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ITS-2 marker for identification of medicinal plants in herbal industry: A supporting tool to differentiate adulterants

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Abstract
Traditional System of Medicine aiming towards treating several diseases and their preventive measures. Such out bursting of pandemics demand promising medication. In this direction, usage and standardization of extractions from medicinal plants would pave an assuring treatment and/or preventing diseases. Since medicinal plants are dynamic in treatment of various diseases especially in India, it becomes challenging task to supply the plants to fulfill the demand. The increasing demand and trade of medicinal plants provide the major source of income to suppliers. To reach the demand in the market, the suppliers depend on adulteration of the product, which misleads the customers. Thus, purposeful adulterations of intended ingredients is a major trouble in recognizing the genuine resources. Validation of therapeutic plants by using molecular techniques is a promising scientific advancement. Though several molecular markers like restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), selective amplification of microsatellite polymorphic loci (SAMPL), simple sequence repeats (SSR), inter simple sequence repeat (ISSR), DNA barcoding and next generation sequencing techniques have been reported to validate the available herbal resources, very less is known about its applications in this field. The present review is an attempt to compile available information and highlight the promising genomic approaches like barcoding towards identifying adulterations among the available medicinal plants.

Keywords: barcoding, medicine, techniques, adulteration, genomic, ingredients

Introduction
Plant and plant product have been utilized since ancient time for the prevention and cure of different disorders in human beings as well as animals. In recent era medicinal plants/herbs are playing key role in world health care system. Around 80% of the total population use herbal medications as their essential medicinal attributes. Such plants are distributed throughout the world, but they are generally abundant in tropical part.

It has been observed that enormous manufacturing units in herbal sector are producing products for consumer. The most important concept is to treat the ailments through herbal plant extracts which is irreplaceable with other alternatives. In this direction, wild resources are to be conserved, collected and supplied with authentication. Routine testing must be carried out to identify such wild species and extracts for assurance, as various plants have similarity morphological traits among species within the class. Adulterated or substitute plant parts are generally validated depending on their phenotypic observations accomplished by an expert taxonomist. Similarly, lack of distinguishing differentiation for the morphological qualities is hindering specialists for guaranteed accuracy.

Misidentification of the essential plants may prompt the consideration of unfortunate, unrelated species, with a potential health issue to the patient. Replacement of the product's either deliberately or coincidentally can have negative impact on both purchasers and makers. The development of reliable methods for species identification must for good manufacturing practice and quality assurance of the products.

In this direction, molecular techniques like DNA barcoding is an incredible asset to identify both animal and plant species. DNA barcode is a short standard DNA region(s) used to distinguish living bodies. A report by Hebert (2003) demonstrated fruitful distinguishing proof of different group of animals like bird, reptile, insect and mammal by targeting a mitochondrial gene cytochrome oxidase I (COI) [3-5]. However, such demonstrating progress among plants is limited.

Various markers are used to obtain suitable results to classify and differentiate plant species and usage of different promising DNA regions like matK, rbcL, trnH-psbA spacer area, trnL and ITS can be useful towards barcoding [6-12]. Massive information on genomic information of various plant species is available and accessible online databases and relevant tools can be explored in this aspect. A combination of barcoding with high resolution melting (HRM) analysis and PCR technique would screen DNA separation. The utilization of BarHRM (Barcoding combined with HRM) has been depicted for species distinguishing and detection in food and plant products [13-18]. Recent reports show a comprehensive investigation on selecting Bar-HRM primer for species distinguish process among various plant groups that can be identified and separated by explicit distinctive markers [19].

Traditionally, herbs confirm by morphological appearance and anatomical characters. Some of these techniques require skill to operate equipment's and identify plant species. Most of the herbs are validated by under microscopes, where the shape, size and organelles of different cells are inspected, analyzed and reported. Other analytical separation techniques including thin layer chromatography, high performance liquid chromatography and liquid chromatography have been utilized for herbal drug validation. Comparatively, molecular techniques using markers are promptly complemented few methods to identify the species. Markers have been broadly utilized in quality control and standardization of herbal medications. Usage of markers dependent on genetic composition and its stability. Such DNA markers are not influenced by age, physiological condition as well as natural factors. Various sorts of DNA based markers *viz*., RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeat), etc., are utilized for species discrimination of plants including scientific classification, physiology, embryology and hereditary designing.

Molecular markers used for authentication

DNA hybridization based markers (Non PCR based markers)

The confinement of this methodology is that it needs high quantity of DNA and utilization of radio level probes for examines [20].

RFLP (restriction fragment length polymorphism)

It is a molecular marker utilized for the isolation and distinguishing proof of desired sections of DNA by restriction enzymes. It is used for detection of polymorphism dependent on DNA differences. Methodology is to isolate DNA, its digestion by restriction enzymes and separation by gel electrophoresis. The ideal fragment is identified by utilizing labeled probes. The primary constraint of this method is, it requires enormous amount of DNA, tedious and labor intensive [20].

Markers based on PCR amplification

PCR method is used for the amplification of required DNA sequences. It requires low quantity of DNA for the experiments.

