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Review Article

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REVIEW ON MOLECULAR MARKER AND METHODOLOGY FOR ITS2 MARKER FOR PLANT IDENTIFICATION

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ABSTRACT:
Plant molecular biology is the most expanding field due to neutraceuticals, herbal medicines and ever-increasing demand of food for the World. Various field including Ayurveda, natural product, food and agriculture needs to identify plants correctly to keep their properties intact for any specific products. To identify correctly, various molecular markers are available, among all RAPD is the most favorable marker due to easiest way of comparative studies among samples. Along with RAPD, AFLP, SSR, ISSR, matK, rbcl, rpoB, Cyt C etc, ITS1 and ITS2 along with flanking regions and 5.8S, 28S are extensively studied. For medicinal plants ITS2 is most favored gene for molecular identification.
KEYWORDS: Plant, Molecular Marker, Identification

Introduction- Plants provide some of our most valuable medicines factors. Plant molecular biology is the most expanding field due to neutraceuticals, herbal medicines and ever-increasing demand of food for the World. Various field including Ayurveda, natural product, food and agriculture needs to identify plants correctly to keep their properties intact for any specific products. However, the lack of capacity to identify the majority of therapeutic plants has resulted from urbanisation and the loss of contact with nature. Organoleptic tactics (through the senses of taste, sight, smell, and touch), naturally visible and microscopic systems, and chemical profiling such as TLC, HPLC-UV, and HPLC-MS are all included in the conventional approaches for identification (Soni et al. 2013). These approaches, however, frequently fail to correctly identify closely related species because physiological and storage circumstances might change the outcome of chemical profiles or biochemical markers. Furthermore, none of these techniques can easily distinguish between phylogenetically similar species of medicinal plants. Additionally, identification of powdered or processed samples using a typical taxonomic method to plant identification is not possible. Therefore, it is vital to create a method for identifying adulterants and authenticating plants that is accepted around the world.

Since DNA is a constant macromolecule that is present in all tissues and is unaffected by environmental factors. To identify correctly, various molecular markers are available.Along with RAPD, AFLP, SSR, ISSR, matK, rbcl, rpoB, Cyt C etc, ITS1 and ITS2 along with flanking regions and 5.8S, 28S are extensively studied. For medicinal plants ITS2 is most favored gene for molecular identification of the plant

DNA based molecular identification:

A DNA-based method would give precise information and make it easier to distinguish between different species. To ensure the quality of particular items, it is crucial to have a strong and trustworthy system for species discrimination. Short DNA sequences are used in barcoding, a technique for identifying species.

Molecular marker is very popular and powerful tool to give information of genetic variance within or among species and it is helpful for identifying species, variety of crop or plant and assessment of genetic variation. "A DNA fragment known as a molecular marker, sometimes known as a genetic marker.

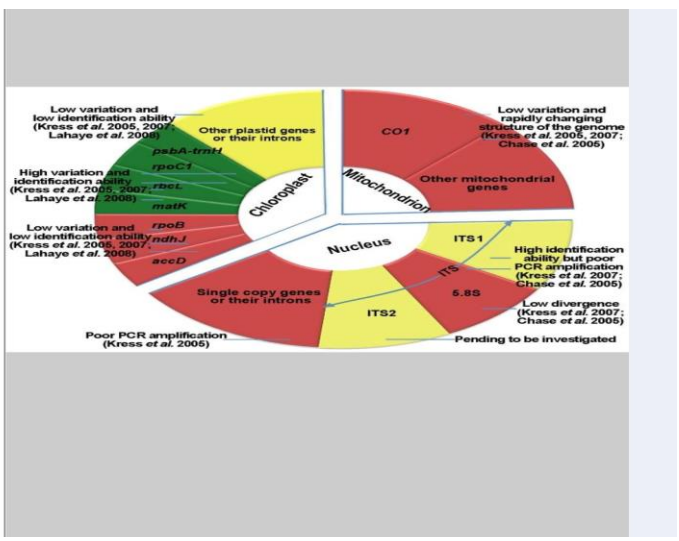
There are various types of marker which are used for various purposes like identification, fingerprinting, forensic etc. These are mainly two type based and type of reaction first Non-PCR based marker that is RFLP (Random fragment length polymorphism) and VNTR (variable number of tandem repeats) and second is PCR based marker that is There are several names for randomly amplified polymorphic DNA, including RAPD (randomly amplified polymorphic DNA), SCAR (sequence characterised amplification region), AFLP (amplified fragment length polymorphism), and ISSR (P.Kumar et al, 2009). The identification of species diversity by using molecular markers has been proposed recently by the use of short DNA fragment of nuclear genome or extra nuclear genome. As diagnostic tools for authentication, several researchers have also sequenced additional DNA sections like the spacer region of the 5s rDNA and the trnK of chloroplasts. The small DNA sequence used for species evolutionary studies at different taxonomic levels or genetic variation etc. referred as DNA barcode gene and region of these genes is called as DNA barcode gene.

