Journal of Academia and Industrial Research (JAIR)
Volume 5, Issue 10 March 2017

ISSN: 2278-5213

REVIEW

Application of DNA Fingerprinting for Plant Identification

E. Selvakumari*, J. Jenifer, S. Priyadharshini and R. Vinodhini
Dept. of Pharmacognosy, College of Pharmacy, Mother Theresa Research Institute of Health Sciences, Puducherry, India
selvakumari_7@yahoo.co.in*; +91 9787760577

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, *Ipomea 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.

Table 1. DNA based markers in medicinal plants.

Sl. No.	Plant name	Techniques used	References
	For Identification and Authentication		
1.	Leaves of *Ocimum sanctum, O. barilicum, O. gratissimum*	RAPD and ISSR polymorphism	Sarwat *et al*. (2016)
2.	*Ipomoea mauritiana*	RAPD and SCAR	Kambiranda *et al*. (2011)
3.	*Andrachne telephioides, Zilla spinosa Caylusa hexagyna, Achillea fragrantissima Bassia eriophora, Lycium llshawii Zygophyllum propinquum, Moricandia sinaica Withania somnifera, Rumx vesicarius Sonchus oleraceus*	RAPD	Arif *et al*. (2010)
4.	*Embelia ribes, E. tsjeriam-cottam*	AFLP	Balakrishna *et al*. (2010)
5.	*Solanum melongena*	RAPD	Srinath *et al*. (2012)
6.	Leaves of 8 varieties of *Zingiber officinale*	RAPD	Harisaranraj *et al*. (2009)
7.	*Citrus volkameriana, C. sinensis, C. reticulate*	RAPD and VNTR	Francois *et al*. (1995)
8.	*Cryptocoryne pallidinervia*	PCR	Ipor *et al*. (2007)
	Species differentiation		
9.	Leaves of *Mentha spicata, M. piperita, M. xgracilis*	RAPD	Fenwick and ward (2001)
10.	Leaves of *Cinnamomum verum, C. citronella, C. camphora, C. glucens*	RAPD	Priya and Maridass (2008)
	Adulteration detection		
11.	Leaves and stem of *Angelica decursiva (Peucedanum decursivum), P. praeruptorum and Anthricus sylvestris*	rDNA-ITS RAPD SCAR	Byung *et al*. (20090
12.	Leaves of *Zanthoxylum acanthopodium* and *Z. oxyphylum*	AFLP	Debmalya and Swati, (20130
	Identification of Phytoconstituents		
13.	Leaves of *Mentha piperita, M. citrate, M. requienii, M. spicata, M. arvensis*	RAPD	Prasad (2014)
14.	*Curcuma aeruginosa*	RAPD and SCAR	Chantana *et al*. (2011)
15.	Leaves of *Aloe arborescens*	$_C$DNA	Hideo *et al.* (1997)

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 temperature variation for Taq DNA polymerase which adds complementary nucleotides one by one to the 3' OH group of the primer. Estimates for the rate of nucleotide incorporation at 72ºC vary from 35-100 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.

PCR based method viz., Inter Simple Sequence Repeat (ISSR), Random Amplification Polymorphic DNA (RAPD)/Arbitrary Primed PCR, Amplified Fragment Length Polymorphism (AFLP), DNA Amplification Fingerprinting (DAF). Sequence based method viz., Simple Sequence Repeats (SSR), Sequence Characterized Amplified Region (SCAR), Cleaved Amplified Polymorphic Sequence (CAPS) and Single Nucleotide Polymorphism (SNP). The following section deals with the DNA fingerprinting methods for herbs by DNA based markers.

