

Original Research Article

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**Genetic Analysis of *Madhuca longifolia* (J. Koenig ex L.) J.F. Macbr.  
Using RAPD Markers**

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*Madhuca longifolia* commonly known as *mahua* belongs to family Sapotaceae. It is an important non-timber economic tree which has two varieties namely *Madhuca longifolia* var. *latifolia* and *Madhuca longifolia* var. *longifoliae*. These two varieties show remarkably similarity in their economic use and phytochemistry. *Mahua* tree grows wildly and is also cultivated in norther and southern part of India. The two varieties differ morphologically with respect to the leaf hence the names. RAPD profile was optimized to study the genetic variability in *mahua* using randomly selected 10 genotypes. The profile was developed using 10 decamer primers. The dendrogram was constructed using UPGMA algorithm. Our results highlighted the use of RAPD markers to gain simple and lucid information about the genetic similarities or dissimilarities in the two varieties that might not be so easily evident from other commonly used techniques.

**Introduction**

*Madhuca indica* and *Madhuca longifolia* were two species found in India which are categorized as two varieties due to remarkable similarities as *Madhuca longifolia* var *latifolia*syn *M.indica* and *Madhuca longifolia* var *longifolia*. Both of these are geographically distinguished as *North Indian mahua* and *South Indian mahua* respectively.

Inter and intra specific components characterize biodiversity and these assessments have been carried out by field study and taxon survey by ecologist and taxonomist. A genetic resource management strategy for such variations in species

needs to be based on research data examining the extent of genetic differentiation within and between populations and on understanding the processes maintaining this variation (Chelliah *et al.*, 2010). Genotype fingerprinting is also an important tool which can remove any duplicates that are introduced through mislabeling or during multiplication in clonal propagation of plants (Shivashankar, 2014).

There is less information available on the existing germplasm diversity in *Madhuca longifolia* and, therefore, cataloguing of natural genetic diversity becomes essential

for its efficient and sustainable germplasm management.

Polymerase Chain Reaction (PCR) based molecular markers such as Randomly Amplified Polymorphic DNA (RAPD) technology has become an efficient tool in investigation of genetic diversity within and between populations and has been explored by molecular biologist (Singh *et al.*, 2002; Belaj *et al.*, 2002; Shan *et al.*, 2005).

*Madhuca longifolia* commonly called *mahua* is one of the most harnessed non timber trees. Several pharmacological activities have been reported about the various parts of the plant. It is also considered as valued and sacred tree by several tribes in different states of India (Patil *et al.*, 2004).

Our research is hence focused on the genetic diversity which was carried out to determine intra –specific or inter-varietal variations in randomly selected genotypes of *Madhuca longifolia*.

## **Materials and Methods**

### **Collection of samples**

Plant samples used in the present study were fresh young leaves of 10 genotypes of *Madhuca longifolia* (*J.Koenig ex L.*) *J.F. Macbr.* belonging to family Sapotaceae. 8 genotypes were randomly selected belonging to *Madhuca longifolia* var. *latifolia* from Bhavans college campus and Wada, Taluka Vasai, Dist. Palghar, Maharashtra, India. 1 genotype was the leaves of *Madhuca longifolia* var. *longifolia* purchased from nursery in Chennai, Tamilnadu. 1 genotype was cultivated genotype of *Madhuca longifolia* var. *longifolia* taken from bhavans college campus. The leaf samples of these 10

genotypes were collected fresh and were carried to laboratory in a zip-lock bag containing packet of silica gel.

### **DNA extraction and quantification**

Optimized protocol comprising of 1% CTAB buffer with homogenization in liquid nitrogen followed by grinding with a pinch of PVP was used for extraction of DNA from the randomly selected 10 genotypes which were further subjected to RAPD analysis.

### **Reagents and the optimal PCR reaction mixture**

PCR amplification was done in 20 µl of reaction volume containing 1x PCR buffer (Kappa, SA); MgCl<sub>2</sub>, 3 mM; dNTP mix, 0.25 mM; *Taq* DNA polymerase, 0.05 U; primer, 1 pmol and template DNA, 50 ng. Sterile nuclease free water is used as negative control.

