EcoRV digestion patterns in ITS region of medicinal plants

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Abstract – The Internal Transcribed Spacer (ITS) is the evolutionary conserved region present on nr- DNA (nuclear ribosomal DNA) sequence. The ITS region sequences are important markers for the identification of plant species, taxonomic clarification and phylogenetic analysis at the molecular level. With the use of Universal ITS primers developed by White et.al, polymerase chain reaction (PCR) can be performed to amplify ITS region. The entire ITS region (ITS 1-5.8S – ITS 2) varies between 600-1000bp depending on plant species. In most of the angiosperms it ranged between 565-700bp. The sequencing of this region can be used for the further analysis and confirmation of species. In this article the data shown is PCR-RFLP with unique EcoRV site. It has been found from the analysis of sequence data using bioinformatics tools that most of the ITS-region of medicinal plants have single EcoRV site. To confirm this restriction digestion has been performed with EcoRV site and analyzed on Agarose gel electrophoresis that has shown two bands in majority that further confirms the unique EcoRV site.

Key words – ITS region, nr-DNA, PCR, RFLP

1. INTRODUCTION:

THE Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA (nr DNA) has been widely used for identification and phylogenetic analysis of plants. The complete ITS region is separated into ITS 1 and ITS 2. The ITS 1 is present between 18S and 5.8S rRNA whereas ITS 2 is present between 5.8 and 26s rRNA. 5.8S rRNA is a highly conserved region. The ITS 1 is generally longer and variable than ITS 2. The ITS 1 and ITS 2 are incorporated into the mature ribosome but undergo a specific cleavage during the maturation of ribosomal RNA. This region is present in high copy number that allows easy PCR amplification and sequencing from total genomic DNA. (Baldwin et al).

