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Documentation and diversity analysis by DNA fingerprinting of the indigenous Mango (*Mangifera indica* L.) germplasm of West Bengal

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Abstract

Mango (*Mangifera indica* L.) has been reported to have extensive diversity due to alloploidy, outbreeding, continuous grafting and phenotypic differences arising from varied agro climatic conditions in different mango growing regions. Characterization and documentation of local mangoes is important for identifying potential candidates for improved utilization of the genetic resource and future breeding. The districts of Murshidabad and Malda once famous for mango are now facing tremendous genetic erosion of the mango germplasm. More than 200 varieties of Mango were recorded during the time of the royals of these districts. This number has sharply declined in recent times. The traditional varieties are low yielding and are replaced by new high yielding hybrids. These varieties are also facing extinction because of the aggressive cultivation of Amrapali, Himsagar, Langra varieties of mango. Conservation of Mango (*Mangifera indica* L.) germplasm is of utmost importance. But for conservation extensive survey and proper documentation is necessary. This study is an attempt of the documentation of Mango varieties from Murshidabad and Maldah districts. The DNA fingerprinting data will also help in proper identification of mango varieties. The dendogram generated from the fingerprint show the genetic distance among the varieties. The mango germplasm diversity data generated will be important for future mango breeding and in implementing conservation strategies.

Keywords: DNA fingerprinting, Malda, mango, Murshidabad, pulp quality analysis.

Introduction

Mango has been reported to have extensive diversity due to alloploidy, outbreeding, continuous grafting and phenotypic differences arising from varied agro climatic conditions in different mango growing regions

(Ravishankar et al., 2000). In addition, mango being highly cross pollinated, open pollination between the cultivars could have resulted in new varieties not yet documented. Subsequently, mango varieties have experienced great confusion in their nomenclature with many synonyms existing for the same varieties. Characterization and documentation of local mangoes is important for identifying potential candidates for improved utilization of the genetic resource and future breeding programmes (Ramessur & Ranghoo-Sanmukhiya, 2011). Use of morphological descriptors is an easy and cheap approach, but might fail to differentiate closely related accessions and the method can only be applied during the mango harvest season. DNA fingerprinting can be applied to any plant tissue, including the leaves, and the analysis is independent from any environmental influences, however, the approach requires sophisticated technologies and is more costly than the morphological characterization (Gitahi et al., 2016).

Detailed and well documented information about the available genetic material together with a broad, well maintained varietal diversity are essential for breeding efforts. This should also include local varieties (Subedi et al*.,* 2005a), which may have a low market, but high breeding value. In addition to using morphological descriptors for variety characterization (IPGRI, 2006), molecular marker and isozyme analysis techniques are increasingly used for describing the genetic diversity of mango cultivars (Karihaloo et al*.,* 2003; Subedi et al., 2005b; Schnell et al., 2006; Yamanaka et al., 2006; Krishna and Singh, 2007; Díaz-Matallana et al., 2009).

Mango (*Mangifera indica* L.) an important natural resource is also known as the 'king of fruits'. Mango a diploid fruit tree with 2n=40 chromosomes (Mukherjee, 1953) originated in the Indo-Burma region during the earlier period of the Cretaceous era (Yonemori et al*.,* 2002) and gradually spread to the tropical and subtropical regions of the world. India is thought to be the primary centre of diversity along with its status as the centre of origin for mango. Presently, India harbours more than 1000 mango cultivars and represents the biggest mango germ pool in the world (Tomar et al*.,* 2011). Expansion of urbanization and cultivation make the plants vulnerable for extinction, but it is the so called plant varieties that reflect the diversity of plants and these are reservoir of alleles. So conservation of varieties are very much important, it also fulfill India's obligations under conventions on biological diversity with special reference to Article 6 and 7 of UNEP (1992).

Mango (*Mangifera indica* L.) is an economically important plant. Murshidabad and Maldah are famous for its mango varieties but this germplasm is under threat (Mukharjee, S. K., 1953). The age old plants are low yielding, those are replaced by new high yielding hybrids. Every year some of these varieties vanish into oblivion. Conservation of Mango (*Mangifera indica*) germplasm is relatively easy. Only open space is required where these germplasm can be conserved. The conserved germplasm can be used for micropropagation, cutting can be used for vegetative reproduction and in this way this wealth can be restored when needed. This can also serve for breeding stalk for varietal improvement programme. For conservation the 1st step is documentation of the varieties (Pandit et al. 2007).

