

# Genetic Diversity in *Pentapetes phoenicea* and *Malachra capitata* Assessed Through RAPD Analysis

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**Abstract:** *Pentapetes phoenicea* is grown from seed. It does not self-pollinate. *Pentapetes* (Indomalayan) is one of the medicinal and poisonous Sterculiads of the world (Caius J.F., 1989). *Malachra capitata* is found in hotter parts of India where it has become naturalised. It is also used as a medicinal plant. The genetic diversity of two plants, *Pentapetes phoenicea* and *Malachra capitata* is studied with the help of RAPD genetic markers and the evaluation of banding pattern was carried out by similarity and distance matrix computed with Jaccard coefficient matrix. The developed phenogram using unweighted pair group method with average (UPGMA) cluster analysis also suggested the two plants species are diverse plants.

**Keywords:** Genetic diversity, Jaccard coefficient, *Malachra capitata*, *Pentapetes phoenicea*, RAPD

## 1. Introduction

*Pentapetes phoenicea* and *Malachra capitata* belonging to the family Sterculiaceae and Malvaceae respectively have been placed in the order Malvales. Autecological and Phytochemical studies highlight the similarities in these two plants. Both the plants also show some similar antibacterial and antioxidant activities.

Plant wealth is greatly exploited for its therapeutic potential and medicinal efficacy to cure various ailments since time immemorial. For this reason, they were identified and defined by taxonomist at the very early ages of human history. The taxonomic studies of these genera are limited so morphological characters were used in the genus key which can be influenced by environmental conditions (Sumer Aras *et al.*, 2003). In the evolutionary history, the distribution of populations could be constricted or expanded due to the environmental changes (Jin-Ming *et al.*, 2008). Molecular markers could reflect the difference between species directly without affecting the environment (Wang *et al.*, 1996). Several molecular markers particularly the Random Amplified Polymorphic DNA (RAPD), Restriction fragment length polymorphism (RFLP) and Variable Number of Tandem Repeats (VNTR) have proved useful in detecting genetic diversity. RAPD technique has several advantages such as speed, low cost and the usage of small amounts of plant materials (Jain *et al.*, 1994; Heun *et al.*, 1994). So far analysis of genetic diversity in *Pentapetes phoenicea* and *Malachra capitata* has not been carried out. The aim of the present study was to analyse the genetic diversity as well as their relationship using the RAPD markers.

### RAPD marker based analysis

Molecular genetic markers have been developed into powerful tools to analyse genetic relationships and genetic diversity. As an extension to the variety of existing techniques using polymorphic DNA markers, the Random Amplified Polymorphic DNA (RAPD) technique may be used in molecular ecology to determine taxonomic identity, kinship relationships, analyse mixed genome samples and

create specific probes. It is a new technology of DNA fingerprinting to the molecular analysis of relatedness between genotypes. It is a type of PCR reaction, but the segments of DNA that are amplified are random.

In this study, the diversity of two plants, *Pentapetes phoenicea* and *Malachra capitata* is studied with the help of RAPD genetic markers.

## 2. Material and Method

Two plants, *Pentapetes phoenicea* and *Malachra capitata* were collected from the field in the month of October and were kept in refrigerator till further use. Healthy tender leaves were selected for DNA extraction. Genomic DNA isolation was done by CATB Plant DNA Extraction Kit (Merk, cat # 612105500011730).

### DNA extraction method

Total genomic DNA was extracted from leaves of two plants i.e. *Pentapetes phoenicea* and *Malachra capitata* using a modified CTAB method based on the protocol of Doyle and Doyle (1990). Quality and concentration of total DNA was verified by UV Spectrophotometry at 260 nm and 280 nm. Further quality of DNA was tested by submerged horizontal agarose gel (1.8%) electrophoresis and visualised under UV light, gel documentation system.

### RAPD analysis:

RAPD reactions were performed by using 3 random primers i.e. RBA-21, RPI-13 and RBA-8 and the PCR condition was set with these three primers having sequence given below.

RBA-21- 5' CCT GGG CCA G 3'

RPI-13- 5' ACG GCA AGG A 3'

RBA-8- 5' ATC GGG TCG A 3'

The PCR amplification was carried out using Applied Biosystem PCR machine. PCR reactions were performed in reaction mixture with a total volume of 25 µl containing different combinations.

