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Molecular Biotyping Studies on the Traditional and Indigenous Relationships of Pants and Bharia tribe of Chhindwara

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ABSTRACT

Human beings always been largely dependent for their food, shelter, and medicine and other needs on plant resources. But the rural and about original folk are very much in harmony with nature for their various needs, they depend largely on plants. But due to rapid modernization, acculturation and resultant greatly enhanced need; this environmental harmony is getting disturbed. Rural folk are often discarding their age-old traditions and are getting absorbed into the process of modernization. However, there still exist many ethnic groups and cultures that strive to maintain their age-old wisdom and culture. Modern science has borrowed much of its basic knowledge from these cultures, particularly that relating to food and medicine from plants. But since the economic, scientific and technological changes too are influencing these cultures; it is essential to study them before their knowledge is lost. The main aim and objective of study the plants preferred by Bharia tribe for medicinal use their , Collection, identification, enumeration, illustration, preservation and documentation of important plants such as Arjun tree (Terminalia arjuna), Imli (Terminalia indica), Haldu (Adina cordifolia) and Burr (Ficus benghalensis) and plant materials with their local and vernacular names, distribution and ethno botanical uses. As well as observation of conservational practices of plant used by Bharia tribe. Molecular characterization of listed medicinal plants using PCR genomic Fingerprinting technique to develop Molecular markers.

INTRODUCTION

The Bharia, another little known tribe of Madhya Pradesh, is concentrated mainly in the districts of Chhindwara, Jabalpur and Bilaspur. As Bharias have lived for generations side by side with the more influential and prosperous Gonds, they have many things in common with them. The total area of the zone is 52,082 square km. A larger part of the zone is covered by the Satpura range which in the east, northeast and the southeast, it is bound by the Maikal hills. The Mahadeo hill lies in the western portion of the zone. About two-third of the zone is covered by these hills and has an altitude between 2,000 to 3,000 feet above sea level. Large quantities of harra are collected from the forest of Chhindwara. Karaya gum, which finds use if food, cosmetics, textile and other industries, is also found in limited quantity in Chhindwara. If organized properly the minor forest produce could contribute substantially to the tribal's income. To protect the most exploited, rare and threatened varieties, the Bharia do follow unwritten rules, in the form of so-called superstitions. when one has to remove the tubers or underground stem after removing them, as a rule, they have to fill the pit with mud and cut the top portion of the tuber/underground stem and put it in the mud. During rainy season it comes up again. In general, the Bharia also follow certain rules regarding collection of the root from any climber or shrub, i.e. takes roots only from one side of the plant; and for removing bark from down to up. All these general rules help in preserving the plants from destruction. However, unscrupulous slashing of forests, felling of trees for wood, etc. still go on. So, there is need for a systematic effort for conservation. The objective of the study is to characterize the medicinal plants from the forest land of Madhya Pradesh. The existence of biochemical characterization of plants appears to be inadequate to establish proper identification of various (*Terminalia arjuna*), Imli *(Terminalia indica*), Haldu *(Adina cordifolia)* and Burr (*Ficus benghalensis*). The medicinmen are the people who know the largest number of names and their applications. And the name may vary from place to place. Among the many names, the most popular name will be chosen and it will be compared with that given in floras. The common name of selected medicinal plants will be obtained from local people.

MOLECULAR BIOTYPING

Four medicinal plants viz. Arjun tree (*Terminalia arjuna*), Imli *(Terminalia indica*), Haldu *(Adina cordifolia)* and Burr (*Ficus benghalensis)* available at different localities of Chinndiwara district of Madhya Pradesh under different soil conditions will taken. The selected plants will be processed for

successful exploitation of genetic diversity among species isolated by using PCR finger printing technique.

It is one of the most rapid simple and highly reproducible methods that are not absolutely dependent on purified DNA. The molecular diversity of plant community is the most dominant biological unit in forest ecosystem hierarchy. In modern context, the diversity stands with reference to genetic variation in biological community.

The main aim and objective of study the plants preferred by Bharia tribe for medicinal use their , Collection, identification, enumeration, illustration, preservation and documentation of important plants such as Arjun tree (*Terminalia arjuna*), Imli (*Terminalia indica*), Haldu (*Adina cordifolia*) and Burr (*Ficus benghalensis)* and plant materials with their local and vernacular names, distribution and ethno botanical uses to confirm the genetic diversity and observation of conservational practices of plant used by Bharia tribes of Chhindwara district.