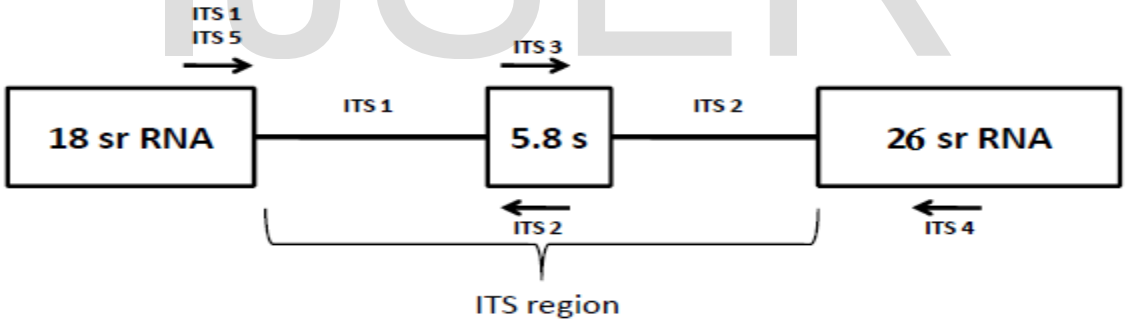

Fig. 1: Internal transcribed spacer region

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2. MATERIALS AND METHODS

2.1 PLANTS SELECTED FOR THE CURRENT WORK

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

S.No.	Botanical name of medicinal plant	Common name	Medicinal use
1	***Michelia champaca*** Linn. (Magnoliaceae)	Champa	Detoxify poison, skin diseases and amenorrhea.
2	***Spinacia oleracea*** L. (Chenopodiaceae)	Spinach, Palak	Natural source of iron, antidiabetic
3	***Jatropa gossypifolia*** L. (Euphorbiaceae)	bellyache bush	Boils, carbuncles, eczema and itches.
4	***Pongamia pinnata*** LPierre (Fabaceae)	Pongam, Indian beech	tumours, cold, cough, diarrhea, leprosy
5	***Cassia tora*** L. (Caesalpiniaceae)	Puwad, Chakunda	Skin diseases
6	***Turnera ulmifolia*** L. (Turneraceae)	Yellow alder	Antibiotic activity against MRSA
7	***Vitex negundo*** Linn. (Verbenaceae)	Chaste tree, nirgundi	Antioxidant, cough, antibacterial
8	***Butea monosperma*** (Lam.) Taub. (Fabaceae)	Palash	Antimicrobial, antifungal, diuretic
9	***Ricinus communis*** L. (Euphorbiaceae)	Castor	cold, gout, nerve pain, convulsion, fever
10	***Crotalaria juncea*** Linn. (Leguminoceae)	Sun hemp, Indian hemp	Antiarthritic, antibacterial
11	***Desmodium gangeticum*** (L.)DC (Fabaceae)	Salparni	Antidysentric, typhoid, antipyretic, asthama, piles, snake bite.
12	***Xanthium indicum*** Koen. (Asteraceae)	Chota dhatura, common cocklebur	Leucoderma, fever, epilepsy, salivation
13	***Cucumis melo*** L. (Cucurbitaceae)	muskmelon; cantaloupe; honeydew	Burns, diuretic
14	***Alstonia scholaris*** R.Br. (Apocynaceae)	Blackboard tree, Indian devil tree, Ditabark, Saptaparni	Malaria, epilepsy, asthama, skin
15	***Dregea volubilis*** Benth ex Hoo.f. (Apocynaceae)	cotton milk plant, akad bel	Indigestion,dyspepsia, dysentery, insomnia, dog bite, insect bite,
16	***Millettia peguensis*** Ali (Fabaceae)	Moulmein rosewood	Antitumor, antibacterial, antiviral
17	***Jacquemontia pentantha*** G.Don (Convolvulaceae)	Sky blue cluster vine	Cathartic
18	***Piper nigrum*** linn. (Peperaceae)	Black pepper	Antitumourigenic, immunostimulatory, anticholestrolemic
19	***Sapindus emarginatus*** Vahl. (Sapindaceae)	Ritha, soapnuts	Eczema, psoriasis

20	***Abrus precatorius*** Linn. (Fabaceae)	Crab's eye, rosary pea, gunja	Purgative, ophthalmic, emetic
21	***Cocculus hirsutus*** (Linn.) Diels (Menispermaceae)	Musakani	Arthritis, leprosy, skin diseases, gout
22	***Pudranjiva roxburghii*** Wall. (Euphorbiaceae)	Putranjiva, Jivanputra	Infertility
23	***Vigna radiata*** (L.) Wilczek (Fabaceae)	Moong	Anti-cancerous
24	***Holarrhena pubescens*** (Buch.-Ham.) Wall. ex DC. (Linn.) (Apocynaceae)	karva indrajau, kutaja	Diarrhoea, gastric, piles, spleen problems
25	***Azadirachta indica*** A.Juss (Meliaceae)	Neem	Antidiabetic, contraceptive, antibacterial,antifungal
26	***Cassia siamea*** (Lamarck) (Leguminosae)	Cassod tree	Depress central nervous system, relieves stress, comstipation
27	***Momordica dioica*** Roxb. (Cucurbitaceae)	Spiny gourd, kantola,	Antimicrobial, antidiabetic, antiallergic

2.2 DNA EXTRACTION

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

2.3 PCR AMPLIFICATION

The polymerase chain reaction was carried out in 50µl reaction, containing 50ng of DNA template, 5µl 10X buffer, 3µl 25mM MgCl2, 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').