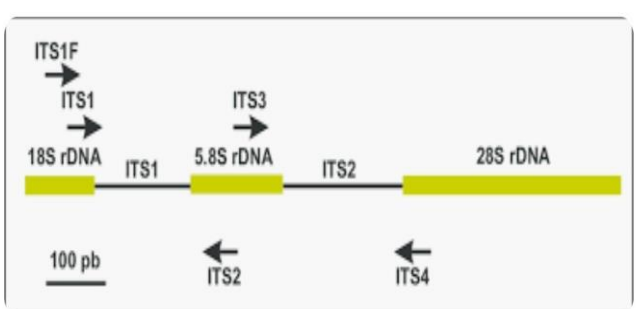
Among several methods, DNA barcoding has been acknowledged as a reliable, quick, affordable, and widely applicable tool for species identification. In order to offer precise identification at the species-level, DNA barcoding uses sequence variation within a brief, standardised section of the genome as a "barcode" (Hajibabaei et al., 2007).

For plant molecular systematic nuclear ribosomal internal transcribed spacer region (ITS) or ITS2, chloroplast internal transcribed spacer region (trnH-psbA, atpF etc) and chloroplast coding region (matK, rpCL) have been used. The sequences used, known as DNA barcodes, are usually short (300-800 bp). Despite being primarily used to identify plant species, chloroplast DNA barcoding has potential applications in the food industry, evolution research, and forensics. It has been proposed that the rbcL, matK, rpoB, and C genes, the non-coding spacers atpF-atpH, trnH-psbA, and psbK-psbI trnL-F, the

trnL (UAA) intron, and the internal transcribed spacer-2 (ITS-2) region of nuclear ribosome all serve as DNA barcodes. ,

ITS2 is considered one of the most significant candidates for DNA barcoding (Jingyuan Song et al., 2010). The 18S, 5.8S, and 28S ribosomal subunit sequences are found in each repeat of the primary ribosomal RNA genes in plants, which are clustered in a cluster of highly repetitive sequences. Within each repeat, these conserved areas are separated by Internal Transcribed Spacers (ITS), which exhibit higher rates of variation. The spacer between the 18S and 5S genes is known as ITS1, and these genes exhibit little sequence divergence amongst closely related species . The spacer between the 5.8S and 28S genes is known as ITS2(Carol E. et al,.1993)





The Present study focused on the identification selected plant by using ITS2 database including ITS2 structure prediction and ITS2 NCBI BLAST.

MATERIALS AND METHODS :

Methodology: -

I) Type of study design- observational

II) Study setting- in authentic genetic lab duration of study- 2 to 3 years

III) Study population- sample of selected plant material e.g.leaves

IV) Sample size- as per requirment

V) Method of sampling- Simple random sampling

VI) Method of selection-

Inclusion criteria- fresh sample

Exclusion criteria -infected sample

DNA ISOLATION BY C-TAB METHOD

Reagent and chemicals: C-TAB extraction buffer, C-TAB wash buffer, Betamercaptoethanol, Chloroform, 10X TE buffer, RNase A (Merck, India), Iso-amyl alcohol, Isopropyl alcohol, 70% ethanol, Tissue roll (Johnson), cotton swab with IPA. Prepared CTAB extraction buffer by preheating the solution to 65oc. (Always prepare in minimum but adequate quality), Chloroform: isoamyl alcohol (24:1) stored in the dark RT solution Prepared and dispense near window with proper ventilation. To the CTAB wash buffer added 360 ml of absolute ethanol and stored at 4^{o}C in a glass bottle. Pre-chilled the CTAB wash buffer and 70% ethanol prior to use.

