AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Usage of DNA Fingerprint for exploring the concept of *Pratinidhi Dravya* (substitution) in Ayurveda -a ray of hope in Demarcation of Substitutes and Adulterants.

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ABSTRACT

In the current status there is no explicit demarcation methodology on finding of substitutes (Pratinidhi Dravya). Thus here an attempt has been made to find out substitute by DNA fingerprinting on the basis of similarity and dissimilarity. In this study two samples *Alysicarpus longifolius* W. & A.Prodr.- Fabaceae, *Desmodium laxiflorum* DC i.e. of same family, same genus with different species were subjected to fingerprints. In this data we find a more similarities characteristics in both the plants i.e. up to 63%. As per the knowledge of research scientist this is a first moral attempt which throwing lights on concept of *Pratinidhi Dravya* (*Substitution*) in *Ayurvedic* science.

Keywords: *Pratinidhi draya*, *Alysicarpus longifolius*, *Desmodium laxiflorum*, RAPD, finger prints.

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Received 05 July 2013, Accepted 26 July 2013

Please cite this article in press as: Joshi PR. *et al.*, Usage of DNA Fingerprint for exploring the concept of *Pratinidhi Dravya* (substitution) in Ayurveda a ray of hope in Demarcation of Substitutes and Adulterants. American Journal of PharmTech Research 2013.

INTRODUCTION

DNA fingerprinting provides an objective evaluation of genetic identity of plants based on species, cultivars, or geographic origin. It can ensure genetic uniformity of raw herbal materials. For medical herbs, synthesis and accumulation of chemical constituents rely on both genetic makeup and environmental conditions[1]. Thus molecular evaluation characterization is an important tool in ensuring that the plant material used is of desired attribute.

In Saurastra region of Gujarat state the plant *Alysicarpus longifolius* , *Desmodium laxiflorum* are substituted for each other both are using as a weed in Gujarat[2]. Rather than throwing these plants if these plants were used in medicinal purpose it will be much beneficial for society.

It is very difficult to differentiate the species level with multi traits. The DNA fingerprints exclusively using for species genus and traits level. To find out similarity and dissimilarity the DNA fingerprint may plays a significant role. The theme of substitution was established by using recent technology through fingerprint. In this study two samples of same family, same genus with different species were subjected to fingerprints, the similar and dissimilar character are expressed, data collected, calculated and scientifically studied. The obtained data explained in the form tables, graphs and with fingerprints.

MATERIALS AND METHODS:

Alysicarpus longifolius W. & A.Prodr.- Fabaceae. And *Desmodium laxiflorum* DC- Fabaceae. Plant DNA isolation and RAPD. The study was carried out at Aristogene Biosciences Pvt, Ltd. Bangalore, Karnataka state, India. 100 mM Tris-HCl (pH8.0), 1.2 M NaCl, 20 mM EDTA, 2% CTAB

DNA extraction and RAPD analysis of plant species

DNA extraction protocol:

DNA was extracted from 2 samples using CTAB Buffer. Tender leaves were selected and crushed to powder using dry ice. 200mg of this powdered leaves was taken in a centrifuge tube. 6 ml of CTAB extraction buffer was added and incubated at 60ºC for 30 min. Centrifuged at 10000 rpm for 10 minutes. Supernatant was transferred to a fresh centrifuge tube carefully. Equal volumes of Chloroform was added and mixed well. Centrifuged at 10000 rpm for 10 minutes. The aqueous layer was pipette out into the fresh centrifuge tube without taking the interface. Equal volumes of Isopropanol and 1/10th volumes of 3M Sodium acetate were added and mixed well. Left at room temperature to stand for 5-10 minutes. Centrifuged at 10000 rpm for 10-15 minutes. The supernatant was discarded. The pellet was washed with 1ml of 70% ethanol. The

pellet air dried and suspended in 400 µl of 1X Tris- EDTA buffer. This was diluted 1:1 using column equilibration buffer and proceeded for column purification[3, 4].

Column purification

The column was placed in collection tube, 400µl of equilibration buffer was added to the column and centrifuged at 10000rpm for 1min. Collected buffer was discarded. DNA sample was loaded on to the column. Flow through was collected. 500µl of wash buffer 1 was added, centrifuge at 10000rpm for 1min and buffer was collected. 500µl of wash buffer 2was added, centrifuge at 10000rpm for 1min and buffer was collected. The column was centrifuged with empty collection tube to completely remove the wash buffer for 2 min. 50µl of elution buffer was added to the column placed in new collection tube. Incubated at room temperature for 2 min and centrifuge at 10000rpm for 1min and eluted sample was saved. Quantizations of eluted DNA samples were done by loading into the agarose gel[5, 6].