MATERIALS AND METHODS

Plant materials

Two samples each of (*Terminalia arjuna*, *Terminalia indica*, *Adina cordifolia, Ficus benghalensis)* collected from native habitats and through the Bharia tribes people , were used in this study (Table 1). The identification of species was performed by the Classification and Identification Medicine, composed of nine experts in the fields of plant taxonomy, botany, pharmacognosy, and herbology.

Table 1 list of plants with their sources and collection date.

Common Name	Scientific Name	Source	Date of Collection	Lane in gel
Arjun tree	*Terminalia arjuna*	Bichua Chhindwara	**9 May 2009**	**1**
Imli	*Terminalia indic*	Jamiya, Chhindwara	**18 May 2009**	**4**
Haldu	*Adina cordifolia*	Tamia, patalkot	**13 June 2010**	**6**
Burr	*Ficus benghalensis*	Harrai Chhindwara	**17 June 2010**	**8**

Preparation of genomic DNA

Genomic DNA was extracted from fresh leaves and stems using the DNA isolation kit (QIAGEN, Germany) according to the manufacturer's protocol. DNA concentration and purity were determined by spectrophotometry (Shimadzu-1000; Nanodrop, japan) and electrophoresis (Bangalore Genie) in a 1.5% agarose gel (Hi-media) with known standards. For PCR (Bio-Rad) amplification, the final concentration of each DNA sample was diluted to approximately 20 ng/µl with TE buffer.

Analysis of RAPD and nucleotide sequences

Four Operon 10-mer RAPD primers, RAPD K (OPA 19–20) and (OPC 19–20), were used to screen the eight samples of the four medicinal plant species to determine the potential of clear polymorphisms and reproducibility (Gel Documentation Bio-Rad). The PCRs were carried out in 30-µl reaction mixtures containing 10 mM Tris–HCl (pH 9.0), 2.5 mM $MgCl_2$, 200 µM of each dNTP, 10 mM $(NH_4)_2SO_4$, 0.5 U *Taq* DNA polymerase (Banglore genie , India), 30 p/mole each primer, and 10–20 ng template DNA. DNA amplification was performed on a DNA Engine PTC-0110 (Bio-Rad). The parameters for RAPD analysis were 95°C for 5 min, followed by 35 cycles of 30 s at 95°C, 1 min at 42°C, 2 min at 72°C, and a final extension for 10 min at 72°C. The amplification products were separated on a 1.5% agarose gel with a 100-bp DNA ladder (Banglore genie) and visualized using ethidium bromide (EtBr) staining.

Genetic Diversity and phylogenic analysis.

The bands originated in a gel as a result of DNA samples was tabulated in the form of matrix (0-1) where 0 indicates absence of band and 1 presence of bands which was then analyzed NTSYS (software clustering of the data which is based on unweighted pair group method with Arithmetic averaging (UPGMA).

Table 2 Polymorphic RAPD amplicons .

Primer	**Polymorhic specificity**	**Size**	**Primer Sequence (5'-3')**
OPA19	*Terminalia arjuna*	439	F (5'-GGT CAA CGA CAT GAT ATT GT-3') R(5'-GTT ATT TGT GCT TAG AGT TA-3')

OPC20	*Terminalia indic*	687	R1 (5′-TCA TAC ATA TCA GGG TAT GT-3′) R2 (5′-ATG TGT CAT TCG AGC TAA TC-3′)
OPC19	*Adina cordifolia*	216	F (5′-AAT CAA CAA TTA CTT GGT GT-3′) R (5′-TCC CTA CAC AAT CCT TTT TC-3′)
OPA20	*Ficus benghalensis*	361	F (5′-GCA AGT GGC AAT GGG ATT GC-3′) R (5′-GGA TGC CTG AGA TAA GAG GA-3′)

