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Analysis of genetic diversity among the *Madhuca longifolia* genotypes from Semi-Arid Ecosystem of Gujarat

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Abstract

An attempt was made to assess the genetic diversity among the elite selections collected from the natural populations in semi-arid parts of Gujarat. A total of 10 accessions of mahua (*Madhuca longifolia*) were subjected to RAPD profiling using 10 primers (OPF). The data from all primers were pooled and subjected to analysis through Nei and Li's coefficient. The results demonstrated that a wide genetic variability exists in the natural population of mahua growing in semi arid parts of the Gujarat. This needs to be conserved and utilized for further improvement of this species.

Key words: *Mahua, RAPD, Biodiversity, genetic diversity*

Introduction

Mahua (*Madhuca longifolia*) of family sapotaceae is a multipurpose tree which is naturally growing in the semi-arid parts of Gujarat. The Indian subcontinent harbours two varieties of this species viz. *Madhuca longifolia* var. longifolia and *Madhuca longifolia* var. latifolia (Roxb.) Cheval. Of the two varieties, var. longifolia is distributed in Sri Lanka, Southern India extending northwards to Maharashtra and Gujrat; var. latifolia is found in some parts of central and north India and Burma (Ramadan *et al.*, 2006; Akshatha *et al.*, 2013). All the parts of the plants are of economic value such as bark, flowers, seeds and leaves are used for food, fodder and fuel (Jayasree *et al.*, 1998). The heartwood of the tree is used for construction of house, flowers are edible and fruits are valued for its seeds, which are the largest source of natural fat known as mahua butter (Yadav *et al.*, 2011, Sastri, 1962; Bringi, 1987; Singh and Singh, 1991). Ramadan *et al.* (2016) have highlighted the nutritional value and industrial application of this crop. The crude oil extracted from the seeds is pale yellow and remains as a semi-solid in the tropical temperatures (Marikkar and Yanty, 2012). According to Ramadan *et al.* (2006), about 46% of the fatty acids present in *M. longifolia* seed oil is saturated, 37.4% mono unsaturated, and 16.5% poly unsaturated. Despite its immense economical value it still remains to be fully utilized agriculturally since no staudard varieties and agro-techniques are available for cultivation of this species.

Being confined to the forests, it has not been subjected to any genetic improvement efforts. The natural populations are seedling populations, therefore they exhibit considerable anatomical, physiological, morphological and genetic variability to survive under varying environmental conditions. As a result of this, wide variations have been observed in sweetness, acidity, size, shape and bearing habits in mahua under Uttar Pradesh and Gujarat conditions (Singh *et al.*, 1999; Anonymous, 2002; Singh and Singh, 2005).

Assessment of genetic diversity based on phenotype has limitations, since most of the morphological characters are greatly influenced by environmental factors and the development stage of the plant. In contrast, molecular markers based on DNA sequence polymorphism, are independent of environmental conditions and show a higher level of polymorphism. Several types of molecular markers such as restriction fragment length polymorphism (RFLP) (Botstein *et al.* 1980), random amplified polymorphic DNA (RAPD) (Welsh and McClelland *et al.* 1990, Williams *et al.* 1990), amplified fragment length polymorphism (AFLP) (Vos *et al.* 1995) and simple sequence repeats (SSRs) are available to assess the genetic diversity and phylogenetic assessment of the crop species. In mahua, very limited work has been done to employ molecular markers in varietal characterization and assessment of phylogenetic kinship among germplasm lines. Accordingly, the objective of the present study was to assess the genetic diversity among the germplasm lines of mahua growing naturally in Gujarat. The results thus obtained constituted the text of the present communication.

Materials and Methods

A total of ten germplasm lines collected from semi-arid region of Gujarat and maintained at CHES, Vejalpur, Godhra constituted the material for present study.

Genomic DNA extraction, PCR amplification and diversity analysis

Young leaves from different genotypes of mahua were collected separately and immediately fixed in ethyl alcohol for 24 hrs. Total genomic DNA was extracted from 100mg of le. afrom each sample seperately by using the DNeasy® Plant Mini kit (QIAGEN, India Pvt. Ltd.) following with some modification. The lysis was achieved by the addition of 400 µl warm (65° C) lysis solution modified by the addition of 10mg/ml PVP (polyvinyl polypyrrolidone), 10mg/ml SDS (Sodium Lauryl Sulphate) and 4 µl of Proteinase K stock solution (100mg/ml) prior to grinding. After grinding the samples were collected into the 1.5 ml eppendorf tube separately. The samples were centrifuged for 30s at low speed (4000g) and 4µl RNase A stock solution (100mg/ml) was added to each tube, followed by mixing until no tissue clumps were visible. Rest of the protocol followed as per standard procedure. To remove RNA, genomic DNA was treated with RNase and stored at -20°C. The quality of DNA was checked by electrophoresis in 0.8 % agarose gel.