RAPD (Random Amplified Polymorphic DNA) assay, detects nucleotide sequence polymorphism in DNA amplification based assay using only a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer binds to the genomic DNA at two different sites on opposite strands of the DNA templates. If these primary sites are

within an amplifiable distance thermo cyclic amplification occurs. The presence of each amplification product identifies complete or partial nucleotide sequence homology, between the genomic DNA and oligonucleotide primer at each end of the amplified product. On an average, each primer will direct the amplification of several discrete loci in the genome, making the assay an efficient way to screen for nucleotide sequence polymorphism between individuals. The DNA amplification product is generated from a region that is flanked by a part of 10-base pair priming site in the appropriate orientation. Genomic DNA from two different individual often produces different amplification pattern (RAPDs). A particular fragment generated from one individual, but not for other represents DNA polymorphism and can be used as Genetic marker (Bhargava and Khorwal, 2011).

Malda is a district in West Bengal, India. It lies 347 km (215 miles) north of Kolkata, the state capital. The latitude range is 24°40’20" N to 25°32’08" N, and the longitude range is 87°45’50" E to 88°28’10" E. The district covers an area of 3,733.66 square kilometres (1,441.6 sq mi). Murshidabad is a district of West Bengal in eastern India. Situated on the left bank of the river Ganges, the district is very fertile. It covers an area of 5,341 km² (2,062 sq mi). The district comprises two distinct regions separated by the Bhagirathi River. To the west lies the Rarh, a high, undulating continuation of the Chota Nagpur plateau. The eastern portion, the Bagri, is a fertile, low-lying alluvial tract, part of the Ganges Delta. The district is drained by the Bhagirathi and Jalangi rivers and their tributaries. Bhagirathi is a branch of the Ganges, and flows southwards from Farakka barrage where it originates from the Ganges. It flows southwards through the district and divides it into more or less equal halves.

Materials and Methods

Quality Characterization

Mango from different market of the district and orchard were collected. The market survey exhibit the availability of that particular breed. The farmers and the traders are the source of information. Naturally ripe Mango were collected from plants, total weight of Mango were measured, pulp were isolated and weighted. The percentage of pulp weight against total weight of Mango was considered as pulp quantity percentage. Pulp P^H was determined by pH meter , total free glucose were measured from the mango sample by Anthron method. (De et al*.,* 2014).

Apparatus Used for Fingerprinting

The apparatus used for the research work were the gel electrophoresis unit, cool centrifuge, program thermo cycler, deep freezer (-20°C and -80°C), refrigerator, hot water bath, hot oven, pH meter, autoclave, gas stove, electronic weighing balance, micropipette, DNA, UV transilluminator, mortar and pestle, tips and Eppendorf tubes

DNA Extraction Solution

It has CTAB, Tris base, NaCl, EDTA (disodium), Chloroform: Isoamyl alcohol, β mercaptoethanol, PVP – 1% (Poly vinyl pyrrolidine).

PCR Ingredients

Template DNA, dNTPs, 10x PCR buffer, $MgCl_2$, Taq DNA polymerase, Mineral oil, Primer OPC 11- AAAGCTGCGG*I.* Reagents: 1.5% agarose, loading buffer – sucrose, Xylene cyanol, Bromophenol blue Ethidium bromide II. Running buffer (5x) or TBE / TAE: Tris Base (pH 8.0), Boric acid, EDTA

Method for RAPD Analysis

DNA is extracted by meshing the leaves of the plant in mortar and pestle and treated

MALDA DISTRICT

MURSHIDABAD DISTRICT

with different reagents, followed by verifying it by electrophoresis in 1.2% agarose gel. DNA is subjected to selective amplification in PCR where primer is amplified and further treated with different reagents of PCR. Target sequence is amplified and is then subjected to agarose gel electrophoresis and stained with ethidium bromide. The gel was photographed and RAPD profile was obtained. The RAPD profile was analyzed by using GEL ANALYZER software for preparation of Dendogram.

Results and Discussion

40 Indigenous mango varieties were studied for different quality parameters and following results were obtained.

Once, more than 200 varieties of mango were known from Malda and Murshidabad districts only as mentioned earlier.

Table 1. Quality characters, distribution and availability of 40 different varieties.

