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Barcoding for authentic identification of medicinal plants

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Abstract
The identification of various species using DNA barcode sequences has been proved to be the most reliable method so far. DNA barcodes allows the identification of species from even a small processed material. In plants the internal transcribed spacer (ITS) region of nuclear ribosomal cistron (18S-5.8S-26S) sequencing is the most commonly used sequence analysis for the species level identification. We therefore used this ITS region sequencing tool for the identification of medicinal plants.

Key Words: DNA barcodes, medicinal plants, ITS region, ribosomal cistron

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1. Introduction

Due to the fewer side effects people have developed trust on use of natural components as a therapeutic agent. An Ayurveda, Unani and Homeopathy medicine uses one or the other part of medicinal plants that have been recognised and accepted all over the world. The major problems to deal with medicinal plants are the correct identification when a small amount of dried/powdered sample provided and adulteration of rare, expensive medicinal plants with easily available local plants. Hence there is a need for the tool that gives correct identification of plant at the molecular level. DNA barcoding system has many prospective uses not only in the identification but also in forensic science, verification of herbal medicines and foodstuffs, resolving ambiguity of species in plant systematic, etc. The Internal Transcribed Region (ITS) is a sequence of primary transcript of RNA and is been removed by splicing during RNA processing. Eukaryotes have two Internal transcribed spacers; ITS-1 located between 18S and 5.8S gene while ITS-2 is located between 5.8S and 28S gene.

Fig: 1

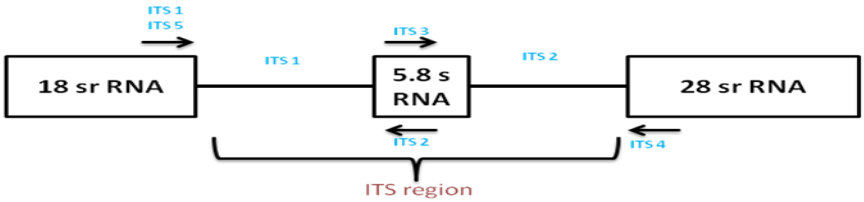


Fig. 1: Internal transcribed spacer region

2. Materials And Methods

Plants selected for the current work

The following nine medicinal plants have been selected for the current study.

Rauvolfia serpentina (L.) Bth. (Apocynaceae), Crotalaria juncea L. (Fabaceae), Jatropa curcas L. (Euphorbiaceae), Jatropa gossypifolia L. (Euphorbiaceae), Pongamia pinnata (L.) Pierre (Fabaceae), Turnera ulmifolia L. (Turneraceae), Butea monosperma (Lam.)Taub Var. Monosperma (Fabaceae), Ricinus communis L. (Euphorbiaceae), Xanthium indicum Koen. (Asteraceae),

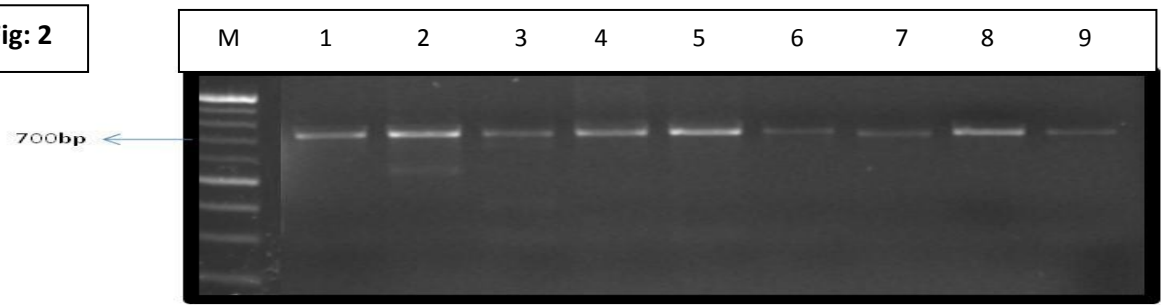
DNA Extraction

The genomic DNA from the above mentioned plants was isolated by Khanuja method; Khanuja *et al* (1999) and then purified with HiPurA spin kit.

PCR amplification

The polymerase chain reaction was carried out in 50µl reaction, containing 50ng of DNA template, 5µl 10X buffer, 3µl 25mM $MgCl_2$, 4µl 10mM dNTPs, 10pmoles of primers and 3 units/µl of Taq polymerase (Eppendorff). The universal ITS primers were used (Sigma) Forward (5'-GGAAGGAGAAGTCGTAACAAGG-3') and Reverse (5'TCCTCCGCTTATTGATATGC-3').

Fig: 2



In the above picture, M is the 100bp ladder (Himedia) and 1 – 9 are the medicinal plants of the same order mentioned in the following table.

Purification of PCR Product (~700bp)

The purification was done with HiPurA spin kit.