RESULT AND DISCUSSION

The conventional parameters to characterize a melting transition are at which the rise in A 260 is half complete. This temperature is called melting temperature. AT and GC pairs are held together by two and three hydrogen bonds, respectively so high temperature is required to disrupt GC pair. For this reason, the value of Tm is related to the base composition of the DNA, and in the solution is standardized with respect to salt concentration and pH, Tm can be used to measure the base composition.Evolutionary significance of any species is defined by the occurrence of organism at various regions of the biosphere bacterial species were considered to maintained mutational resistance as they survived longer duration. Frequency of mutation is determined by exposure of organism to any mutagenic source and its duration. Genomic approach to taxon exploit diversity among DNA sequences, apart from orientation of A, T, G, C content. In very real sense this sequence can review as "genetic Bar codes" that are found in the cell. The discrimination of life diversity, combinational perspective is a task of scientist. Each Barcode consist of combination of 4 bases which define the pattern of orientation of bases.

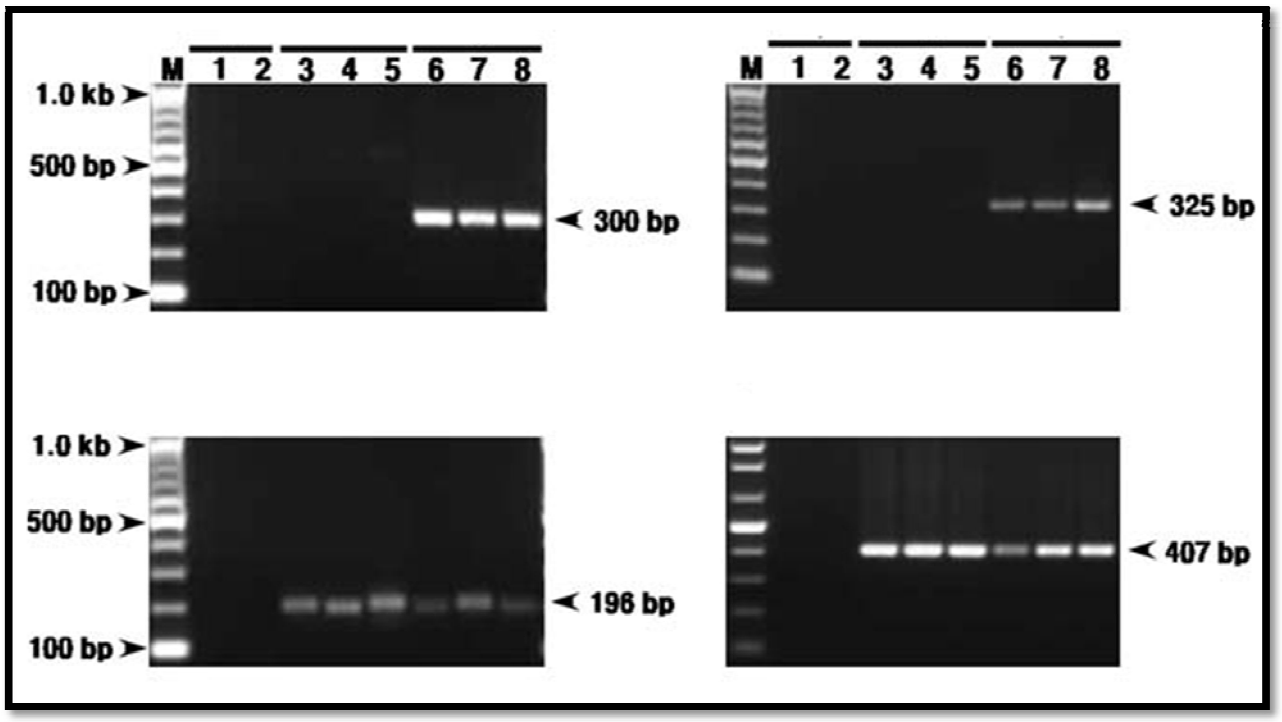

Fig 1. PCR -16S rDNA Amplification , Highly conserve sequence .M represent a 100 bp DNA ladder.

