Application of DNA Fingerprinting for Plant Identification

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Abstract

DNA fingerprints led to the identification of closely related plant species and it is one of the tool for assessing genetic diversity and species relationship. DNA is most stable and does not vary seasonally and with age of the plant. DNA based fingerprinting techniques plays greater role in authentication of botanicals. This review gives an outline about the importance of DNA fingerprinting, DNA fingerprinting methods, procedure for DNA fingerprinting and DNA based markers. In addition, this review will provide the comprehensive data on the DNA based markers for the identification and authentication of medicinal herbs such as Ocimum species, Ipomoea mauritiana, Embelia species, Solanum species, Zingiber species, Citrus species and Cryptocoryne pallidinervia, for species differentiation of Cinnamomum species and Mentha species, for adulteration detection of Angelica species, Zanthoxylum species and for identification of phytoconstituents of Curcuma species, Mentha species and Aloe species. This review emphasize on the importance of DNA fingerprinting for the medicinal plants.

Keywords: DNA fingerprints, medicinal herbs, genetic diversity, techniques, identification tools.

Introduction

DNA fingerprints are a bar code like patterns generated by amplification of chromosomal DNA of an individual which can distinguish the uniqueness of the individual from another. Proper identification is necessary for the closely related taxon of the botanicals. Morphological identification such as shape, size, colour, texture, fracture characteristics, odour and taste are used for discrimination of botanicals. Microscopic evaluation includes comparative microscopic inspection of the crude drugs. Chemical profiling establishes a characteristics chemical pattern for a plant material. Chromatographic tools like Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) are used for qualitative and quantitative determination of impurities. However, these methods have limitations because of the composition and relative amount of chemicals in particular species of plant varies with growing condition, harvesting period, post-harvesting period and storage conditions. Each herb contains large number of compounds and therefore, it is also not possible to analyze or trace the presence or absence of all the compounds of interest either qualitatively or quantitatively. These serious difficulties in testing for active principles or chemical constituent are well known. In spite of these difficulties, DNA fingerprinting plays a very important role in the authentication of botanicals.

DNA Fingerprinting

DNA fingerprints are also called as DNA typing, genetic fingerprinting and DNA profiling. DNA in cell is made of nucleotide such as adenine, guanine, thymine, cytosine and pentose sugar joined by phosphate bonds. DNA fingerprinting is based on the identity of organism at molecular level i.e., genetic characteristics. DNA profiling is primarily used in botanicals for protection of biodiversity, identifying markers for traits, identification of gene diversity and variation etc. DNA markers in molecular biology and biotechnology are used to identify the particular sequence of DNA from group of unknown and for the protection of biodiversity.

DNA Fingerprinting Methods

The basic methods of DNA fingerprinting in plants involve the isolation of DNA from plant cell, quantification and quality assessment of isolation. Further the fingerprinting can be done by PCR method like Random amplification polymorphic DNA (RAPD), Inter simple sequence repeat (ISSR), Amplified fragment length polymorphism (AFLP), DNA amplification fingerprinting (DAF) and non-PCR method like restriction fragment length polymorphism (Santhosh et al., 2014).

Procedure for DNA Fingerprinting

The procedure for DNA fingerprinting involves isolation of DNA from plant parts like leaves, roots and stem is done by removal of cell wall and nuclear membrane around the DNA and separation of DNA from cell debris, proteins, lipids and RNA by the common CTAB method. Quality and quantity of isolated DNA was checked by UV-Visible spectrometry. The quality check is done through A260/A280 ratio where 1.8 value shows the highest purity and more than 1.8 shows the RNA contamination and less than 1.8 shows the protein contamination.
Genomic hybridization with Micro and Minisatellite, Random Variable Number Tandem Repeat (VNTR), Probe polymorphism. DNA based techniques are used to evaluate DNA salt concentration and nature of DNA template (Santhosh nucleotides per second depending upon the buffer, nucleotide incorporation at 72ºC. Estimates for the rate of primer addition, which deals with the following steps: Taq DNA polymerase which varies from 35 to 100 nucleotides per second depending upon the buffer, pH, salt concentration and nature of DNA template (Santhosh et al., 2014).

DNA Based Markers
DNA based techniques are used to evaluate DNA polymorphism. These are hybridization based methods viz., Restriction Fragment Length Polymorphism (RFLP), Variable Number Tandem Repeat (VNTR), Probe hybridization with Micro and Minisatellite, Random Genomic Clone, C-DNA Clone.