### **Agarose gel electrophoresis**

Agarose gel electrophoresis is the most simplest and economic way for visualization and analysis of DNA to quantify it or to isolate a particular band. 1 g agarose was dissolved into 50 ml TAE (Tris Acetic acid Electrophoresis) buffer to get a 2% gel. The agarose was digested in microwave oven so as to get a translucent solution. 2 µl of ethidium bromide (DNA staining dye) was added to it, mixed properly before pouring into the gel casting tray containing the comb and allowed to set for 45 min. The Comb was carefully removed to avoid damage to the wells. About 300 ml of 1X solution of TAE buffer was added to the buffer tank of horizontal electrophoretic set up. 10µl of sample with the gel loading dye containing bromophenol blue as the

tracking dye was loaded in the wells of the gel. 8 µl of 100 bp DNA ladder (Bangalore genei) was also loaded into one well as standard. The gel electrophoresis was carried out using Electrophoresis unit. Power supply was turned on to about 50 volts for 30 minutes and then to 100 volts. The gel was viewed in UV-illuminator. The DNA band pattern was observed and photographed using digital camera.

### **Genetic Diversity DATA Analysis**

The 10 RAPD markers resulted in different banding pattern ranging from 100-2000bp. For construction of a dendrogram, presence and absence of bands were scored as 1 and 0 respectively. Using the UPGMA statistical software, diversity between the samples was analyzed using Paired group algorithm with similarity matrix based on Jaccard coefficient.

### **Results and Discussion**

The 10 genotypes yielded good quality DNA ranging from 530 (µg/gm) to 812 (µg/gm) and the ratio of (260/280) also ranged from 1.7 to 1.8. Extraction of DNA from *Madhuca longifolia* was carried out by optimized DNA method using CTAB buffer system. The extracted DNA was further used to carry out PCR amplification using 10 Decamer primers to generate RAPD profile discussed as follows

Genetic relationship among the ten randomly selected genotypes of two varieties *Madhuca longifolia* was carried out using RAPD finger printing analysis. 10 random primers selected generated

reproducible, informative and easily analyzable RAPD profiles. 4 primers gave 6 unique bands primer 1 – 1 unique band, primer 5 and 6 two unique bands and primer 9 1 unique band was scored. Only Primer 8 gave monomorphic bands whereas rest of the 9 primers no monomorphic bands were scored.

In cluster analysis of the dendrogram revealed that S-5 the genotype collected from Wada formed a separate clade, this genotype grows wild in the industrial area shows delayed flowering and fruits of smaller size. The second cluster showed similarity between S6 and S3 and a clade of S7 and S9. The innermost cluster revealed the similarity between S1 and S2 and its relatedness to S4. The two genotypes of *Madhuca longifolia* var. *longifolia* S8 and S10 were geographically distinct but indicate similarity in the genetic constitution. The variety *Madhuca longifolia* var. *longifolia* differs from variety *Madhuca longifolia* var. *latifolia* but is not very significantly different genetically.

The genotypes taken for study suggested that *Madhuca longifolia* var. *longifolia* and *Madhuca longifolia* var. *latifolia* undergoes a major part of genetic variation by environmental factors. The genetic diversity of *Madhuca longifolia* varies with the geographic distance and environmental factors. Genetic diversity refers to the variation at the level of polymorphism in individual genes, and provides a mechanism for populations to adapt their ever-changing environment (Chelliah *et al.*, 2010). Variation in the genetic constitution may result in offspring with better chances of changing environmental conditions.