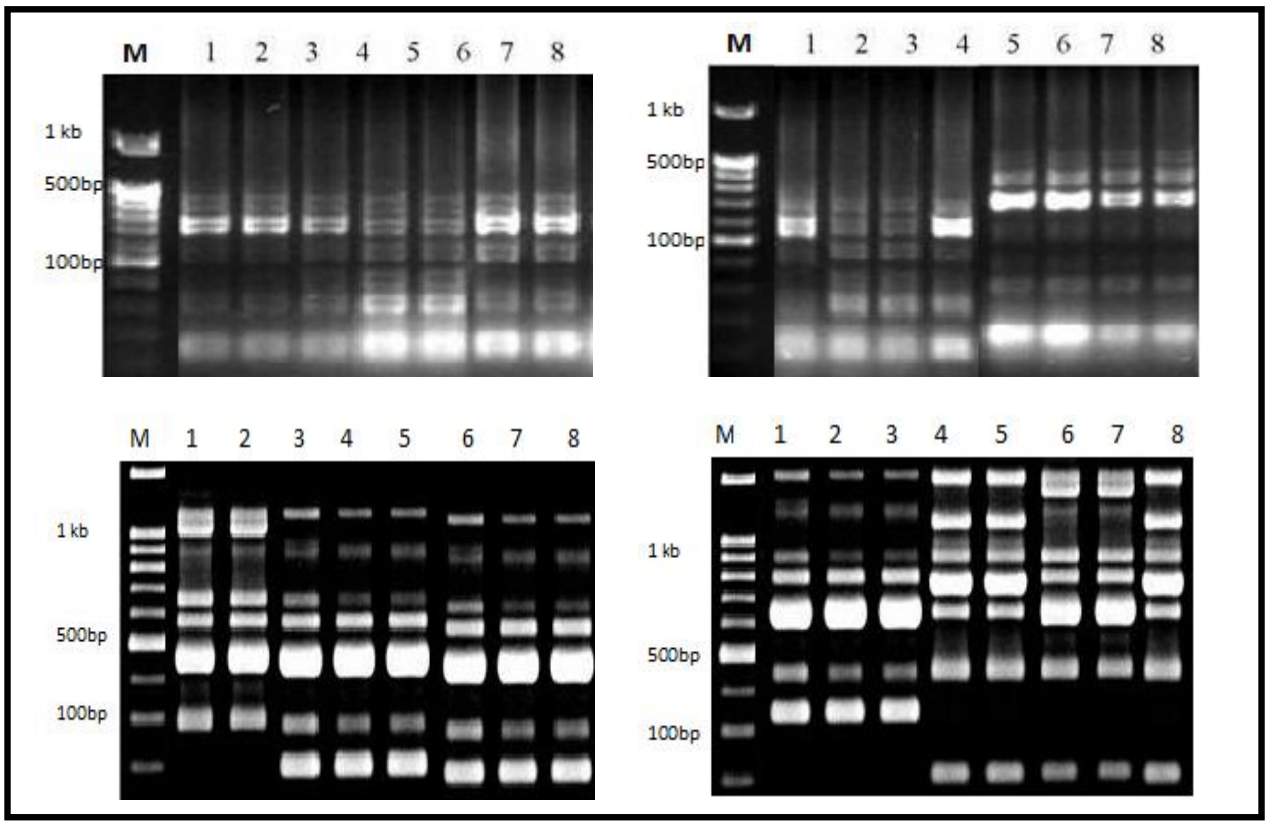

Fig-2. RAPD primer a. OPA19, b. OPC 19, c.OPA 20, d.OPC 20 indicate the species, specific RAPD primers M represent a 100 bp DNA ladder.

