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Morphological characterization and Simple Sequence Repeats (SSRs) based DNA fingerprinting of selected mango (*Mangifera indica* L.) genotypes in Bangladesh

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Nineteen genotypes of mango including nine released varieties viz. BARI Aam-1, BARI Aam-2 (Laxmanbhog), BARI Aam-3, BARI Aam- 4 (Hybrid), BARI Aam-5, BARI Aam-6, BARI Aam-7, BARI Aam-8, BARI Aam-9; one parental line viz. M- 3896 and nine Geographical Indication Crops (GIs) viz. Haribhanga, Surjapuri, Fazli, Gourmoti, Ashwina, Khirsapat, Gopalbhog, Langra and Ranipasand were characterized with a view to identifying the degree of morphological and molecular variation of mango within genotypes with their historical background their historical background, and to establish a permanent database for documentation of mango in Bangladesh. Wide variations were observed among GI crops and released varieties included in this study for plant, leaf, flower and fruit characters. Among 19 mango genotypes, eight were distinct by two traits and 11 by only single character. Molecular characterization was carried out with SSR markers. Using 21 primers across 19 genotypes a total of 80 alleles with an average number of 3.81 alleles per locus were found of which MIAC-6 and MIAC-11 showed the highest number of alleles (6) (size ranging from 244 to 312 and 133 to 167 bp, respectively). However, the lowest number of allele (2) with size ranging 237 to 366 and 118 to 125 bp was observed in the locus MiSHRS-39 and MIAC-11, respectively. The polymorphic information content (PIC values) ranged from 0.349 to 0.781, with a mean value of 0.602 for all loci. Of the 21 SSR primers, 13 were highly informative (PIC value ≥0.6). The distinct level of heterozygosity indicated higher level of diversity among the genotypes. Band patterns corresponding to individual genotype have been identified to discriminate the genotype. The genotypes presented genetic distances between 0.260 and 1.557. The dendrogram generated from UPGMA cluster analysis broadly placed 19 mango genotypes into two major groups, "A" and "B" in which only one poly-embryonic genotype namely BARI Aam-8 congregated in a distinct group "B" and other 18 mono-embryonic genotypes clustered in group "A". The dendrogram revealed that Gourmoti and Ashwina were the most similar hybrids with 21% similarity. Contrary to this, hybrids BARI Aam-5 and BARI Aam-8 were the most divergent with a diversity value of 1.56.

Key words: Geographical Indication Crops (GIs), historical background, morphology, identity.

INTRODUCTION

Mango (*Mangifera indica* L. 2n=40) is a member of the family Anacardiaceae in the order Sapindales, a family of