2.4 PURIFICATION OF PCR PRODUCT (~700BP)

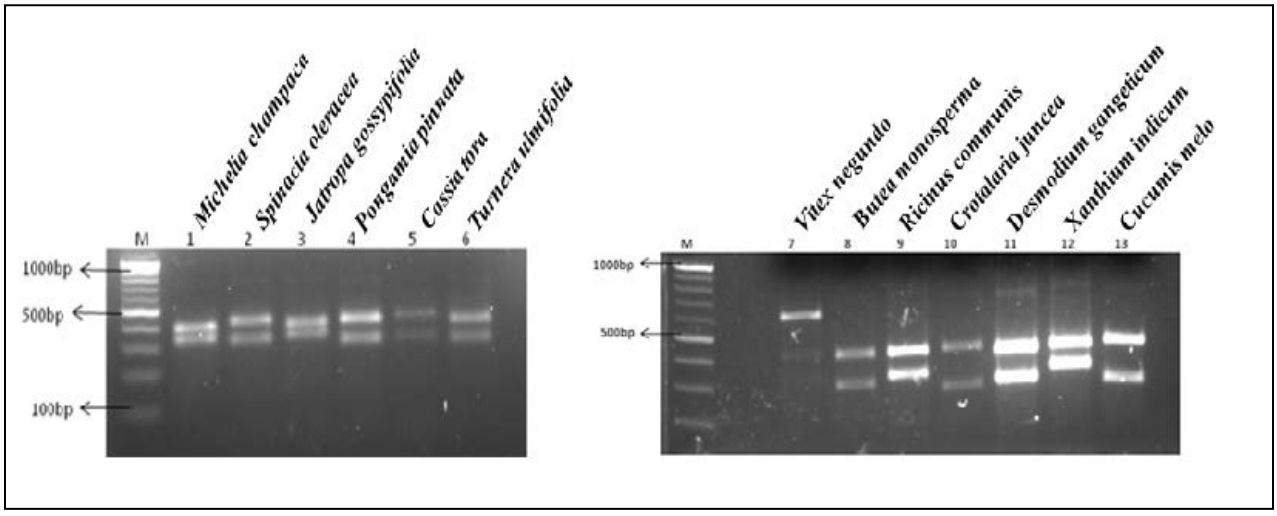
The purification was done with HiPurA spin kit (Himedia).

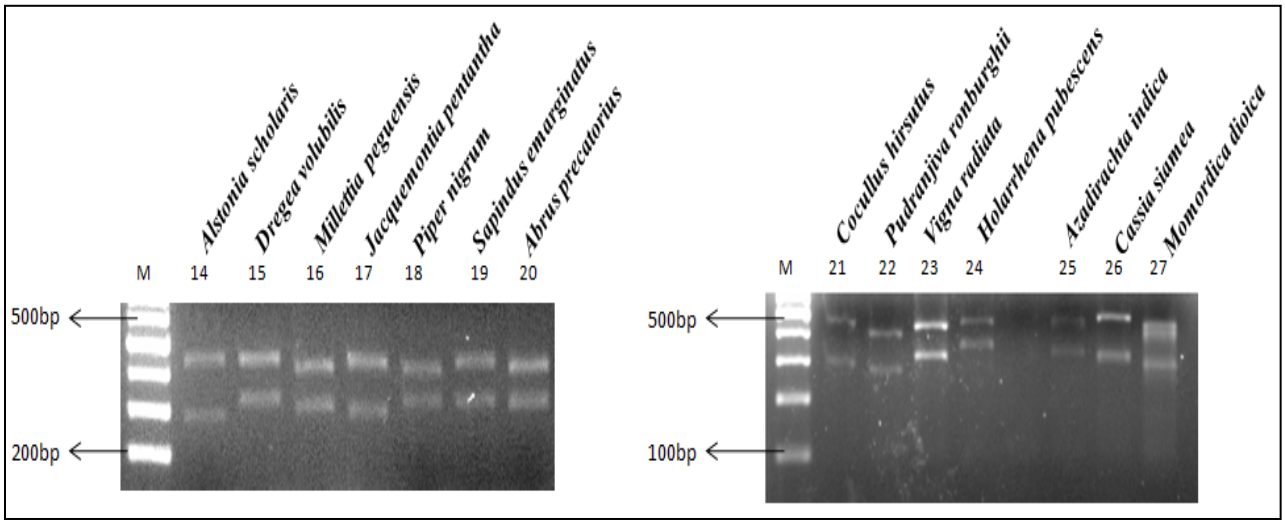
2.5 RESTRICTION DIGESTION WITH ECO RV:

The digestion of purified PCR product (10µg) was performed in 50µl as per the instructions (Himedia).

2.6 RESULT:

The following bands patterns have been observed.





Discussion

The observed restriction band patterns clearly show that ITS region of most of the mentioned medicinal plants has only one site for EcoRV.

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Conclusion:

As most of the ITS region of medicinal plants contains a single restriction site for EcoRV enzymes, this can be used as a marker in confirmation of the plant species.

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