Random amplified polymorphic DNA (RAPD)

RAPD is a kind of PCR reaction, however the segment of DNA that are intensified are arbitrary. The researcher performing RAPD makes several random, short primers (8−12 nucleotides), at that point continues with the PCR utilizing a huge template of genomic DNA, trusting that fragment will amplify. By resolving the subsequent patterns, a semi-novel profile can be gleaned from a RAPD reaction.

No information on the DNA sequence for the targeted gene is required, as the primer binds randomly in the sequence. However, the specific binding site is unknown. This makes the process well known for comparing the DNA of biological system that have not had the consideration of researcher's community, or in a system wherein moderately scarcely any DNA sequences are compared. Since it depends on an enormous and intact DNA template sequence, it has a few confinements in the utilization of debased DNA sample. Its resolving power is much lower than targeted, species explicit DNA comparison process. In recent years, RAPD has been utilized to portray and race the phylogeny of differing plant and animal species. When developing locus-specific and co-dominant markers from RAPDs, new longer and explicit primers are intended for the DNA sequence, which is known as the SCAR [21-24].

Amplified fragment length polymorphism PCR (AFLP)

AFLP is a PCR-based instrument used in energetic research, DNA fingerprinting and in the practice of genetic engineering. Developed in the mid 1990's by Keygene (http://www.keygene.com). AFLP utilizes restriction enzymes to cut genomic DNA and is trailed by ligation of adaptors to the sticky end of the limitation fragment. A subset of the restriction sections then intensified utilizing primers integral to the adaptor and part of the limitation site fragments. The intensified fragments are pictured on denaturing polyacrylamide gels either through auto-radiography or fluorescence approaches.

There are many advantages of AFLP when contrasted with other marker technologies including randomly intensified polymorphic DNA (RAPD), RFLP and microsatellites. AFLP not just has higher reproducibility, resolution and affectability at the entire genome level contrasted with other techniques [25]. There is no prier sequence data is required for amplification [26]. Therefore, AFLP has become incredibly valuable in the investigation of taxa including microorganisms and plants, where much is as yet unknown about the genomic makeup of different organisms [27-30].

Inter-simple sequence repeat (ISSR) and simple sequence repeats (SSR)

Microsatellites, SSRs, or tandem repeats, are repeating sequences of 1−6 base sets of DNA. Microsatellites are regularly neutral and co-dominant. They are used as molecular markers in genetics, for kinship, populace, and different studies. ISSR is a general term for a genome district between microsatellite loci. The corresponding sequences of two neighboring microsatellites are utilized as PCR preliminaries. The variable regions between them get intensified. The restricted length of amplification cycles during PCR block extreme replication of excessively long contiguous DNA sequences, so the outcome will be a mixture of an assortment of intensified DNA strands which are commonly short but vary in length.

Sequences intensified by ISSR-PCR can be utilized for DNA fingerprinting. Since an ISSR might be a preserved or non-preserved region, this method isn't helpful for recognizing individuals, but instead for phytogeography analysis or possibly delimiting species. Sequence decent variety is lower than in SSR-PCR. Furthermore, microsatellite sequencing and ISSR sequencing are commonly helping, as one produces primers for the other [31-34].

Internal transcribed spacer (ITS) based sequence characterized amplified regions (SCAR)

Internal transcribed spacer (ITS) region of rDNA, characterized as the unit containing the ITS1 spacer, 5.8S rDNA gene and ITS2 spacer have been demonstrated to be a helpful succession to examine phylogenetic connections in many plant species. This region can be promptly amplified by polymerase chain reaction (PCR) with explicit primers and sequenced. In view of their different rates of evolution, ITS region has become the favored markers in evolutionary investigations at various taxonomic levels. This procedure is additionally proved a valuable molecular marker for screening various species in a genus.

Technically, building up a SCAR marker requires the two specific primers structured from nucleotide sequences built up in cloned gene of intrigue. Once it is grown, enormous number of samples can be screened at a time thus decreases the time and improves reliability. In this manner, several number of reactions are required to carry out the fingerprinting process. SCAR marker partition proficiency is better than that of RAPD or ISSR markers dependent on the number of reactions required per sample. Furthermore, the SCAR marker gels are cleaner and simpler to score assisting PC computerization. The main limitation of its development might be the sophisticated laboratory facilities like sequencing.

Conclusion

Genomic fingerprinting can differentiate between individuals, species and populaces and has been demonstrated helpful for the portrayal of sample homogeneity and identification of adulterants. DNA based confirmation of therapeutic plants is a work in progress that offers incredible new tools and entry points for measures aimed on quality control and quality affirmation in medicinal plant research as well as the production, clinical use, and forensic assessment of herbal drugs. However, very limited studies have been reported to discriminate the herbal plant species. It is important to examine the failure rate of different DNA techniques, particularly for those high-value therapeutic plants. DNA-based species differentiation is important to identify the species. Perhaps, exploring the whole genome and epigenetic information is highly essential for quality assurance. Metabolomics approach would also play significant role in mediating the pharmacologic impacts of herbal drugs. Thus, the present review emphasizes the marker-based molecular approach to identify the herbal plant away from its adulterants. Based on this information, an array of authentication and assessment tools would be explored and used in future to conserve the herbal plant resources and discriminate them from their adulterants for the benefit of human kind.

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