PCR conditions and DNA amplifications:

Kit F comprising 20 decamer random primers (Operon Technologies, Alameda, CA, USA) was screened out for the present investigation. To optimize the PCR amplification conditions, experiments were carried out with varying concentrations of DNA template, primers, Taq polymerase, as well as dNTPs. A total of 10 primers were used for PCR-RAPD analysis (Table 1). To determine the optimum amplification conditions and also to ensure the reproducibility of the results, the reaction conditions were standardized using a study and tested at least twice. This study was carried out with two primers (OPF-01 and OPF-02) using a range of DNA concentrations (1, 1.5, 2 and 2.5 µl from the diluted DNA Stock of 200ng/µl) and three different cycles (35, 40 and 45 cycles). After ascertaining the optimum quantity of DNA, annealing temperature and PCR cycles, all the PCR reactions, with 10 primers(OPF 1 to OPF 10), were carried out in 25 µl varying concentrations of template DNA, 1x TopTaq PCR buffer, 1x Q-Solution, 200 µM of each dNTP, 1.25 U TopTaq DNA polymerase, 10 pmol of primer (QIAGEN, India Pvt. Ltd.) and the reaction programmes were set at 94°C for 3 min followed by 40 cycles of 94°C for 30 Sec, 36° C for 1 min and 1 min elongation at 72°C, and a final extension at 72°C for 7 min in a thermal cycler Genemate Series (Analytica Biotech, USA). After completion of the amplification, 2.5 µl CoralLoad dye was added to the samples, and the amplified DNA was analyzed on 1.2% agarose gel pre-stained with ethidium bromide in 1X TAE buffer at 65 - 90 V for 2.5 h, along with O "GeneRuler 100 bp DNA ladder Plus (Thermo Scientific, India Pvt. Ltd.) The gels were observed on the gel documentation system (Gene Genius Bioimaging System) and photographed.

RAPD data analysis:

All the amplified bands were counted manually along with their size. Computer analysis of RAPD patterns were performed as described by Halmschlager et al. (1994) in which the band pattern obtained from agarose gel electrophoresis was digitalized by hand to a two-discrete-character-matrix (0 and 1 for absence and presence of RAPD-bands, respectively) The data of all primers were combined. The analysis data was based on the Nei and Li's coefficient (Nei & Li, 1979). Dendrograms were constructed by the unweighted paired group method of arithmetic average (UPGMA) based on Jaccard's similarity coefficient by using NTSYSpc-2.02e version 2.0.1.5 software (Applied biostatistics, Inc).

Results and Discussion

Optimization of the concentration of DNA for PCR reaction set-up:

Genomic DNA was successfully extracted from ethanol dried young leaf of mahua (*Madhuca longifolia*), using a modifications from the commercial the DNeasy® Plant Mini kit procedure. The approximate DNA concentration in the extracts was 1000 ng/µl. The original concentration of the DNA extracts was too high for reliable amplification, showing poor amplification with faint, smeared products. The optimal DNA concentration for amplification of *Madhuca longifolia* DNA was found to be 4.0 µl of 1:3 dilutions (approximately 200 ng), whilst the optimum annealing temperature was 36° C and the optimum no. of PCR cycles was 40.

Polymorphism and Marker Efficiency:

The largest fragment amplified was in the range of 250 to 3000 bp while the smallest but easily recognizable fragment was approximately of 250 bp. Most bands were concentrated between 500 to 2000 bp. An example of RAPD pattern, obtained with different primers (OPF-01 to OPF-10), is shown in Fig. 1. The number of bands scored for each primer varied from 6 to 17. The highest number of bands (17) was generated with Operon primers OPF 2, OPF 6, and OPF 10, while the lowest number (6) was obtained with Operon primers OPF 3 and OPF 8. The 10 primers yielded a total of 129 fragments, of which 79 amplicons (61.24%) were polymorphic, the number of polymorphic bands per primer ranged from 2 to 14 (Table 1). It was observed that the scored 50 RAPD-PCR fragments were monomorphic (38.75%) The primer OPF-6 is the highly polymorphic primer as 82.35% and gel image of RAPD profiling of this primer is given in Figure 1. However the five primers such as OPF-1, OPF-2, OPF-05, OPF-7, and OPF-10, were also informative primers as 60% or more of the amplicons were polymorphic. The results of the RAPD-PCR analysis indicated that some polymorphic RAPD bands are present were found to be shared among more than one genotype .