**Table.1** PCR-MIX

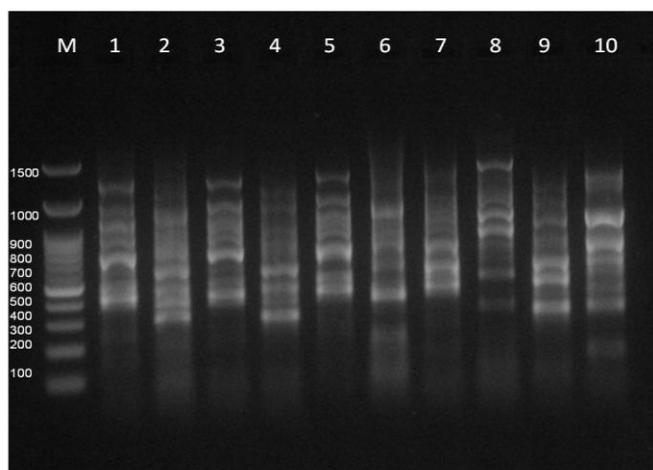
PCR components	Volume (µl)
Nuclease free water	10.75
10X reaction buffer with MgCl <sub>2</sub> (1.5mM)	2.00
dNTP mix (2.5mM)	2.00
Primer 16S FP (10picomoles/ µl)	2.00
Primer 16S RP (10picomoles/ µl)	2.00
<i>Taq</i> DNA polymerase (5U)	0.25
Template DNA (50ng/ µl)	1.00
Total volume	<b>20.0</b>

**Table.2** List of RAPD primers used

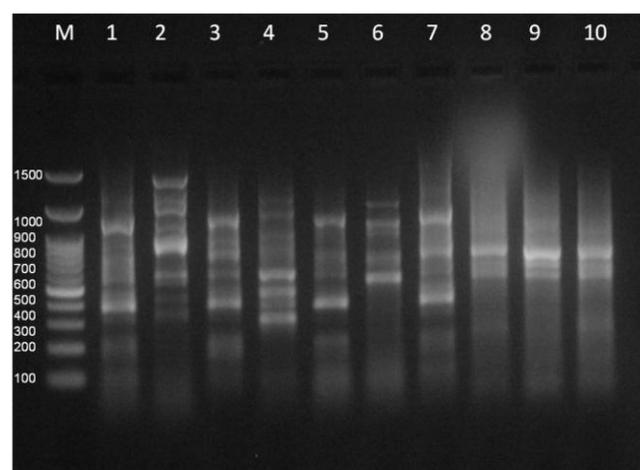
SR. No	Sequence (5' – 3')	SR. No	Sequence (5' – 3')
1	AGACGGCTCC	6	CTACGCTCAC
2	GAGACCAGAC	7	AGATGGGCAG
3	TTAGCGCCCC	8	TGGTCGGGTG
4	AGGACTGCTC	9	GGACCCAACC
5	GTGGGTGCCA	10	CAGGCCCTTG

**Table.3** PCR temperature profile

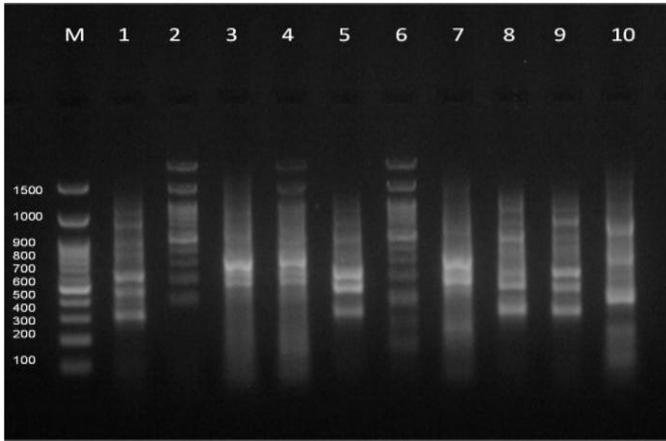
Initial denaturation	Denaturation	Annealing	Extension	Final Extension
94°C	94°C	37°C	72°C	72°C
5 min	1 min	1 min	1 min	10 min
40 cycles				