mainly tropical species with a few representatives in temperate regions (Viruel, 2005). The mango is considered as one of the oldest cultivated trees in the world. Historical records provide conflicting accounts for origin and distribution of mango. Although some authors have considered India as the centre of origin due to the high degree of mango diversity observed in that country (Ravishankar et al., 2000), taxonomic and molecular evidence also supports an evolution of mango within a larger area including northwestern Myanmar, Bangladesh and Northeastern India (Mukherjee 1997). Total world mango production is 26 million tons, and it is one of the most important fruits in the world, along with bananas, oranges, grapes and apples (Ukoskit, 2007). Its popularity and importance can easily be realized by the fact that it is often referred as the “the king of fruits” in the tropical world (Purseglove, 1972). The King among fruit is thriving very well in Bangladesh. Mangoes grow widely all over Bangladesh and there are innumerable varieties to charm the connoisseur. Each variety has its own admirers. Each has its distinctive flavour and arguments about the superiority of one over the other can get very serious. Though these are table varieties, meant to be relished as cut fruit, there are others that are used for making jam, jelly, squash, chutney, and pickle. The raw green mango is even added to dal or curry to enhance the flavour. According to the latest statistics provided by BBS (2017), indicated that, the production of mango in Bangladesh is1288000 metric tons. Mango contributes 21.77% to total fruit production in Bangladesh. Several local and exotic cultivars are grown in the country. The cultivars are mostly location specific. Bangladesh Agricultural Research Institute (BARI) has developed eleven improved varieties. Some cultivars have been originated in some localities and are being cultivated in those areas from more than several hundred years, which can be termed as geographical indication crops (GIs) for those localities. It is essential to characterize the GIs and released varieties of mangoes both in morphological and molecular level for establishment of Intellectual Property Right (IPR). Moreover, selection and correct identification of genotypes is essential for any breeding and improvement effort, is difficult, inefficient and inaccurate when based on morphological traits only. Even though a high number of descriptors are used (Thomas et al., 1994), this is due to some phenotypic traits are difficult to describe, and phenotypic data may be influenced by environmental factors and growing conditions, in addition to quantitative inheritance, or partial and complete dominance often confound the expression of genetic traits. Recently, as in other fruit tree species molecular identification of mango cultivars has been carried out with different molecular systems as isozymes, minisatellites (Jintanawongse and Changatragoon, 2000), RFLPs (Chunwongse et al., 2000; Capote et al., 2003; Ravishankar et al., 2004), AFLPs (Kashkush et al., 2001; Yamanaka et al., 2006), ISSRs (Singh et al., 2007; Bajpai et al., 2008; Samant et al., 2010). While DNA profiles based on polymorphic band patterns from Random Amplified polymorphic DNA (RAPD) analysis have been described for several fruit species including mango (Rahman et al., 2007; Abirami et al., 2008; Bajpai et al., 2008; Pruthvish and Chikkaswamy, 2016). But these markers have some limitation like RFLPs requires the use of radioactivity and is labour intensive. RAPDs and AFLPs identify only dominant alleles and are sensitive to PCR amplification. Different thermocyclers, Taq polymerases, DNA primer concentrations and even the skill of the experimenter can influence the results of RAPD marker (Sefc et al., 2001). Considering these aspects, a project was undertaken by Agricultural Research Council (BARC) to characterize GIs and released varieties of some crops grown in the country with the financial support of Sponsored Public Goods Research-National Agricultural Technology Project (SPGR-NATP) Phase-1. Bangladesh Agricultural Research Institute (BARI) has been assigned with 10 of its mandated crops and mango was one of them. Nineteen mango genotypes (one advanced line, nine released varieties and nine GIs) have been included in this programme. The present study was, therefore, undertaken to identify distinct morphological characteristics along with establish allelic patterns and estimate genetic distances based on microsatellite markers for 19 mango genotypes to generate a reference database to support cultivar protection and settle possible commercial disputes as well as to guide breeding programmes and genetic resources of the species.

MATERIALS AND METHODS

Selection of trees for GI released varieties

Nineteen genotypes of mango including nine released varieties viz. BARI Aam-1, BARI Aam-2 (Laxambogh), BARI Aam-3, BARI Aam-4 (Hybrid), BARI Aam-5, BARI Aam-6, BARI Aam-7, BARI Aam-8, BARI Aam-9; one parental line viz. M-3896 (Male parent of BARI Aam-4) and nine GIs viz. Haribhanga, Surjapuri, Fazli, Gourmoti, Ashwina (female parent of BARI Aam-4), Khirsapat, Gopalbhog, Langra and Ranipasand were characterized both at morphological and molecular level. Historical background was not recorded in two genotypes viz Gourmoti and Ranipasand. Morphological characterization of seventeen genotypes was done from standing trees. The centre of diversity of most concentrated with experienced fruit of the respective GI was identified through discussion with experienced fruit scientists and Department of Agriculture Extension (DAE) Officials at district and upazila level. A team of scientists, involved in this programme, visited particular geographical locations

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Table 1. List of mango genotypes used in this study with their existing tree age and locations of sites in Bangladesh.