Sl. No.	VARIETY NAME	PULP QUANTITY (%)	pH	FREE SUGAR (%)	LOCALITY	AVAILIBILITY
1	ALAPATI	62.76	5.63	17.28	SAHAPUR, MALDA	RARE
2	ARAJANMA	54.9	3.89	19.02	MALDA	RARE
3	BADSABHOG	51.78	6.03	22.97	LALBAG, MURSHIDABAD	MODERATE
4	BAISHAKGUTI	45.78	3.32	16.89	LALBAG, MURSHIDABAD	MODERATE
5	BHARATI	46.86	5.90	17.25	MALDA	MODERATE
6	BIMLI	57.87	5.03	20.06	AJIMGAUNGE, MURSHIDABAD	RARE
7	BRINDABANI	43.34	5.87	23.34	SAHAPUR, MALDA	RARE
8	CHAMPA	41.23	4.87	22.01	AJIMGAUNGE, MURSHIDABAD	MODERATE
9	CHINICHAMPA	42.67	6.04	29.07	JIAGAUNGE, MURSHIDABAD	MODERATE
10	DILSWAD	47.6	6.04	26.89	LALGOLA, MURSHIDABAD	MODERATE
11	CHOTOLAKSMAN	43.4	5.07	21.21	LALGOLA, MURSHIDABAD	MODERATE
12	DUDHKUMAR	42.85	5.02	19.09	MALDA	RARE
13	KRISHNABHOG	47.8	4.07	21.26	MALDA	MODERATE
14	ANARASI FAJLI	46.91	4.01	17.21	MALDA	AVAILABLE
15	FANIA	51.6	5.06	17.67	MALDA	AVAILABLE
16	GOLACHOKA	53.4	5.07	16.56	MALDA	AVAILABLE
17	GOPALBHOG	56.32	4.93	21.96	CHACHOL, MALDA	AVAILABLE
18	JILEPIKERA	49.09	4.73	14.78	RAIPUR, MALDA	MODERATE
19	KACHAMITHA	42.67	3.92	17.77	SHAKTIPUR, MURSHIDABAD	AVAILABLE
20	KHIRSAPATI	51.7	5.05	17.45	MALDA	AVAILABLE
21	GUTI KHIRSAPATI	47.9	4.83	19.57	MALDA	AVAILABLE
22	KOPAI	43.6	4.87	18.98	NASIPUR, MURSHIDABAD	MODERATE
23	LAKSHMANBHOG	46.97	5.03	20.03	SAHAPUR, MALDA	AVAILABLE
24	MADHUCHUSKI	49.3	5.89	20.91	MURSHIDABAD	MODERATE
25	MADHUGULGULI	51.6	3.94	22.01	MURSHIDABAD	RARE
26	MISRIKANTA	53.7	6.1	20.9	LALBAG, MURSHIDABAD	MDERATE
27	MOLAMJAM	47.3	5.01	18.8	MURSHIDABAD	AVAILABLE
28	RAKHALBHOG	41.7	5.03	17.67	MALDA	AVAILABLE
29	RANI	43.7	5.97	24.03	MURSHIDABAD	AVAILABLE
30	SHADULLA	47.8	4.23	17.88	MURSHIDABAD	AVAILABLE
31	SINDURIA	51.4	4.56	16.76	MALDA	MODERATE
32	VABANI	56.29	4.38	15.97	MURSHIDABAD	MODERATE
33	SURIKHAS	42.01	4.72	17.07	ARAPUR, MALDA	RARE
34	SARENGI	43.78	4.7	17.92	AJIMGAUNGE, MURSHIDABAD	MODERATE

35	ASWINA	56.23	5.01	16.53	MALDA	AVAILABLE
36	MOHANBHOG	51.03	6.01	19.68	MALDA	MODERATE
37	LANGRA	56.07	4.02	16.93	MALDA, MURSHIDABAD	AVAILABLE
38	MUCHI SAMANIA	47.91	5.78	14.87	MALDA	MODERATE
39	RASI	45.92	5.03	16.78	MURSHIDABAD	MODERATE
40	VADARIA	47.01	4.01	16.41	MALDA	MODERATE

Altapati, Sahapur, Malda

Arajanma, Malda

Bharati, Malda

Aswina

Bimli, Ajimgaunge, Murshidabad

Brindaboni, Sahapur, Malda

	
Champa, Ajimgange, Murshidabad	**Dudhkumar, Malda**
	
Fajli, Anarasi, Malda	**Fania, Guthi, Malda**
	
Golachoka, Guthi, Malda	**Gopalbhog, Chanchol, Malda**

	
Jilepikera, Raipur,Malda	**Khirsapati, Malda**
	
Khirsapatia, Guthi, Malda	**Krisnabhog, Malda**
	
Laksmanbhog, Malda	**Langra, Malda, Murshidabad**

	
Misrikanta, Lalbag, Murshidabad	**Mohonbhog, Malda**
	
Muchi Samania, Malda	**Nawab Bhog, Lalbag, Murshidabad**
	
Rakhalbhog, Malda	**Sarengi, Ajimgange, Murshidabad**

Sinduria, Guthi, Malda

Suri Khas, Arapur Kotwali, Malda

Vadaria, Guthi, Malda

Figure 1. Fruits of Different Mango varieties (Shape and Colour).