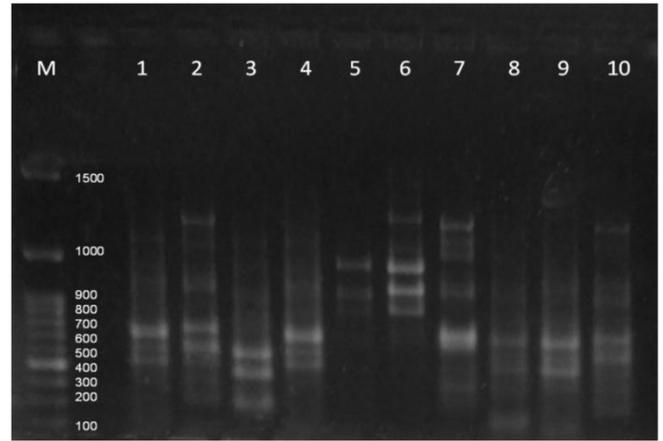
**Figure 1:- RAPD PRIMER  
5'AGACGGCTCC3' GEL IMAGE**



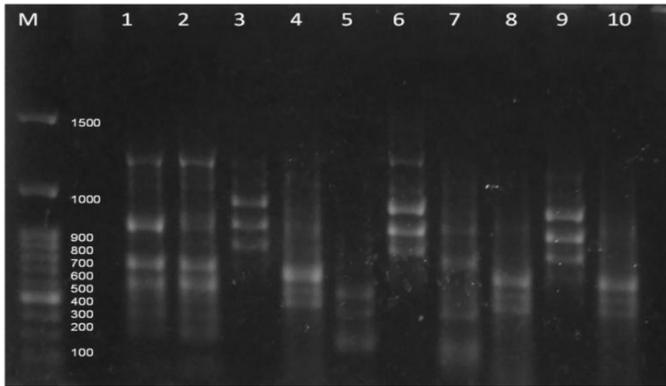
**Figure 2:- RAPD PRIMER  
5'GAGACCAGAC3' GEL IMAGE**



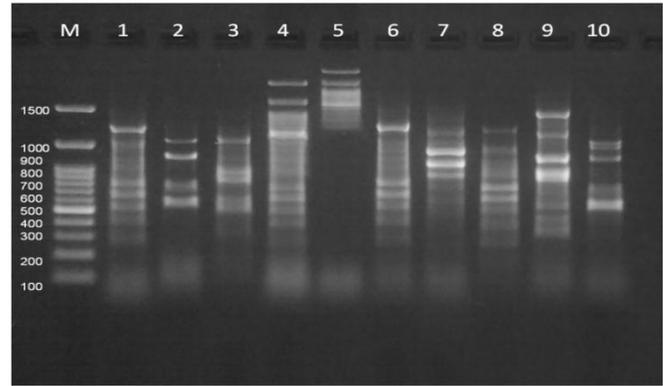
**Figure 3:- RAPD PRIMER  
5'TTAGCGCCCC 3' GEL IMAGE**



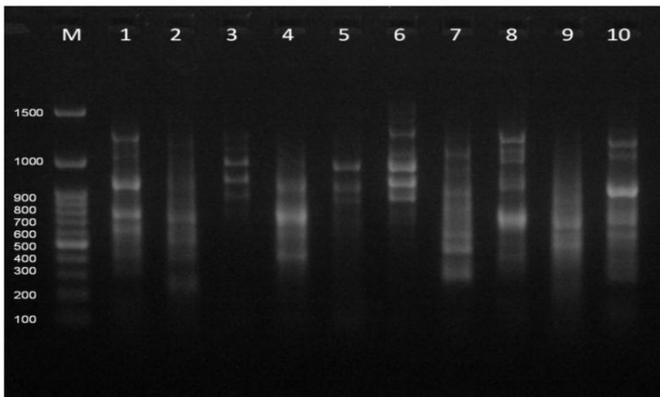
**Figure 4:- RAPD PRIMER  
5'AGGACTGCTC3' GEL IMAGE**