Lab Facilities and instrumentation: The DNA isolation was carried out in separate working table along with separate set of micropipettes only meant for plant DNA isolation, micro pestle, Spinwin MC01 micro centrifuge with 10,000 rpm, Spin win MC02 micro centrifuge with 13,000 rpm, Spinix shaker.

POLYMERASE CHAIN REACTION (PCR) - Reagents and Chemicals: Nuclease free (DNase, RNase, Proteinase free) water, 10X PCR buffer (Taq Polymerase buffer B), 50 mM MgCl2, 10 mM dNTP mix, 1U/ul Taq polymerase Enzyme, genomic DNA for each PCR reaction, primer for PCR: 10 uM i.e. 10 picomoles / ul (stock concentration was diluted 1:10 to get 10 pmoles/ul from 100 pmoles/ul). For all PCR reactions, 10 pmoles /ul primer concentrations were used.

Lab facilities and instrumentation: Thermal cycler Gradient (Bio-Rad,USA); Spinwin, Micro centrifugation machine (10,000 rpm) for 1.5 ml eppendorf tubes, Microspin centrifugation machine (6,000 rpm), 200 ul PCR tubes, Spinix shaker (Tarson, India);

Laminar air flow (microfilt, India) devoted set of micropipettes for PCR master mix preparation.

AGAROSE GEL ELECTROPHORESIS -Reagent and Chemicals: Tissue roll (Johnson); Autoclaved Double distilled water (from our Lab); 50X TAE (stock which is diluted into 1X for working) , 6X DNA loading dye/buffer i.e. orange or BPB with

Xylene Cyanol (Merck, India), 1 mg/ml EtBr (sd fine chemicals, India), PCR products, double distilled water for cleaning Gel Docment surface after gel visualization.

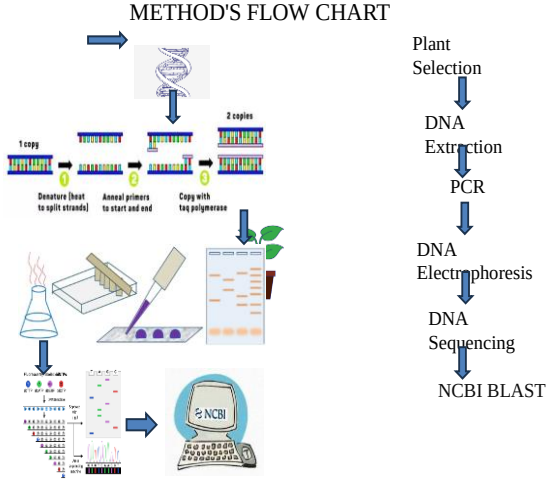
Lab facilities and instrumentation: Clean working table devoted only for DNA electrophoresis with separate set of micropipettes and plastic wares, Gel casting tray and gel casting assembly with 8 well/ 9 well combs, Gel lifting tray, Mini sub cell GT submarine agarose gel electrophoresis unit (Bio-Red, USA), Universal power pack (Bio-Red, USA), Gel documentation system XR and Quantity one software for analysis of gel (Bio-Rad, USA), Personal computer with 1GB RAM, Graphic card and Intel Core2Duo Microprocessor.

Applied Bio-informatics for data analysis and initial molecular identification: Hardware: High speed Personal computer with 1GB RAM Graphic card and IntelCore2Duo Microprocessor or higher/ present days laptop and broadband internet for genomic data download and online BLAST analysis.

Conclusion-The ITS2 gene, one of the most critical indicators in molecular systematics and evolution, exhibits high sequence variation at the species level, reconstruct phylogenetic trees. ITS2 may be helpful as a common DNA barcode to distinguish between plant species because of its beneficial characteristics, including the availability of conserved regions for designing universal primers, the ease of its amplification, and enough variability to distinguish even closely

related species, ITS2 is considered one of the most significant candidates for DNA barcoding

METHOD'S FLOW CHART



Plant Selection
↓
DNA Extraction
↓
PCR
↓
DNA Electrophoresis
↓
DNA Sequencing
↓
NCBI BLAST

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