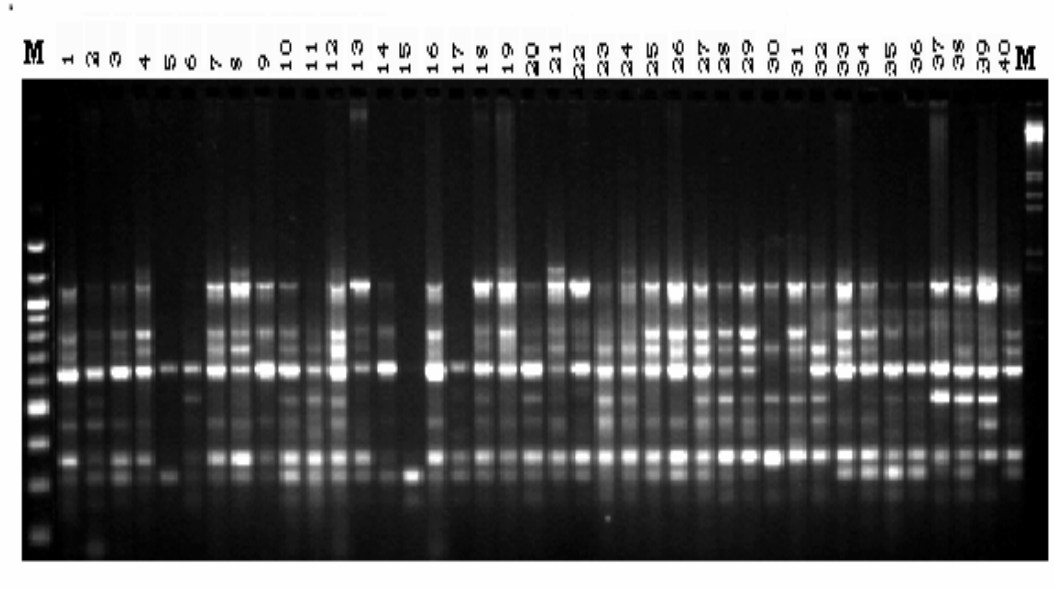

Figure 2. DNA fingureprint of 40 Mango varieties using RAPD.