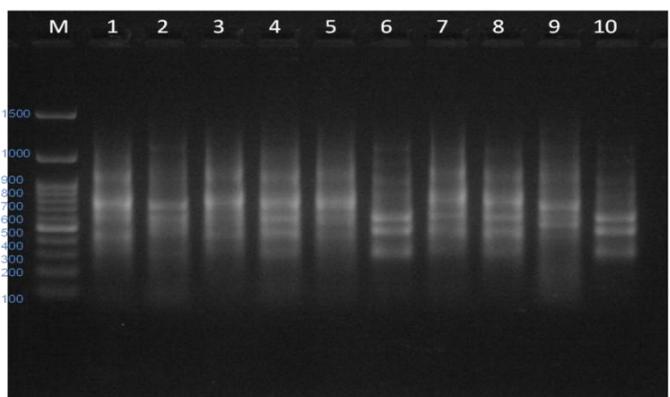
**Figure 5:- RAPD PRIMER  
5'GTGGGTGCCA3' GEL IMAGE**



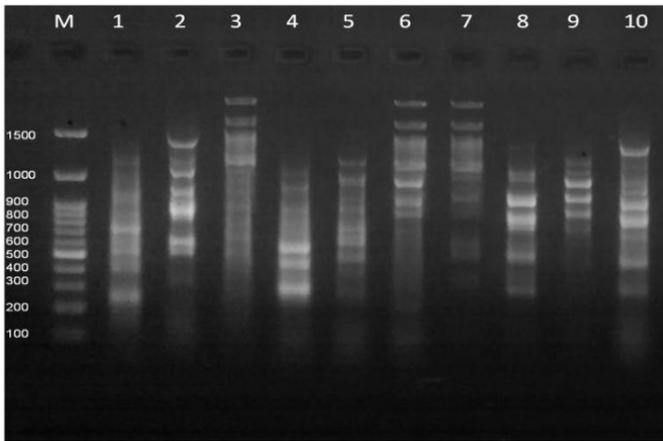
**Figure 6:- RAPD PRIMER  
5'CTACGCTCAC3' GEL IMAGE**



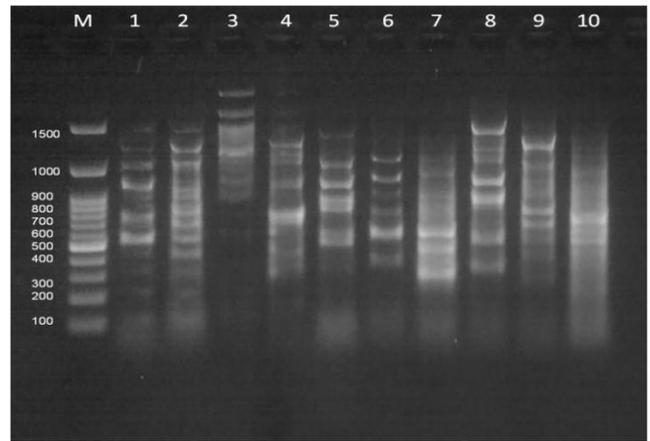
**Figure 7:- RAPD PRIMER  
5'AGATGGGCAG 3' GEL IMAGE**