Our results are in agreement with those reported earlier on other taxa. It has been demonstrated by Dangi *et al.* (2004) that *Trigonella foenum-graecum* shows 70.12% polymorphism, Rao *et al.* (2006) reported 77.8% polymorphism in chick pea by using 10 decamer primers, Thomas *et al.* (2006) reported 78.8% in wheat by using 50

decamer primers. Patra and Chawla (2010) recorded 76.5% in rice by using 12 decamer primers and Skaria *et al.* (2011) reported 72.27% polymorphism in rice genotypes. This high level of polymorphism in mahua is indicative of fact that during the course of evolution a large number of genetic manipulations have taken place such as single nucleotide change or insertion or deletion of nucleotide or complete loss of complimentary sites. All these have generated genetic variability and got fixed in natural population since the species is a perennial tree and can be propagated by vegetative means.

Genotype identification using RAPD profiles

The data generated on 10 germplasm lines of mahua showed that the RAPD profiles developed by using primers OPF 1 to OPF 10 can be employed for cultivar identification. This is illustrated by the fact that the line MH 5 can be identified by using OPF 2 profile. The line has typical band combination being represented by 350, 2000 and 2200 bp. Similarly, line MH 6 can be identified by using profile developed by OPF 4 having band combination of 780 and 1900bp. Among other lines, MH 1 can be identified using band combination of 500 and 1700 bp in OPF 4, 1150 and 2000bp in OPF 6, combination of 550 and 1000bp with OPF 10 and sole band at 530bp with OPF 9, Line MH 2 can also be identified using combination of bands at 400, 680, 7650, 1000, 1100 and 1500bp with OPF 10.

Our results are in line with those reported in Mulberry (Bhattacharya and Ranade, 2001), *Gymnema sylvestre* (Balamurali Krishna *et al.*, 2012), *Madhuca longifolia* (Gavankar and Chemburkar, 2016 and *Trigonella* (Raja *et al.*, 2015).

Genetic relationships among accessions and cluster analysis

Cluster analysis based on similarity values (Table 2) classified genotypes into two distinct clusters (I & II, Figure 2). The first cluster (I) included only four genotypes MH-14, MH-2, MH-6 and MH-1, whereas the second cluster (II) included six genotypes (MH-10, MH-3, MH-15, MH-8, MH-5 and MH-4) and was further divided into three sub-clusters (Figure 2). Both of the cluster I and II were further divided into two sub-clusters labeled (I-A, I-B) and (II-A, II-B) respectively. The sub-cluster I-B and II A are monophyletic branches including MH-1 and MH-10 genotypes respectively. The sub cluster II-B was further grouped into two sub-sub clusters II-B_1 (MH-3, MH-8 and MH-15) and II-B_2 (MH-5 and MH-4) Similarity coefficients between Madhuca longifolia ranged from 0.619 to 0.900 (Table 2). Among the similarity coefficients of the genotypes, the similarity coefficient MH-2 and MH-6 was highest (0.900), which indicates closer relationship among these accessions, while the similarity coefficient of MH-1 and MH-4 was observed lowest (0.619) (Table 2).

Similar work has been carried out on other plant species also. A wide genetic variability has been reported by Rout and Das (2002) in *Plumbago*, Gilani *et al.* (2009) in *Wathania somnifera*, Smita and Keshvachandran (2015) in *Trigonella* cultivars. Gavankar and Chemburkar (2016) also made an attempt to assess the genetic variability in some accessions of Mahua. Our results highlight that a wide genetic variability exits in the natural population of Mahua in Panchmahal region of Gujarat. This calls for a systematic collection, evaluation and conservation of this species so that trait based breeding programme can be initiated to develop an elite forms for commercial cultivation.

Table 1: List of primers and degree description of the polymorphism obtained among 10 mahua genotypes.

S. No.	Primer code	Sequence 5' to 3'	Sizes (bp) min-max	Total band No.	Polymorphic bands	Polymorphism ratio (%)	PIC
1	OPF-1	ACGGATCCTG	330-1800	15	11	73.33	0.391
2	OPF-2	GAGGATCCCT	290-2200	17	11	64.71	0.378
3	OPF-3	CCTGATCACC	600-1300	6	3	50.00	0.406
4	OPF-4	GGTGATCAGG	450-2500	15	6	40.00	0.343
5	OPF-5	CCGAATTCCC	400-1800	15	10	66.67	0.260
6	OPF-6	GGGAATTCGG	280-2800	17	14	82.35	0.467
7	OPF-7	CCGATATCCC	600-2000	10	6	60.00	0.241
8	OPF-8	GGGATATCGG	500-1500	6	2	33.33	0.153
9	OPF-9	CCAAGCTTCC	550-3000	11	5	45.45	0.388
10	OPF-10	GGAAGCTTGG	440-2500	17	11	64.71	0.389

Table 2: Dice Jaccard's similarity co-efficient matrix of 10 different genotypes of mahua.

