part of the plant at any stage of its life cycle. In fact, identification of species adulteration in traded raw drugs is one of the most useful applications of plant DNA barcoding. Though several chloroplast markers have been used for DNA barcoding of plants (Sucher and Carles (2008), Techen *et al*.

Table 1. The identification percentage of BLAST and BOLD for matk and rbcl

S.No.	Blast for matk	Bold for matK	Blast for rbcl	Bold for rbcl
1	*Anisomeles indica* - 99.6%	*Anisomeles indica* - 99.6%	*Anisomeles indica* - 99.6%	*Anisomeles indica* - 99.6 %
2	*Microtoenaurticifolia* - 97.99%	*Anisomelesmalabarica* - 98.38%	*Microtoenaurticifolia* - 97.99%	*Anisomelesmalabarica* - 98.38 %
3	*Microtoenarobusta* - 97.99%	*Rostrinuculasinensis* - 97.08%	*Microtoenarobusta* - 97.99%	*Rostrinuculasinensis* - 97.08 %
4	*Microtoenadelavayi* - 97.99%	*Anisomelesindica* - 99.3 %	*Microtoenadelavayi* - 97.99%	*Anisomelesindica* - 99.3 %
5	*Colebrookiaoppositifolia* - 97.35%	*Cymariaacuminata* - 96.82 %	*Colebrookiaoppositifolia* - 97.35%	*Cymariaacuminata* - 96.82 %

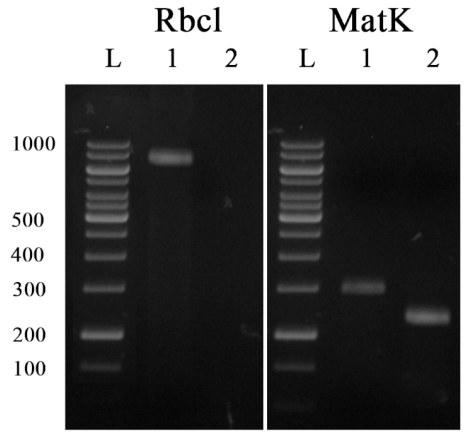

Fig. 1. PCR profile of *A.malabarica*

(2014) the Consortium for the Barcode of Life Plant Working Group has recommended rbcL and matK as core DNA barcode markers for plants CBOL Plant Working Group (2009).

The power of DNA barcoding to identify species has been demonstrated in several studies. Bruni *et al*. (2010) have demonstrated that DNA barcoding can be used for rapid and accurate identification of poisonous plant materials. De Mattia *et al*. (2011) have employed a DNA barcoding approach for species identification in processed plant materials of commercial kitchen spices. Cornara *et al.* (2013) have successfully used DNA barcoding in a multidisciplinary approach for the identification of ingredients in commercial plant mixtures. However, there are only a few DNA barcoding studies in which a large number of medicinal plant raw drugs and natural health products were tested for species adulteration (Kool *et al*. (2012); Wallace *et al*. (2012); Newmaster *et al*. (2013). The present study focuses on the identification of species of *Anisomelesmalabarica* using BLAST and BOLD databases. The two primes used were matK and rbcl. The present study identifies the difference in the study using BLAST and BOLD databases.

The species similar to *Anisomelesmalabarica* identified from BLAST and BOLD differ significantly. Homology greater than 95 % identification are considered as efficient. The sequence identified from BLAST using matk and rbcl are same which are as follows, *Anisomelesindica* - 99.6%, *Microtoenaurticifolia* - 97.99%, *Microtoenarobusta* - 97.99%, *Microtoenadelavayi* - 97.99% and *Colebrookiaoppositifolia* - 97.35%. Similarly, the sequence identified from BOLD using matk and rbcl are same which are as follows, *Anisomelesindica* - 99.6%, *Anisomelesmalabarica* - 98.38%, *Rostrinuculasinensis* - 97.08%, *Anisomelesindica* - 99.3 % and *Cymariaacuminata* - 96.82 %. The sequences identified from rbcl and matK are same in both BLAST and BOLD databases.

Taxonomic analysis of controversial drug plants (Nair 2004) that are often found as adulterant species show that 78% of them belong to other genera and families. The conserved rbcL marker, which is capable of mostly genus-level taxonomic discrimination but credited with good PCR amplification and sequencing success (Bafeel *et al*. (2012), Parmentier *et al*. (2013), Chen *et al*. (2010) would be sufficient to establish species adulteration in most of the traded raw drug. The *rbcL* locus reveals more potential for classification of jackfruit than the matK locus Ho *et al.* (2021).