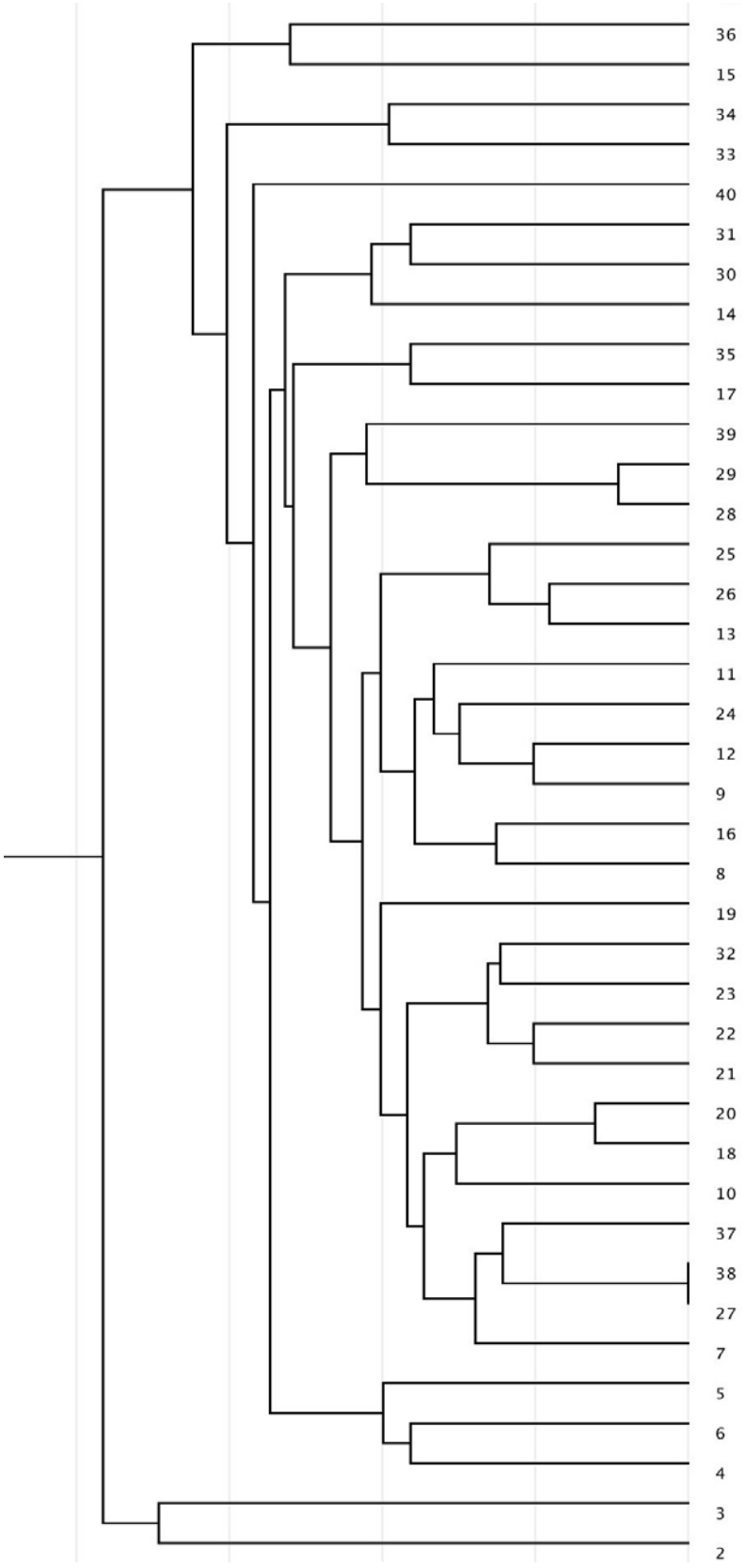

Figure 3. Dendogram showing genetic distance of 40 indigenous Mango varieties.

But extensive survey in 2011-2014 showed that only 53 varieties are now available. Of these, 40 different mango varieties are studied. These include assessment quality parameters like Pulp, pH, pulp quantity and total free glucose. The pulp quantity was found highest in Chandankhosa (79.05%) and lowest in Champa (41.23%), while free sugar was highest in Chinichampa (29.07%) and lowest in Jilipkhera (14.78%). Utilization of the conserved germplasm in the breeding programme requires precise information on the genetic relationships among the accessions. Information on the genetic distance among the germplasm accessions will also help avoiding duplicates, thus clearing the nomenclature ambiguity, widening the genetic base of the core collections and ultimately helping in preserving the valuable diversity (Singh, 2012; Vasugil, 2012**)**. But in West Bengal proper documentation of the mango varieties is lacking. For assessment, distance can be measured from the dendogram prepared. the genetic diversity is measured using RAPD. The quality characters are very much important (Dash and Hota, 1997; Majumder, 2013), because the quality characters are parameters of selection of proper plants for propagation. The marketable quality characters are only considered when assessing the indigenous varieties. From the study it is found that the juiciness is very high in 11 varieties, much higher than Amrapali, which is most cultivated variety. The yield cannot be compared, because the indigenous plants are age old and not also well maintained. Among the indigeneous varieties, 19 of 52 varieties contain 20% or more sugar in the pulp and the pH is in some cases it is near neutral. That means these are very sweet in test and less sour. It is found that nine mango varieties are already rare among the documented 40 varieties. These varieties are having very less number of plants, restricted to certain pockets on Malda and Murshidabad. The commercial cultivation is the main reason, besides the plants are age old and need to be replaced by their cuttings. The glory of 'Mango Districts' is already at stake. The rest of the germplasm requires immediate attention. Characterization of diversity is a necessary requirement for the improvement, use and conservation of plant genetic resources (Archak et al., 2003; Krishna and Singh, 2007). The present attempt may serve as baseline data for further investigation of already at stake germplasms of Mango. These varieties require immediate restoration and attention, otherwise the genetic diversity of mango in these two districts will be lost in near future.

RAPD analysis is shown to be very efficient in identifying markers linked to the targeted region of the genome. The extent of polymorphisms detected by RAPD method is better than that of RFLP since it is a dominant marker. RAPD is less expensive, fast and reliable when compared to RFLP, which is more expensive, tedious and involves radioisotopes. The results of the present study indicated that the RAPD analysis could be utilized by breeders for further improvement of mango varieties. The dendogram constructed from RAPD shows the genetic distance among the varieties. Mango is highly heterogeneous plant. The varieties were developed from selection of the segregating germplasms. Suitable varieties are cloned by cuttings. The origin of different varieties can be traced back from the dendogram created.

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