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. *Ipomea mauritiana* known as Vidari in Ayurveda are authenticated using RAPD (600bp amplicon primer) and SCAR (323bp amplicon primer) which is specific to *Ipomea 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 offinale* using RAPD markers.
7. PCR markers are used for genetic 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

1. Arif, I.A., Bakir, M.A., Khan, H.A., Al-Farhan, A.H., Al-Homaidan, A.A., Bahkali, A.H., Al-Sadoon, M. and Shobrak, M. 2010. Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. *Gene. Mol. Res.* 9(4): 2191-9198.
2. Balakrishna, G., Chandrika, K., Prasanna, K.T. and Kirana. V.C. 2010. AFLP Authentication of *Embelia ribes* Burm. F and *Embelia tsjeriam-cottam* A.DC. *Int. J. Sci. Nat*. 1(1): 58-60.
3. Byung, C., Byeong, C.M., Yunui, J., Bo-Be, K., Goya, C., Taesood, Y. and Ho-Kyoung, K. 2009. Development of SCAR markers for the Discrimination of Three species of Medicinal plants, *Angelia decursiva (Peucedanum decursivum), Peuceedanum praeruptorum* and *Anthricus sylvestris*, Based on the Internal transcribed spacer (ITS) sequence and Random Amplified Polymorphic DNA (RAPD). *Biol. Pharm. Bull*. 32(1): 24-30.
4. Chantana, A., Senee, P. And Preeya, W. 2011. Constituents of the rhizome of *Curcuma aeruginosa* and its DNA. *Int. J. Phytomed.* 3: 198-203.
5. Das, G. and Mandi, S. 2013. Species specific AFLP markers for authentication of *Zanthoxylum acanthopodium* and *Zanthoxylum oxyphylum. J. Med. Pl. Stud*. 1(6): 1-9.
6. Fenwick, A.L. and Ward, S.M. 2001. Use of Random Amplified polymorphic DNA markers for cultivar Identification in Mint. *Hort. Sci.* 36(4): 761-764.
7. Francois, L., Frederic, L., Joseph-Marie, B. and Patrick, O. 1995. DNA amplified fingerprinting, a useful tool for determination of genetic origin and diversity analysis in *citrus. Hort. Sci.* 30(5): 1063-1067.
8. Harisaranraj, R., Suresh, K. and Saravanababu, S. 2009. DNA fingerprinting analysis among eight varieties of *Zingiber officinale* Rosc. by using RAPD markers. *Glob. J. Mol. Sci.* 4(2): 103-107.
9. Hideo, H., Hiroaki, S. and Hiromori, A. 1997. Isolation of $_{C}$DNA for an NADP-malic enzyme from *Aloe arborescence. DNA Res.* 4: 397-400.
10. Ipor, I.B., Ho, W.S., Tawan, M.S., Salmizana, M.S. and Noryatimah, M. 2007. Ecology and DNA fingerprinting of *Cryptocoryne pallidinervia Engler (Araceae)* Acessions using polymerase chain reaction M13 Universal primer. *Malay. Appl. Bio.* 36(2): 21-31.
11. Kambiranda, D., Subramani, P. and Padma, V. 2011. Development of Randomly Amplified Polymorphic DNA Based SCAR Marker for Identification of *Ipomea mauritiana* Jacq (Convolvulaceae). *Evid. Compliment. Alt. Med*. 10: 1-6.
12. Prasad, M.P. 2014. Phytochemical analysis and DNA fingerprinting of *Mentha* species using RAPD molecular markers. *Int. J. Adv. Biotechnol. Res.* 5(1): 31-35.
13. Priya, J. and Maridass, M. 2008. Inter species relationship of *Cinnamomum* species using RAPD marker Analysis. *Ethnobot. Leaflets*. 12: 476-480.
14. Santhosh, P., Pushkar, K., Shraddha, N. and Subarna. R. 2014. Application of DNA fingerprinting tools for authentication of Ayurvedic herbal medicines. *J. Sci. Innov. Res*. 3(6): 606-612.
15. Sarwat, M., Srivastava, S. and Khan, T.H. 2016. RAPD and ISSR polymorphism in the Medicinal plants: *Ocimum sanctum, Ocimum basilicum* and *Ocimum gratissimum. Int. J. Pharmacog. Phytochem. Res.* 8(8): 1417-1424.
16. Srinath, R., Shivaraj, G., Kaviraj, C., Surender Reddy, P. and Kavi Kishor, P.B. 2012. RAPD based genetic diversity analysis within the Genus Solanum (Solanaceae). *Curr. Trends Biotechnol. Pharm.* 6(1): 78-83.