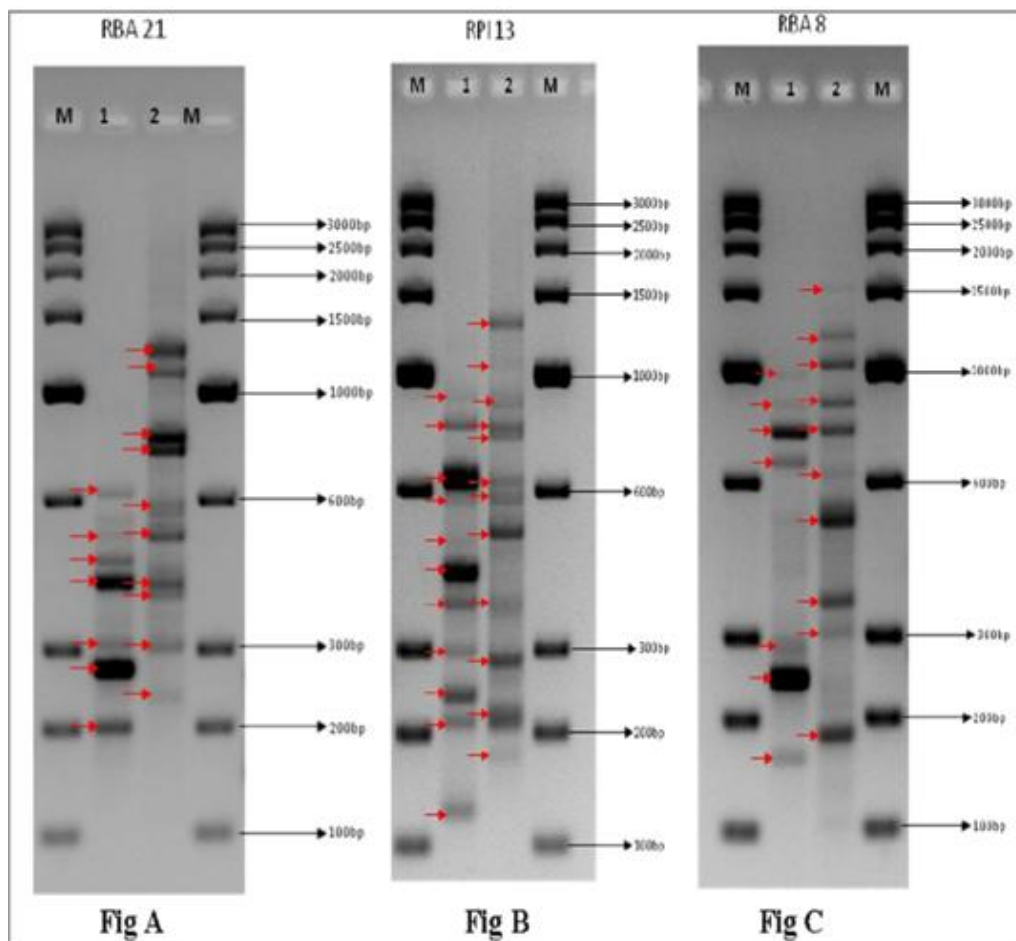
**The PCR mix composition:**

Genomic DNA:	~20ng
dNTP mix (2.5mM each) :	1.5µl
Random Primer:	100ng
TaqDNA Polymerase Assay Buffer A (10X):	1X
TaqDNA Polymerase enzyme:	1.5U
Glass distilled water:	Made up
the volume to 25µl	

The PCR was set as per following cycle condition.

94°C	94°C	38°C	72°C	94°C	45°C	72°C	72°C	4°C
5min	45 sec	1 min	1.5min	45 sec	1 min	1 min	10 min	∞
		10 Cycles			35 Cycles			

PCR amplification was carried out in two sets with pre-denaturation at 94°C for 5 min, denaturation at 94°C for 45 sec, primer annealing at 38°C for 1 min and primer extension at 72° for 1.5 min followed by 10 cycles of amplification. In second set, denaturation at 94°C for 45 sec, primer annealing at 45°C for 1 min and primer extension at 72° for 1 min followed by 35 cycles of amplification and final extension at 72°C at 10 min. PCR products were kept at 4°C. Gel electrophoresis was carried out on the 12 µl of PCR products using 1.8% agarose, stained with ethidium bromide and visualised under UV illumination. The 1kb DNA ladder was used as a molecular weight marker and the amplifications were repeated twice to confirm the results.