RESULTS AND DISCUSSION:

Amplification:

38µl of this was aliquoted into 6 different labeled PCR vials and to this 2 µl of different template DNA was added. The PCR was set.

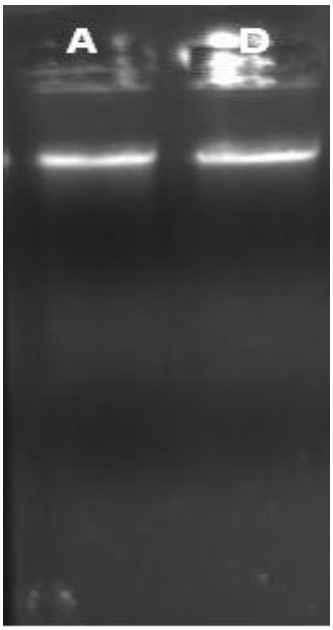
Table 1: A cocktail was made with PCR master mix and respective Random primer.

	For 1 reaction	Cocktail	Notes
Double Distilled water	19 µl	114 µl	-
2X PCR master mix	20 µl	120µl	1X Contains 100µM each of dATP, dGTP, dCTP and dTTP. Assay buffer with 15mM $MgCl_2$, 3U/reaction Taq Polymerase.
Random Primer	1 µl	6µl	10pM used for each reaction
Total Volume	**40 µl x 6**	**240µl**	-

PCR Conditions:

Table 2: PCR conditions

Temperature	Time	No. of cycles
94˚C	2 minutes	1
94˚C	30 seconds	40
45˚C	1 minute	
72˚C	1min 30 sec	
72˚C	7 minutes	1



2 ul of DNA was 100ng

Figure 1: Quantitation of DNA Samples

Marker (M) : PhiX/Hae III: 72,118,194,234,271,281,310,603,872,1078 and 1351bp. Sample A and Sample D

