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DNA Barcoding Identification of Endangered *Dipcadi saxorum* Blatt. Species of Gujarat

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ABSTRACT

DNA barcoding is an appropriate molecular method which uses a short sequence as a barcoding region precise for identified species. It has the capability to fast the discovery of new species. In this study, the potential of DNA barcoding to approve the identities of endangered plant species in Dediyapada, Gujarat was assessed using DNA barcode *rbcL*. *rbcL* marker was successful in amplifying target regions for *Dipcadi saxorum* Blatt. species. *RbcL* primer resulted in cleanest reads. Correct identification of any plant is a complete requirement. DNA barcoding is a reliable tool in methodically identifying unknown endangered plants. The current study explains how DNA barcode analysis of the plant *Dipcadi saxorum* Blatt. helps in the correct identification based on nucleotide diversity of short DNA segments. DNA from the leaf of the plant were extracted. The chloroplast gene rbcL were amplified by PCR and sequenced. The sequence was subjected to a BLAST analysis to compare it with that of other species and a phylogenetic tree was constructed. The results established that the plant belonged to the family Asparagaceae. Overall, the endangered species were precisely identified to the species level. It is positively amplified and sequenced using *rbcL* marker. DNA barcoding can contribute to taxonomic and biodiversity research. It will complement efforts to select taxa for various molecular ecology and population genetics studies.

INTRODUCTION:

The genus *Dipcadi* is considered in India by seven species viz. *Dipcadi concanense* (Dalz.) Baker, *Dipcadi erythreum* Webb. Et Berth., *Dipcadi minor* Hook. f., *Dipcadi montanum* (Dalz.) Baker, *Dipcadi saxorum* Blatter, *Dipcadi serotinum* (L.) Medik. And *Dipcadi ursulae* Blatter (Deb and Dasgupta 1981). Because of the taxonomically wicked morphological characters, the taxonomy of the genus *Dipcadi* is poorly defined (Dixit *et al.*, 1992). Undistinguishable and overlying morphology of changed species makes the genus taxonomically tough at species level. Besides, the characters show countless plasticity with environmental conditions. However, *Dipcadi concanense* has ascended to be a very discrete species from the other species of Western Ghats namely *D. montanum, D. minor, D. saxorum* and *D. ursulae* which is grouped together. Among the other Western Ghat species, *Dipcadi ursulae* populations were discrete while *Dipcadi montanum* and *Dipcadi minor,* former measured being a narrow leaved form of the former, grouped together beside with *Dipcadi saxorum.* With the publication of Red Data Book of Indian Plants six species of *Dipcadi* were measured as threatened in India and thus prioritized for conservation (Dasgupta and Deb 1981). *Dipcadi saxorum* Blatter is one of the six threatened plants in India which has been reported as endemic to Maharashtra earlier (Gaikwad *et al.*, 2014). DNA barcoding has been positively applied in identification of *Dipcadi saxorum* Blatt. plant species as well as in reanalyzing taxonomic status. DNA barcoding region rbcL have been selected as universal barcodes for this plant (CBOL Plant Working Group 2009). The rbcL region is easy to amplify and sequenced over a broad spectrum of taxa (CBOL Plant Working Group 2009). It is one of the most quickly developing coding sections of the plastid genome. This study is to determine the current *Dipcadi saxorum* Blatt. taxonomy and delimitations of this species with the core DNA barcodes rbcL. In improver, the efficacy of these barcode for this species identification is measured.

MATERIAL AND METHODS:

DNA extraction: *Dipcadi saxorum Blatt.* Was identified. Leaf were cut and collected from whole plant sample. Stored in zip lock bag contains silica gel with sample id. After that sample were transported to the laboratory and kept in (−20 °C) refrigerator. Total genomic DNA was extracted from freshly collected leaf material according to the modified CTAB protocol (Bishoyi*et al.*, 2016).

PCR Amplification: DNA extracts were diluted to 10 ng/µl and this served as the initial working DNA concentration for PCR amplification. rbcL marker was assessed for species identification. PCR amplification reagents and thermal conditions were used. PCR was performed using isolated genomic DNA of this study to determine the optimum annealing temperatures of the Primers used, namely rbcL-F(5'-ATGTCACCACAAACAGAAAC-3') rbcL-R (5'-TCGCATGTACCTGCAGTAGC -3') primer was obtained from Xcelris Lab Ahmedabad. The PCR reaction mixture was consisted of 12.5µl2X PCR Master Mix, 1.0µlPrimer (10pmole), 25-50ng of template DNA.

Thermal cycling conditions for gradient PCR were as follows: Initial DNA denaturation at 95°C for 5 min, followed by 25/30 cycles of 95°C for 30 sec 45°C for 30 sec, DNA strand extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. The PCR products were verified by electrophoresis in 1.5 % agarose gel stained with ethidium bromide. PCR products were mixed with 1µl of gel loading dye (Bromophenol dye) in a 1.5 % agarose gel containing ethidium bromide along with 5 µl of DNA ladder. Electrophoretic separation was performed at 100 v for 30 min. the resulting DNA fragments were visualized using an ultraviolet Trans-illuminator.

Sequencing: According by provided protocol, All the DNA regions were sequenced by using the Big Dye Terminator v3.1 cycle sequencing Kit. Cycle sequencing was performed by the 10µl volume. Reaction mixture was prepared for each reaction by adding reagents. Two reaction tubes were prepared. One is for forward sequencing primers and other one is for reverse sequencing primers. RbcL gene amplification primer (rbcLF and rbcLR) served as sequencing primers in this experiment.