Conclusion

The discrimination power of *matK* and *rbcL* markers in this jewel orchid study displayed different efficiency level. The *rbcL* gene has higher distinguishing potential than either *matK* gene alone or the combination of both genes. These results are contrast to our present study in which the distinguishing power of the both primers are same.

Acknowledgement

The authors are grateful to the Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Tamil Nadu for supporting and providing all the necessary facilities to conduct this research.

Conflict of Interest- None

References

Bafeel, S.O., Arif, I.A., Al-Homaidan, A.A., Khan, H.A., Ahamed, A. and Bakir, M.A. 2012a. Assessment of DNA Barcoding for the identification of *Chenopodium murale* L. (*Chenopodiaceae*). *Int. J. Biol.* 4:66.

Bruni, I., De Mattia, F., Galimberti, A., Galasso, G., Banfi, E., Casiraghi, M. and Labra, M. 2010. Identification of poisonous plants by DNA barcoding approach. *Int. J. Legal. Med*. 124(6): 595–603.

CBOL Plant Working Group, 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America.* 12794-12797.

Chase, M.W. and Fay, M.F. 2009. Barcoding of plants and fungi. *Science.* 325: 682-683.

Chen, S., Yao, H., Han, J., Liu C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T. and Pang, X. 2010. Validation of the ITS2 Region as a Novel DNA Barcode for Identifying Medicinal Plant Species. *PLoS ONE.* 5: e8613.

Cornara, L., Borghesi, B., Canali, C., Andrenacci, M., Basso, M., Federici, S. and Labra, M. 2013. Smart drugs: green shuttle or real drug? *Int. J. Legal Med.* 127: 1109–1123.

De Mattia, F., Bruni, I., Galimberti, A., Cattaneo, F., Casiraghi, M. and Labra, M. 2011. A comparative study of different DNA barcoding markers for the identification of some members of Lamiacaea. *Food Res. Int*. 44: 693-702.

Doyle, J.J. and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissue. *Focus.* 12**:** 13-15.

Ho, V.T., Tran, T.K.P., Vu, T.T.T. and Widiarsih, S. 2021. Comparison of matK and rbcL DNA barcodes for genetic classification of jewel orchid accessions in Vietnam. *Journal of Genetic Engineering and Biotechnology*. 19(1): 93.

Kool, A., de Boer, H.J., Kruger, A., Rydberg, A., Abbad, A., Bjork, L. and Martin G. 2012. Molecular identification of commercialized medicinal plants in southern Morocco. *PLoS ONE.* 6: e39459.

Maloukh, L., Kumarappan, A., Jarrar, M., Salehi, J., El-Wakil, H. and Rajya Lakshmi, T.V. 2017. Discriminatory power of rbcL barcode locus for authentication of some of United Arab Emirates (UAE) native plants. *3 Biotech*. 7: 1-7.

Nair, R.V. 2004. *Controversial drug plants*. Universities Press.

Newmaster, S.G., Grguric, M., Shanmughanandhan, D., Ramalingam, S. and Ragupathy, S. 2013. DNA barcoding detects contamination and substitution in North American herbal products. *BMC Med*. 11: 222.

Parmentier, I., Duminil, J., Kuzmina, M., Philippe, M., Thomas, D.W., Kenfack, D. 2013. How effective are DNA barcodes in the identification of African rainforest trees? *PLoS One*. 8: e54921.

Ratnasingham, S. and Hebert, P.D. 2007. The Barcode of Life Data System (http://www.barcodinglife.org). *Mol Ecol Notes*. 7(3): 355-364. doi: 10.1111/j.1471-8286.2007.01678. x. PMID: 18784790; PMCID: PMC1890991.

Sucher, N.J. and Carles, M.C. 2008. Genome-based approaches to the authentication of medicinal plants. *Planta. Med*. 74: 603-623.

Tandon, V. and Gupta, R.K. 2004. Histomorphological changes induced by Vitex negundo in albino rats. *Indian Journal of Pharmacology*. 36(3): 176.

Techen, N., Parveen, I., Pan, Z. and Khan, I.A. 2014. DNA barcoding of medicinal plant material for identification. *Curr. Opin. Biotechnol*. 25: 103-110.

Wallace, L. J., Boilard, S.M.A.L., Eagle, S.H.C., Spall, J.L., Shokralla, S. and Hajibabaei, M. 2012. DNA barcodes for everyday life: routine authentication of natural health products. *Food Res. Int.* 49: 446-452.