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<tr>
<td>1.</td>
<td>Leaves of Ocimum sanctum, O. barilicum, O. gratissimum</td>
<td>RAPD and ISSR polymorphism</td>
<td>Sarwat et al. (2016)</td>
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<td>2.</td>
<td>Ipomoea mauritiana</td>
<td>RAPD and SCAR</td>
<td>Kambiranda et al. (2011)</td>
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<td>3.</td>
<td>Andrachne telephioides, Zilla spinosa</td>
<td>RAPD</td>
<td>Arif et al. (2010)</td>
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<td>5.</td>
<td>Solanum melongena</td>
<td>RAPD</td>
<td>Srinath et al. (2012)</td>
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<td>6.</td>
<td>Leaves of 8 varieties of Zingiber officinale</td>
<td>RAPD</td>
<td>Harisaranraj et al. (2009)</td>
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<td>8.</td>
<td>Cryptocoryne pallidinervia</td>
<td>PCR</td>
<td>Ipor et al. (2007)</td>
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Species differentiation

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Adulteration detection

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<td>11.</td>
<td>Leaves and stem of Angelica decursiva (Peucedanum decursivum), P. praeruptorum and Anthricus sylvestris</td>
<td>rDNA-ITS</td>
<td>Byung et al. (20090</td>
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<td>12.</td>
<td>Leaves of Zanthoxyllum acaanthopodium and Z. oxyphyllum</td>
<td>AFLP</td>
<td>Debmalya and Swati, (20130</td>
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Identification of Phytoconstituents

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<td>14.</td>
<td>Curcuma aeruginosa</td>
<td>RAPD and SCAR</td>
<td>Chantana et al. (2011)</td>
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<tr>
<td>15.</td>
<td>Leaves of Aloe arborescens</td>
<td>cDNA</td>
<td>Hideo et al. (1997)</td>
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Respective genotyping, result interpretation and matching with sample recovered and control sample of suspected herb involve the following steps which includes heat denaturation of double strands at particular temperature, annealing that includes the one primer binds with the 5’ end of one DNA strand and the other primer binds with 3’ end of its complementary strand. Annealing is hybridization of primers to single stranded DNA and the length of time required for primer annealing depends on the base composition, length and concentration of primer. Primer extension is the following steps for Taq DNA polymerase which varies from 35 to 100 nucleotides per second depending upon the buffer, pH, salt concentration and nature of DNA template (Santhosh et al., 2014).

Identification and Authentication
1. Leaves of Ocimum sanctum, O. barilium and O. gratissimum species are authenticated using DNA based markers like ISSR and RAPD.
2. Ipomoea mauritiana known as Vidari in Ayurveda are authenticated using RAPD (600bp amplicon primer) and SCAR (323bp amplicon primer) which is specific to Ipomoea mauritiana.
3. RAPD marker was used for the estimation of genetic diversity in various endangered plant species.
4. Embelia ribes and E. tsjeriam-cottam were analyzed for their genetic diversity using AFLP marker.
5. RAPD markers are used for genetic diversity analysis within the genus Solanum (Solanum melongena and S. violaceum).
6. Identification of genetic variation within eight high yielding varieties of *Zingiber officinale* using RAPD markers.
7. PCR markers are used for forensic analysis and individual identification of *Citrus volkameriana*, *C. sinensis* and *C. reticulate*.
8. PCR method with M13 universal primer is used to distinguish *C. pallidinervia* accessions with high efficiency (Table 1).

Species differentiation
Genetic inter-relationship of various *Cinnamomum* species was estimated using RAPD marker. RAPD marker was used for identification of *Mentha* species yielding high volatile oil from its various species.

Adulteration detection
The roots of Angelica species known as Jeonho in Korean and Qianhu in Chinese are authenticated using SCAR marker and identified 273bp amplicon primer which is specific to *A. sylvestris*, 363bp amplicon primer which is specific to both *A. decursiva* and *P. praeruptorum* and 145bp and 305bp amplicon primer are specific to *Peucedanum praeruptorum*. AFLP markers are used for authentication and identification of genuine and adulterant samples of *Zanthoxylum acanthopodium* and *Z. oxyphyllum*.

Identification of Phytoconstituents
RAPD technique was employed for determination of phytochemical content and genetic similarity between five species of mentha. DNA fingerprinting of *Curcuma aeruginosa* using PCR analysis and the chemical constituents are detected using thin layer chromatography and gas chromatography. Isolation of NADP-malic enzyme from *Aloe arborescens* was carried out using DNA method.

Conclusion
DNA fingerprinting is used in medicinal plants for identification and authentication, for species differentiation, for adulteration detection and for identification of phytoconstituents. The DNA based markers are the most important tools for the above said techniques. This review outlines the importance of DNA fingerprints for the future scientific researches in the field of Pharmacognosy.

References