Genotypes	MH-14	MH-10	MH-2	MH-1	MH-3	MH-5	MH-4	MH-6	MH-8	MH-15
MH-14	****									
MH-10	0.622	****								
MH-2	0.820	0.661	****							
MH-1	0.741	0.687	0.678	****						
MH-3	0.636	0.670	0.689	0.630	****					
MH-5	0.678	0.684	0.718	0.644	0.730	****				

MH-4	0.652	0.643	0.692	0.619	0.766	0.817	****			
MH-6	0.763	0.681	0.900	0.669	0.681	0.754	0.669	****		
MH-8	0.681	0.734	0.650	0.675	0.783	0.750	0.691	0.669	****	
MH-15	0.670	0.645	0.624	0.634	0.740	0.645	0.664	0.643	0.780	****

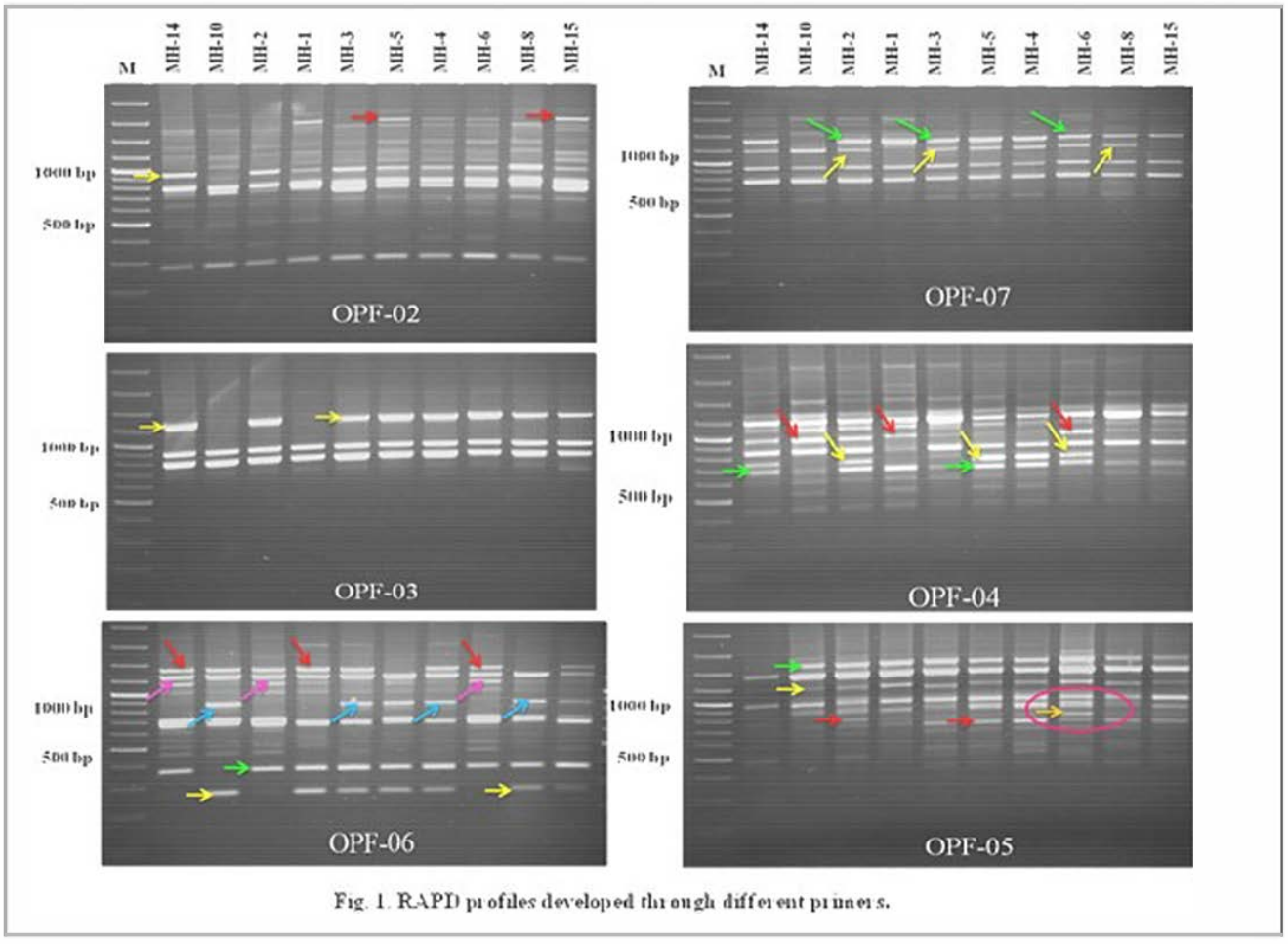

Fig. 1. RAPD profiles developed through different primers.

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