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EANDANGERED TYLOPHORA ROTUNDIFOLIA BUCH.-HAM.EX WIGHT AND TYLOPHORA FASCICULATE BUCH-HAM.EX WIGHT SPECIES IDENTIFICATION THROUGH DNA BARCODE

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Abstract: DNA barcoding is a modern and broadly used molecular-based identification system that aims to identify biological specimens, and to allocate them to a given species. Though, DNA barcoding is even more than this, and moreover many practical uses, it can be measured the core of an integrated taxonomic system, where bioinformatics plays a key role. DNA barcoding data could be interpreted in diverse ways depending on the examined taxa but the technique relies on standardized approaches, methods and analyses. We tested two medicinal endangered plants *Tylophora rotundifolia* Buch.-Ham. ex Wight and *Tylophora fasciculate* Buch-Ham.ex Wight using two DNA barcoding regions (matK and rbcL). The matK showed poor results for both the plants and rbcL region showed good universality, and consequently the efficiency of these loci as DNA barcodes. We consequently tested the potentiality of the rbcl primer for the identification of two medicinal endangered species, which were collected from Shoolpaneshwar Sanctuary, Dediyapada and Gujarat. In this work rbcl primer were used to discriminate and confirm the identification of two medicinal endangered plants, it was found that, the potentiality of rbcL region in identification process for the two medicinal plants used is more efficiency than matK, where rbcl confirm the identification of two plants at species level.

Keywords: DNA Barcode, rbcL, Barcode, Endangered medicinal Plants

I.Introduction: *Tylophora rotundifolia* Ham.ex Wight and *Tylophora fasciculate* Ham.ex Wight are significant local medicinal plants found in restricted localities and facing threats of dissimilar proportions. However, *Tylophora indica* has a spatially structured population and is found in the plains, hilly slopes and the outskirts of the forests of Gujarat; the other two species do not have a scattered population and are listed as endangered species. *Tylophora rotundifolia* and *Tylophora fasciculata* are over exploited to an extent that now it can be found in restricted regions in the inner recesses of Shoolpaneshwer sanctuary in South Gujarat (D'Cruz, 2003). *Tylophora rotundifolia* is a twining perennial, mostly unbranched, hairy herb. Its leaves are coriaceous, broadly ovate or subrotund, and petiolate (Cooke, 1908; Shah, 1978; Almeida, 2001). The ethnic medicinal men of Gujarat have been using *Tylophora rotundifolia* root extracts for indigestion, as an emetic for insect bite, chest pain. *Tylophora fasciculate* is a stout, tuberous, tall, slightly twining herb. The leaves are ovate-lanceolate. It produces chocolate brown small flowers from August to September, long, ovate or oblong, flat seeds (Cooke, 1908; Shah, 1978; Almeida, 2001). Treatment of Leprosy has been treated with the root extracts of *Tylophora fasciculata*. The root and leaf extracts are used as a nasal drop for snakebite (D'Cruz, 2003). Although DNA barcoding provides quick species identification, its precision relies on PCR technology by using a standardized DNA region as a tag (Hebert and Gregory, 2005). This technology itself has been cited as producing artifacts and inconsistent data. This study is to determine both *Tylophora rotundifolia* Buch.-Ham. exWight and

Tylophora fasciculate Buch.-Ham. exWight taxonomy and delimitations of both species with the core DNA barcodes rbcL. In improver, the efficacy of these barcode for this species identification is considered.

II.Materials and Methods: Field work was carried out during mid-July-August in the year of 2018. Both plant samples were collected from Shoolpaneshwar forests area, Dediyapada, Gujarat. Plants were identified by their morphological characters. It was compared with the Flora of Gujarat (Shah, 1978). Leaves were cut through scissor. Without harming the whole plant from tuber collectedleaves sample were stored in zip lock bag contains silica gel with sample id. After that plant sample were kept in (-20˚C) refrigerator.

2.1 Isolation of Genomic DNA: DNA was isolated from both the plants usingQIAGEN kit based method (DNeasy Plant Mini Kit)

2.2 Polymerase Chain Reaction (PCR): Solution and Reagents: Taq Buffer, Mgcl2, Deoxynucleotide triphosphate (dNTPs), twoprimers (Forward and Reverse), Template DNA, miliQ water.

2.3 PCR Reaction mix: Nuclease free water To makeup volume for 25µl, DNA 25-50ng, Primer (10pmole) 1.0µl**,** 2X PCR Master Mix 12.5µl, Total Volume 25µl

2.4 Primer used: rbcL-F ATGTCACCACAAACAGAAAC

rbcL-R TCGCATGTACCTGCAGTAGC

2.5 Procedure:

➢ Take 12.5 µl 2X PCR Master Mix in autoclave Eppendorftubes.Add 1µl of dH2O and 1 µl of 10X primers, 2µl of DNA. For mixing primers and DNA give short spines then tap it again.Kept tubes into PCR thermal cycler machine and run.