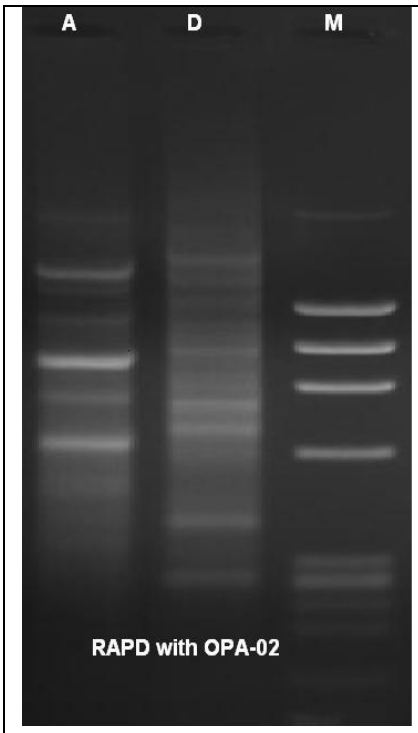


Figure2: RAPD with Random primer-OPA-02

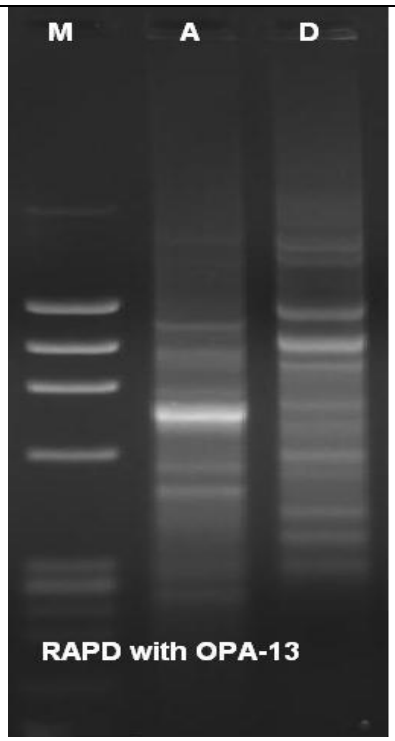


Figure 3: RAPD with Random primer-OPA-13

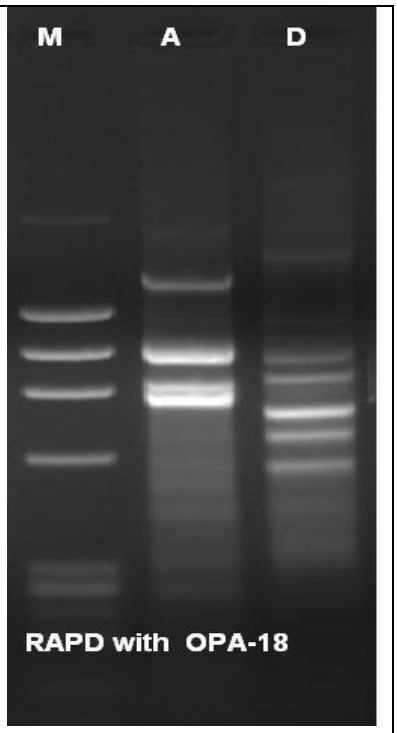


Figure 4: RAPD with Random primer-OPA-18

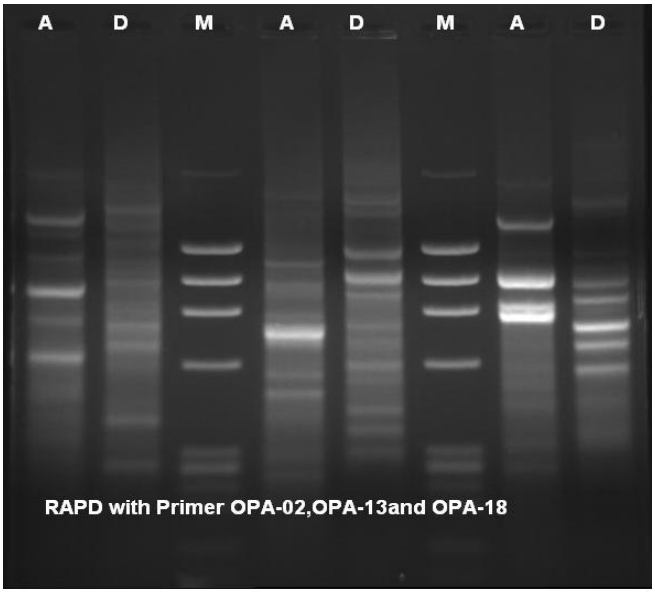


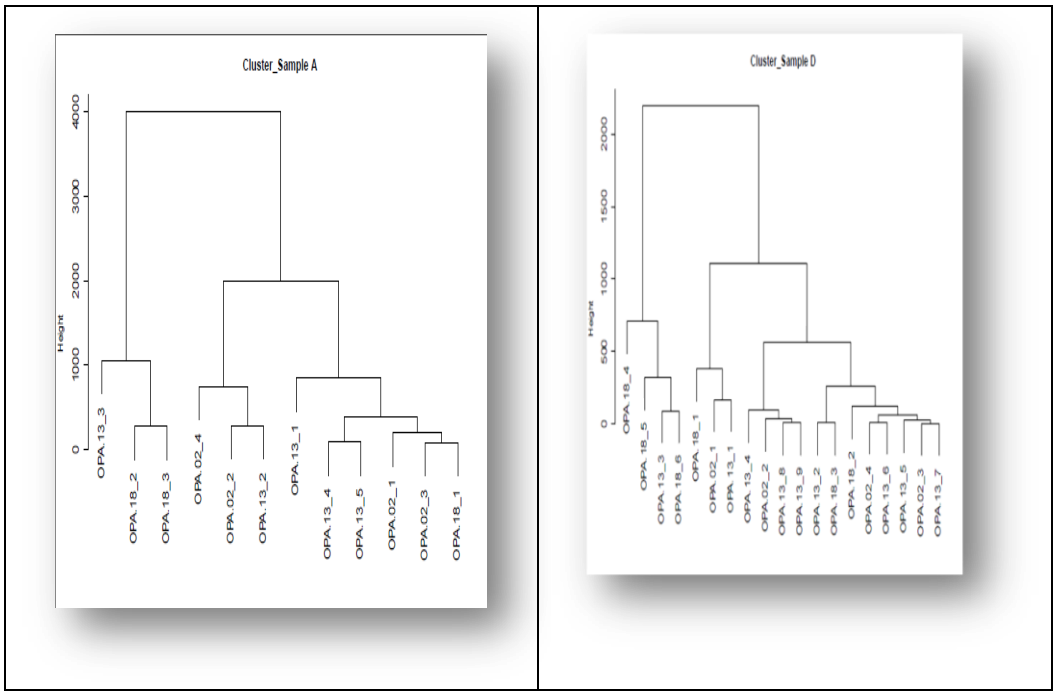
Figure 5: RAPD with all the three primers