3. Sequencing Data

All the sequencing reactions for described medicinal plants in both the direction (Forward and Reverse) are performed by Sanger method at Xcelris Labs Ltd. Ahmedabad, The sequencing data has been aligned and analysed with bioinformatic tools like Chromas lite and ApE software. The total number of base pair alignment in respective plants has shown below. (The sequence data is unpublished here).

S.No.	Plant name	Align data (base pair)	Total number of base pair (from sequencing data)	Restriction digestion with Eco RV	GC%
1	***Rauvolfia serpentina*** (L.) Bth. (Apocynaceae)	646	710	444,266 / 710	62
2	***Crotalaria juncea*** L. (Fabaceae)	644	712	429,283 / 712	56
3	***Jjatropa curcas*** L. (Euphorbiaceae)	646	707	404,303 / 707	64
4	***Jatropa gossypifolia*** L. (Euphorbiaceae)	645	714	403,311 / 714	62
5	***Pongamia pinnata*** (L.) Pierre (Fabaceae)	622	699	422,277 / 699	55
6	***Turnera ulmifolia*** L. (Turneraceae)	603	601	359,242 / 601	61
7	***Butea monosperma*** (Lam.)Taub Var. Monosperma (Fabaceae)	614	670	418,252 / 670	61
8	***Ricinus communis*** *L*. (Euphorbiaceae),	653	704	428.276 / 704	59
9	***Xanthium indicum*** Koen. (Asteraceae)	651	732	434,298 / 732	54

The above table shows the aligned data in base pair out of the total number of base pair sequencing data. The software ApE was used to analyse digestion pattern from total number of base pair sequencing data. Also GC% has been calculated in the ITS region by using same software.

4. Restriction digestion pattern

The PCR product was purified by HiPurA spin column kit and then digested with Eco RV enzyme. 1µg of PCR product was digested with 3U/µl Eco RV (Himedia) enzyme at 37^0C for 1hr that are loaded and checked on 2% Agarose gel. The following digestion band pattern has been obtained for the above mentioned plants.

Fig: 3

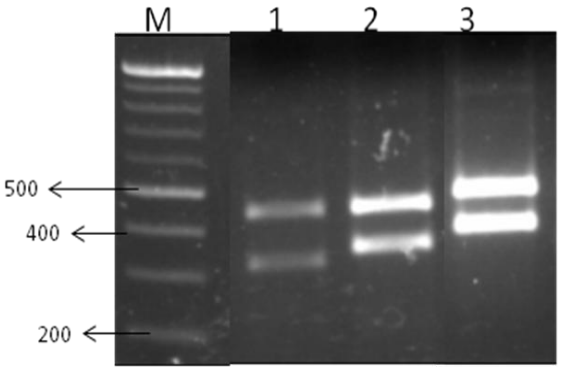


Fig: 4

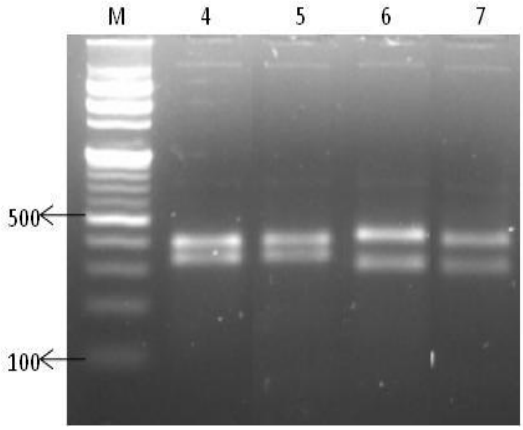
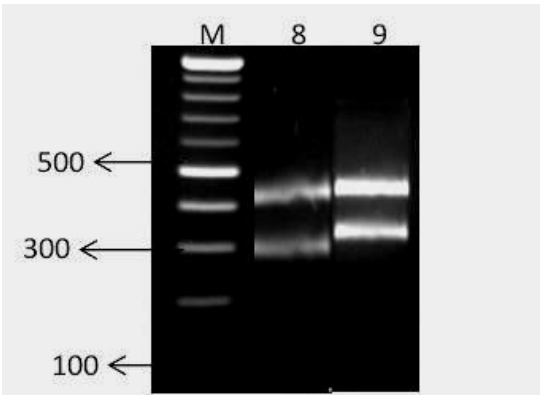


Fig: 5



It has been observed that the ITS region of the above mentioned plants has unique restriction site for Eco RV, and showed two bands in all the reactions set up.

5. Result And Discussion

The ITS region barcode sequencing has been used for the identification of medicinal plants at the molecular level. Also each of the plant specific ITS region gives unique digestion pattern with Eco RV that can be again used for identification of above mentioned medicinal plants.

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