2.5.1 Program setup:

➢1 min 95˚C for 5 min (initial denaturation)

➢ 35 cycles

➢95˚C for 30 sec denaturation

➢45˚C for 30 sec annealing at standardized (Gradient PCR)

➢72˚C for 1 min extension

➢ Repeat 35 cycles

➢Final extension 72˚C for 10 min

➢4˚C ∞.

III.Sequencing and Sequence alignment: Sequencing of DNA was done using ABI-3730 X1 sequencer (Applied Biosystems).Forward and Reverse sequence were generated in ab.1 file format after sequencing. Contig preparation and fasta format sequence were saved in for further bioinformatics analysis.

IV.Results and Discussion: In the current study both *Tylophora fasciculata* and *Tylophora rotudifolia* collected from Dediyapada, Gujarat were assessed for identification and classification using DNA barcoding data although, the general protocol for the isolation and amplification of DNA remained the same but, in some cases, the PCR conditions were optimized. Rigorous optimizations for the isolation of DNA and the PCR annealing temperature for this both sample were performed. rbcL nucleotide sequences were found in good order to be further used for the phylogenetic analysis for both sample.

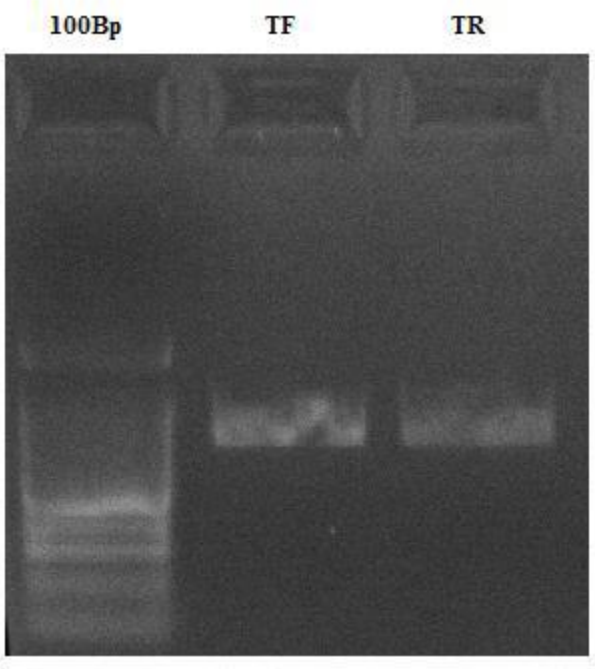

Fig.4.1: 1.5 % Agarose gel image of *Tylophorarotundifolia*Ham.ex Wight and *Tylophorafasciculata*Ham.exWight shows good quality results

V.Agarose Gel Electrophoresis after PCR Product: 1.5 % agarose gel using 1X TAE buffer containing 0.5 µg/ml ethidium bromides was used for electrophoresis of 25 µl PCR products with 3µl gel dye. Amplified PCR products were measured on gel for the presence or absence of the band. Size of PCR products resulting from the primers pairs of the specific barcoding gene weredetermined by using 5 µl of standard 100 bp DNA ladder. Electrophoretic separation was performed at 100V for 30 min. Resulting DNA fragments were visualized using ultraviolet trans illuminator.

VI.Barcode of TF and TR:The amplification of Gradient PCR product was strong enough for isolation of bands or direct sequencing and inthe present study, the DNA sequences were done at Xcelris Labs Ltd. Ahmadabad, India. The methods of ABI – 3730 Xl sequencer gave a success rate of 90-95 %and read length of 700 bases or more.