**Figure 8:- RAPD PRIMER  
5'TGGTCGGGTG3' GEL IMAGE**

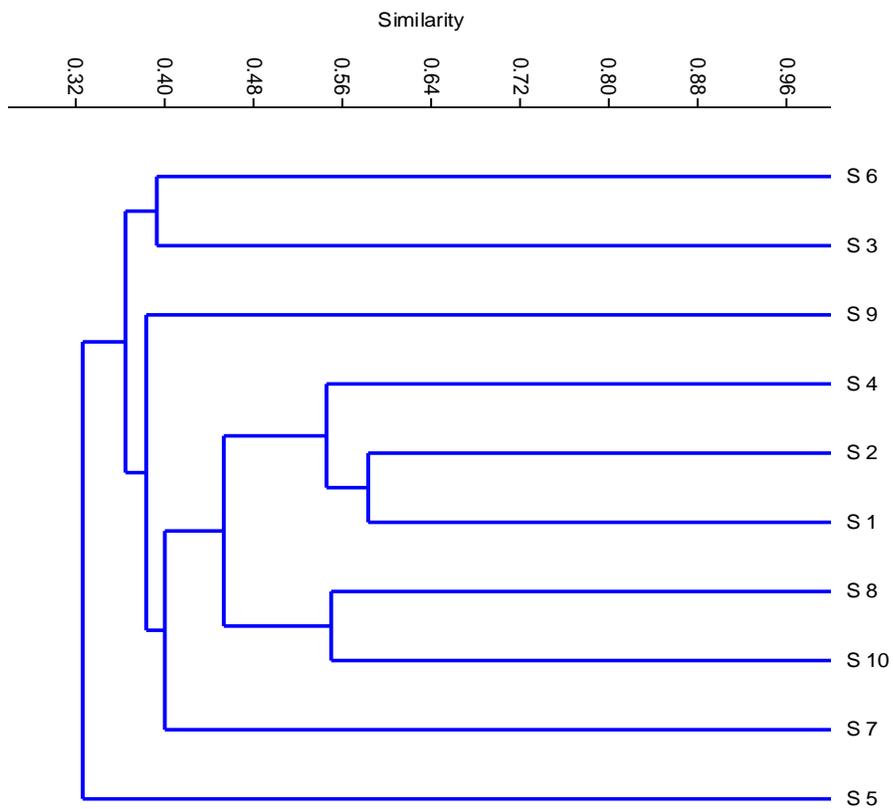


**Figure 9:- RAPD PRIMER  
5' GGACCCAACC 3' GEL IMAGE**



**Figure 10:- RAPD PRIMER  
5' CAGGCCCTTG3' GEL IMAGE**

**DENROGRAM (Figure 11)**



**Table.4** DNA extraction - Ratio and yield of 10 genotypes used for RAPD profile

Sample (Genotypes)	Location	Ratio (260/280)	Ratio (260/230)	Yield (µg/gm)
1	Wada, Tal. Vasai Dist. Palghar, Maharashtra	1.781	0.732	636
2	Wada, Tal. Vasai Dist. Palghar, Maharashtra	1.818	0.699	548
3	Wada, Tal. Vasai Dist. Palghar, Maharashtra	1.854	0.854	561
4	Wada, Tal. Vasai Dist. Palghar, Maharashtra	1.897	0.832	691
5	Wada, Tal. Vasai Dist. Palghar, Maharashtra	1.732	0.751	789
6	Bhavan's College campus Andheri (Mumbai) Maharashtra	1.826	0.711	610
7	Bhavan's College campus Andheri (Mumbai) Maharashtra	1.881	0.810	812
8	Bhavan's College campus Andheri (Mumbai) Maharashtra	1.719	0.891	716
9	Dahanu Dist. Palghar, Maharashtra	1.829	0.858	632
10	Chennai	1.845	0.692	530

**Table.5** Similarity matrix computed with Jaccard coefficient

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
S1	1	0.583	0.439	0.508	0.400	0.419	0.421	0.500	0.364	0.377
S2		1	0.349	0.583	0.311	0.422	0.448	0.448	0.368	0.434
S3			1	0.367	0.367	0.393	0.365	0.365	0.354	0.235
S4				1	0.400	0.375	0.350	0.500	0.415	0.460
S5					1	0.304	0.320	0.320	0.224	0.289
S6						1	0.375	0.375	0.340	0.327
S7							1	0.373	0.391	0.409
S8								1	0.362	0.550
S9									1	0.400
S10										1

Selection of more geographically diverse genotypes from North and south part of India will help to find out the individual heterozygosity to estimate the best

individuals in the population and this data may be correlated with other features of the variety to identify the superior variety.

In conclusion, *Madhuca longifolia* commonly called as North *Indian mahua* or *mahua* in north and *South Indian mahua* or *illupai* is an important non-timber tree which is economically viable but is often neglected due to its common exploitation in production of liquor. *Madhuca longifolia* is rich in interfering agents such as polyphenols and polysaccharides hence PCR amplification is a difficult task as DNA extracted is of poor quality. The current study focused on generating an RAPD profile using PCR, thus revealing valuable information and forming the basis of a powerful tool for further research. Genetic variation estimated in this report provides the basis for *in situ* and *ex situ* conservation programme of genetic resources. Information on the spatial structure of natural populations of *Madhuca longifolia* throws insights into the history, isolation, and diversification of its two varieties.

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