**RAPD Profile of Two samples generated using 3 RAPD primers**

**Fig A**

Lane M: Low Range DNA Ruler (Cat# 612652370501730)  
 Lane 1: RAPD of Sample 1 (*Malachra capitata*) with Primer RBA-21  
 Lane 2: RAPD of Sample 2 (*Pentapetes phoenicea*) with Primer RBA-21

**Fig B**

Lane 1: RAPD of Sample 1 (*Malachra capitata*) with Primer RPI -13  
 Lane 2: RAPD of Sample 2 (*Pentapetes phoenicea*) with Primer RPI -13  
 Lane M: Low Range DNA Ruler (Cat# 612652370501730)

**Fig C**

Lane 1: RAPD of Sample 1 (*Malachra capitata*) with Primer

**RBA-8**

Lane 2: RAPD of Sample 2 (*Pentapetes phoenicea*) with Primer RBA-8  
 Lane M: Low Range DNA Ruler (Cat# 612652370501730)

**Similarity Matrix computed with Jaccard coefficient:**

**Table 1**

	Sample 1	Sample 2	Sample 1R	Sample 2R
Sample 1	1	0.19	1	0.19
Sample 2		1	0.19	1
Sample 1R			1	0.19
Sample 2R				1

Distance matrix based on Jaccard coefficient: Table 2

	Sample 1	Sample 2	Sample 1R	Sample 2R
Sample 1	0	0.81	0	0.81
Sample 2		0	0.81	0
Sample 1R			0	0.81
Sample 2R				0

Distance matrix based on RMSD coefficient

	Sample 1	Sample 2	Sample 1R	Sample 2R
Sample 1	0	0.9	0	0.9
Sample 2		0	0.9	0
Sample 1R			0	0.9
Sample 2R				0

**Note:** Sample1R, sample2R are the repeat of Sample1, sample2 which were considered for the statistical compilation.

Phylogenetic Tree Analysis: Phenogram:

### 3. Result and Discussion

The RAPD technique had been successfully used in variety of taxonomic and genetic diversity studies (Hepsibha *et al.*, 2010). Genetic diversity is of great importance to the sustainability of plant populations. In this study, three primers were screened and the evaluation of banding pattern was carried out by similarity and distance matrix computed with Jaccard coefficient matrix. The two plant samples were used to perform the RAPD analysis. Based on the obtained banding patterns for polymorphism along the plant genomes, several polymorphic and monomorphic bands were obtained as in Fig A, B and C for three RAPD primers used. Accordingly sample 1 is polymorphic in banding pattern when compared with patterns of sample 2 as highlighted in similar index table 1 and Distance matrix table 2. While the developed phenogram using UPGMA also suggested the two plants species are diverse plants.

### 4. Conclusion

The two plant species are diverse in relation and belongs to different group of evolution.

### References

- [1] Causis JF (1989). The Medicinal and Poisonous Plants of India, Scientific Publishers, Jodhpur, India, 445-488.
- [2] Doyle JJ and Doyle JJ (1990) Isolation of plant DNA from fresh tissue. *Focus*. 12, 13-15.
- [3] Hepsibha BT, Premalakshmi V and Sekar T (2010). Genetic diversity in *Azima tetraantha* (Lam) assessed through RAPD analysis, *Ind. J. of Science and Technology*, vol. 3 (2), 170-173.
- [4] Heun M, Murphy JP and Phillips TD (1994) A comparison of RAPD and isozyme analysis for determining the genetic relationships among *Avena sterilis* L. accessions. *Theor. Appl. Genet.* 87, 689-696.
- [5] Jain A, Bhatia S, Banga SS, Prakash CS and Lakshmi Kumaran (1994) Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis. *Theor. Appl. Genet.* 88, 116-122.

- [6] Jin-Ming Chen, Fan Liu, Qing-Feng Wang and Timothy J Motley (2008) Phylogeography of a marsh herb *Sagittaria trifolia* (Alismataceae) in China inferred from cpDNA *atpB-rbcL* intergenic spacers. *Mol. Phylogenetics & Evol.* 48, 168-175.
- [7] Sumer Aras, Ahmet Duran and Gulay Yenilmez (2003). Isolation of DNA for RAPD Analysis from Dry Leaf Material of some *Hesperis* L. specimens. *Plant Mol. Bio. Reporter.* 21, 461a-461f.
- [8] Wang X.P, Zou Y.P, and Zhang D.M (1996) Problems in the use of RAPD to the study of genetic diversity and systematis. *Acta Botanica Sinica.* 38(12), 954-962.