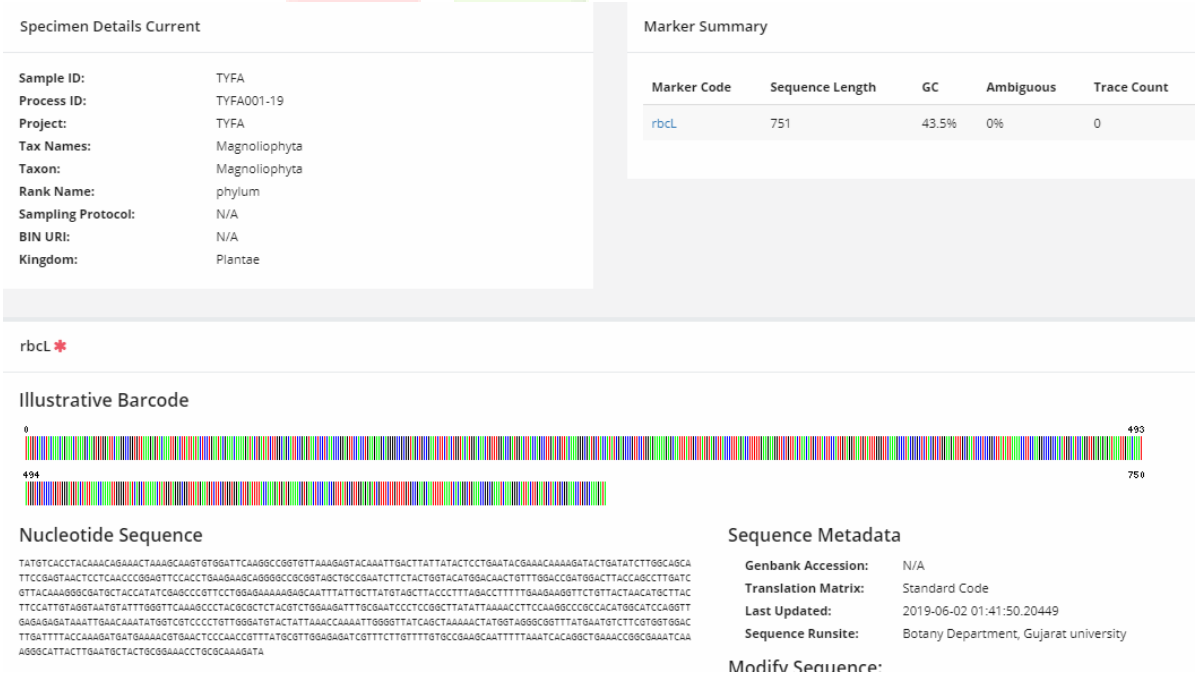

Fig4.2: *Tylophora fasciculate* Ham.ex Wight Barcode generated from BOLD Data System

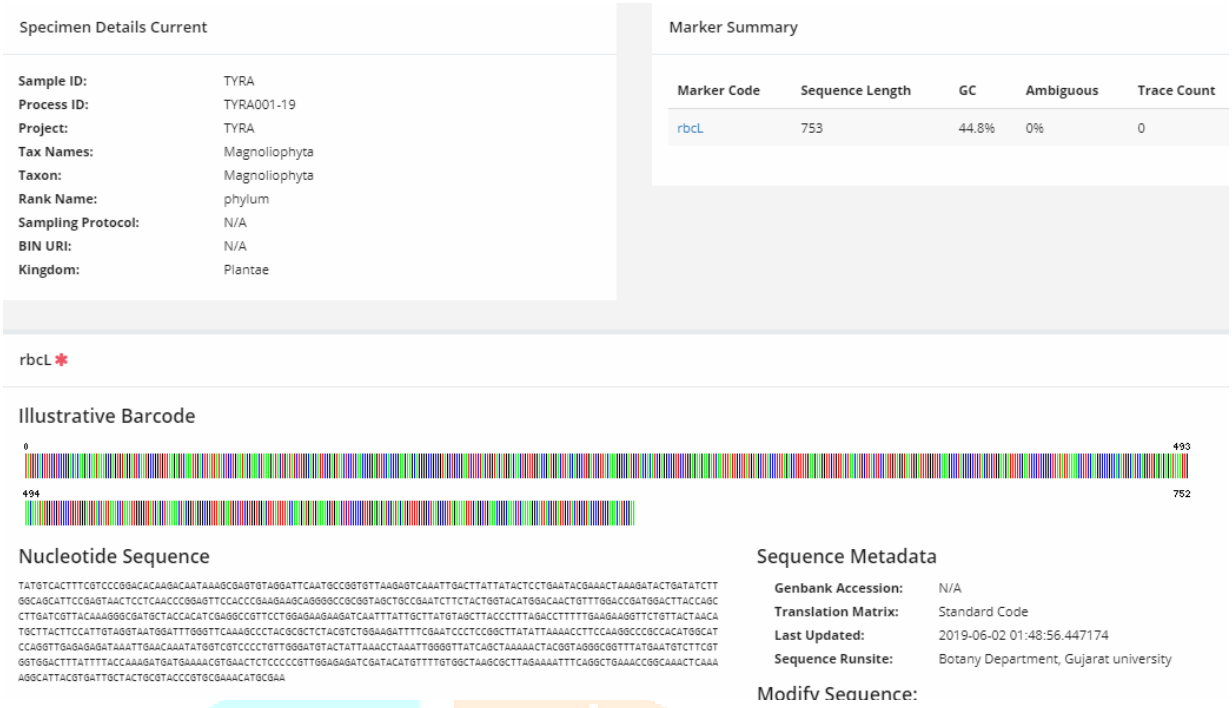

Fig 4.3: *Tylophora rotundifolia* Ham.ex Wight Barcode generated from BOLD Data System

VII. Conclusion: In conclusion, Barcoding of both Endangered Plant species will be useful for quick identification in future for taxonomist. With the current development of primers, we found that rbcL will be very useful for the Barcoding of plant species than matK Primer. On the other hand further procedure will be used to improve clean DNA extraction as well as the development of new primers, PCR amplification approaches, and local authenticated databases. It could play significant roles in effective utilization of Plant DNA Barcoding. In current study the possible DNA Barcode has been generated for two different plant species but comes from same family Apocynaceae from Dediyapada, Gujarat. DNA Barcode has been generated from BOLD Data System. Barcode of both plant species has been successfully submitted to the BOLD data system. Though, the presence of rbcL and matK and other plant gene sequences in the databases has been more and more rapid. As a result, future work on this species will provide trustworthy information regarding the phylogeny of this species.

VIII. Acknowledgment: we are thankful to the local fellows of Dediyapda village for providing me plant materials and we are thankful to Forensic science lab from Gujarat University School of Science. We are thankful to the Xcelris lab.

IX. References:

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