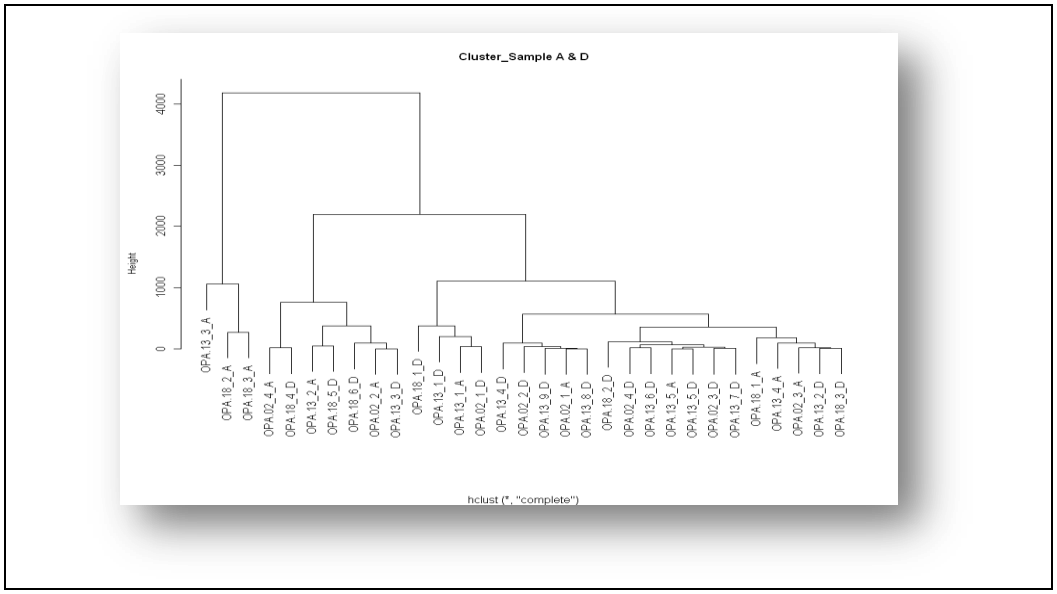
Comparing the similarity and diversity of sample sets with Jaccard coefficient.

The gels were scored by using binary coding method and uploaded to the software. Presence of band is noted as '1' and absence of the band is considered as '0'. The data was uploaded to the Statistical software Bioconductor R, where the analysis was done based on the clustering method. Jaccard coefficient is a statistic used for comparing the similarity and diversity of sample sets.

Statistical Software Used: Biocnductor R

Table3: showing cluster sample A, cluster sample D and cluster sample A and D.





Similarities and differences between the samples:

Table 4: Showing similarity and variation in sample A & D.

Samples		**D**	
	Primer	**Similarity**	**Variation**
A	OPA-02	0.80	0.2.
	OPA-13	0.56	0.44
	OPA-18	0.50	0.50
	Polled data	**0.63**	**0.37**

The binary scoring of primer OPA_02, OPA_13 and OPA_18 with two samples are shown were, horizontally marked as number of number of wells loaded and vertically marked as number of Samples.

Medicinal plant species were authentified by RAPD analysis the advantages of RAPD technique include their simplicity, rapidity and low amount of genomic DNA required. RAPD marker provide to easily reproducible under a wide variation of amplification conditions as it was clearly visible up to an nailing temperature of 38^0 C and result were not affect with changes in the origin of the primer.

Plant DNA extraction was done using CTAB method. The DNA was further purified using spin columns. RAPD done with 3 primers showed clear differentiation between Sample A and Sample D. Each primer showed entirely different pattern for the 2 samples. The binary scoring of primer OPA-02, OPA-13 and OPA-18 showed 63% of similar characters and 37% different character between the two samples (*Alysicarpus longifolius* W. & A.Prodr. - Fabaceae and *Desmodium laxiflorum* DC-Fabaceae.) Thus the DNA fingerprint result shows most of all similar genomic character that expressed in pharamacognostical study. From the aspects of categorization of *Pratinidhi Dravyas* in *Ayurveda*, derived on the basis of comparative analysis

of DNA fingerprints of selected samples can contribute significantly which further need to be elaborated.

CONCLUSION:

In case of same fame family members many genus and again many species create same identical morphology when it scientifically subjected to the phrmacognosy, phytochemistry and the advanced DNA finger printing gives the specific differentiate and similarity characters it is scientifically necessary in this type of works. This work is an superficial work to implicate for the further research works.

ACKNOWLEDGEMENT:

The authors are thankful to the authorities of IPGT&RA, and Gujarat Ayurved University for providing facilities to publish the research work.

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