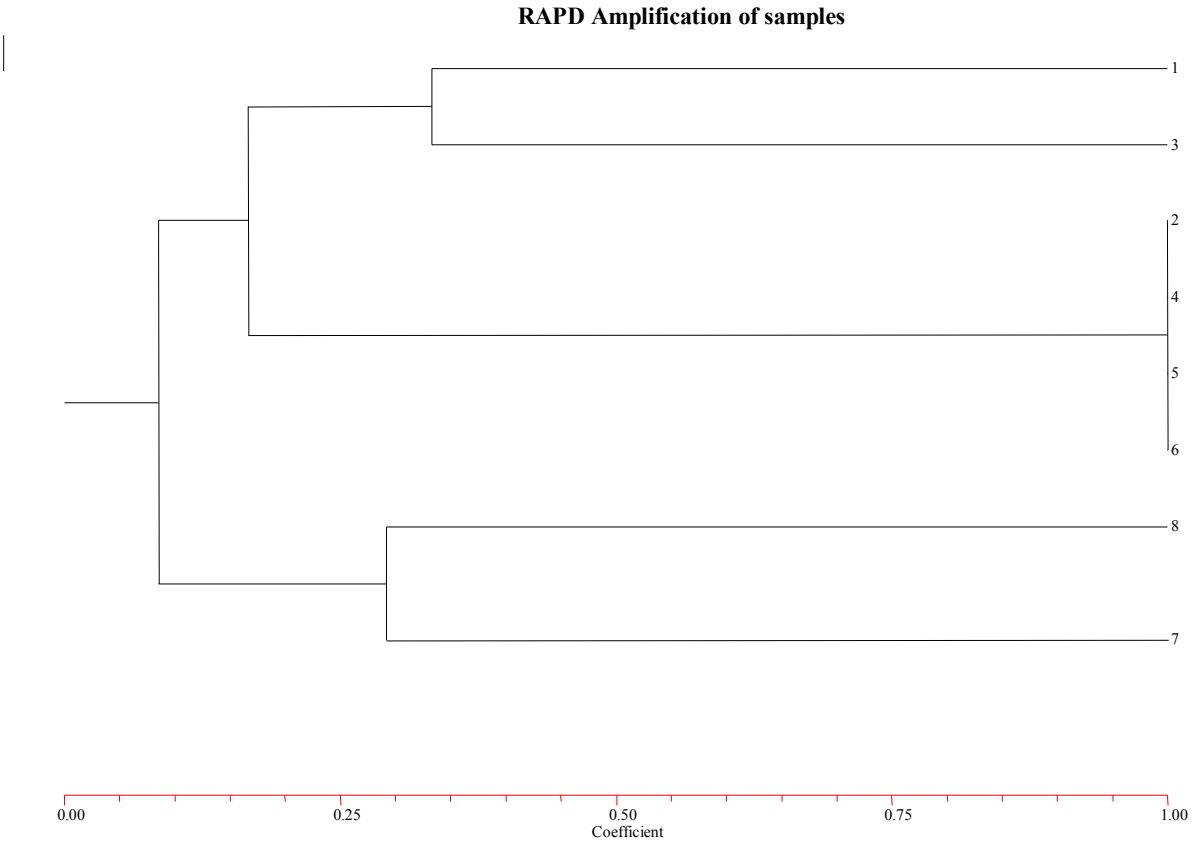

Fig 3. Dendogram based on RAPD-PCR of *medicinal plant* isolates showing genetic relatedness using Jaccord's similarity coefficient and UPGMA cluster method.

We used a PCR-based RAPD method to find species-specific sequences of *Terminalia arjuna, Terminalia indic, Adina cordifolia, Ficus benghalensis* at the genomic level. To control for individual variations, we examined two or three samples per species. In this study, we used four Operon 10-mer random primers to analyze polymorphic patterns, and identified 4 distinct species-specific DNA fragments from 2 of the primers. These polymorphic fragments, designated 1–7 DNA fragments, varied in size, ranging from 200 to 2,100 bp. This study was designed to produce a rapid genetic test for identification and characterization Potential thus, to prevent indiscriminative distribution and prescription of *Terminalia arjuna, Terminalia indic, Adina cordifolia, Ficus benghalensis*, we used the analysis of polymorphic patterns based on RAPD. In the RAPD analysis, we detected polymorphic amplicons among *Terminalia arjuna, Terminalia indic, Adina cordifolia, Ficus benghalensis.* However, greater homology between *Terminalia arjuna and Terminalia indic,* was seen, than with *Adina cordifolia, Ficus benghalensis* . Sample 1 and 3 has 70% similarity sample 2,4,5,6 are 100 % similar to each other and sample 7 and 8 is 75 % similar to each other and dissimilarity coffiecient between all the sample is approximately 25%. The amplification products from this analysis exhibit polymorphism and thus can be used as genetic markers. The presence of a RAPD band, however, does not allow distinction between hetero- and homozygous states repeated appearance of common sequence and in the present of observation occurrence of such bands for more prevalent. The site of action of enzyme most preferably more available thus, by implementing Jaccard's similarity coefficient and UPGMA cluster method minute degree of deviation resultant hieracy of polymorphism.

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