RESULT AND DISCUSSION:

In the current study *Dipcadi saxorum Blatt.* collected from Dediyapada 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 sample were performed. *Dipcadi saxorum Blatt.* For *rbc*L nucleotide sequences were found in good order to be further used for the phylogenetic analysis.

DNA Sequence:

>Contig

CCAACAGAAACTAAAGCGGGTGTTGGATTTAAAGCTGGTGTTAAAGATTACAGA
TTGACTTATTATACTCCTGATTACGAAACCAAAGATACTGATATTTTGGCAGCAT
TCCGAGTAACTCCTCAACCTGGAGTTCCCGCTGAAGAAGCAGGGGCTGCGGTAG
CTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAG
TCTTGATCGTTACAAAGGACGATGCTACCACATTGAGGCTGTTGTTGGGGAAGAA
AATCAATATATTGCTTATGTAGCCTATCCTTTAGACCTTTTTGAAGAAGGTTCTGT
TACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGTTTCAAAGCCCTACGAG
CTCTGCGTCTGGAGGATCTGCGAATTCCCCCTTCTTATTCCAAAACTTTCCAGGGC
CCGCCCCATGGAATCCAAGTTGAAAGAGATAAATTGAACAAGTACGGTCGTCCC
CTATTGGGATGTACTATTAAACCAAAATTGGGATTATCCGCAAAAAACTACGGTA
GAGCGGTTTATGAATGTCTACGCGGTGGACTTGATTTTACCAAGGATGATGAAAA
CGTGAACTCGCAACCTTTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCTGAAG
CAATTTATAAAGCACAAGCCGAAACAGCGAATCAAAGACATTCTTTAATGCTCT

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.

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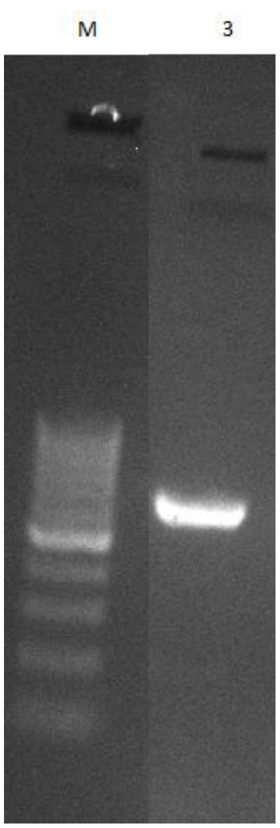

FIG 1: 1.5% agarose gel electrophoresis image of *Dipcadi saxorum* Blatt. M. DNA ladder 3. *Dipcadi saxorum*Blatt.

Gel electrophoresis image shows that *Dipcadi saxorum Blatt.* have good quality and quantity DNA too with another DNA ladder. Using rbcL gene for *Dipcadi saxorum Blatt.* gives better result.

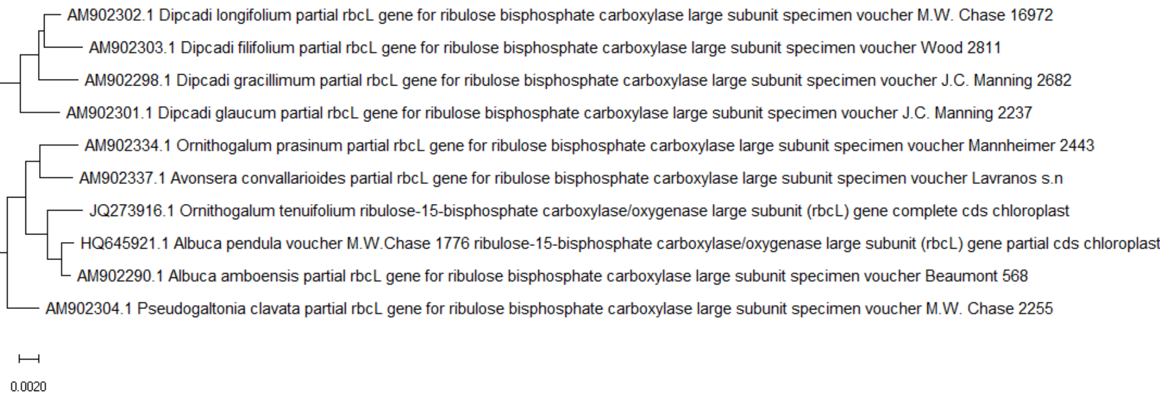

Fig 2: Overall Phylogenic tree represents the evolutionary relationship between the plant species *Dipcadi saxorum*Blatt. and other neighbor joining species.

In the present study, the phylogenetic trees were constructed for the species studied and their related taxa by using Neighbor joining (NJ) method to study the identification, discrimination, closeness and the evolutionary trend among them and the constructed trees. Morphologically it was identified that the *Dipcadi saxorum* Blatt. is from Asparagaceae family. The rbcL gene used for *Dipcadi saxorum* Blatt. The present study sequence showed more similarity to the *Dipcadi* species, belonged to Asparagaceae family. The other species used in the present study were *Dipcadi longifolium, Dipcadi filifolium, Dipcadi gracilimum, Dipcadi glaucum, Ornithogalum prasinum, Avonseracon vallarioides, Albuca pendula, Albuca amboensis, Pseudo galtonia.* All these species were belonging to Asperagaceae family.

Barcode of *Dipcadi saxorum* Blatt. :

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