S/N	Plant designation	Tree age (Years)	Location of collecting site (Upazila and District)	Latitude and Longitude
1	BARI Aam-1	80	RHRS, Chapainawabganj	24º35.5' N and 88º16.8' E
2	BARI Aam-3	18	RHRS, Chapainawabganj	24º35.5' N and 88º16.8' E
3	BARI Aam-4 (Hybrid)	19	RHRS, Chapainawabganj	24º35.5' N and 88º16.8' E
4	BARI Aam-5	8	FRF, BARI, Gazipur	23º59.595' N and 90º24.874' E
5	BARI Aam-6	20	RHRS, Chapainawabganj	24º35.5' N and 88º16.8' E
6	BARI Aam-7	20	RHRS, Chapainawabganj	24º35.5' N and 88º16.8' E
7	BARI Aam-8	8	FRF, BARI, Gazipur	23º59.595' N and 90º24.874' E
8	BARI Aam-9	10	RHRS, Chapainawabganj	24º35.5' N and 88º16.8' E
9	M-3896	19	RHRS, Chapainawabganj	24º35.5' N and 88º16.8' E
10	Laxambogh	80	RHRS, Chapainawabganj	24º35.5' N and 88º16.8' E
11	Haribhanga	65	Mithapukur, Rangpur	25º45.117' N and 89º15.176' E
12	Surjapuri	200	Baliadangi, Thakurgaon	26º09.14' N and 88º12.31' E
13	Fazli	100	Shibganj, Chapainawabganj	24º48.5' N and 88º08' E
14	Gourmoti	50	Shibganj, Chapainawabganj	24°48'49.18'N, 88° 8'35.74'E
15	Ashwina	100	Shibganj, Chapainawabganj	24º48' N and 88º07' E
16	Khirsapat	100	Shibganj, Chapainawabganj	24º48.5' N and 88º08' E
17	Gopalbhog	100	Shibganj, Chapainawabganj	24º48.5' N and 88º08' E
18	Langra	80	Shibganj, Chapainawabganj	24º48' N and 88º07' E
19	Ranipasanda	20	Shibganj, Chapainawabganj	24º48.5' N and 88º08' E

RHRS: Regional Horticulture Research Station, FRF: Fruit Research Farm, BARI: Bangladesh Agricultural Research Institute.

Figure 1. Ashwina (more than 100 years old tree).

and located the targeted trees of selected GIs. Then three plants were labeled with laminated paper sheet as plant number 1, 2 and 3 for each GI. For released varieties and parental line, the team visited Regional Horticulture Research Station (RHRS), Chapainawabganj and Fruit and conserved original plant. Discussing with the station heads and working scientific personnel, the original mother tree (s) (OMT) was identified and selected for data collection. In cases where there was only one OMT, daughter mother trees (DMT) were also selected for data collection. Each OMT and DMT was labeled as plant number 1, 2 and 3. The list of mango genotypes used in this study with its data collecting site is given in Table 1.

Recording historical background

Scientists discussed with aged people of the growing areas to find out the historical background of the respective GIs of mango. The team also located some very old trees (≥100 years) as indicated by the nearby people and symptoms on the tree like canopy coverage, trunk circumference, extra-rough trunk surface and galls on trunk etc. Typical plant photographs of Ashwina and Surjapuri were shown in Figures 1 and 2.

Management practices

Farmers normally sell their crop as total plantation either in orchard or in homesteads just after harvest of the previous year's fruit. Then entire liability of the plantation goes to the traders. Management practices like pruning, weeding, irrigation and fertilizer and pesticide application etc. were done by the traders. Chemical fertilizers like urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum etc. were applied at different rates. None of the traders used recommended doses of fertilizers. Pesticides as prescribed by the dealers or experienced traders were used indiscriminately even one or two days before harvesting especially for controlling fruit fly. In the research stations application of fertilizers and other cultural practices like ploughing, weeding, irrigation, pruning etc. were done as per recommendation of BARI.

Observation, data collection and record keeping

The selected trees were visited frequently at different stages of growth, flowering and fruiting. Passport information and morphological data in respect of plant, leaf, flower/inflorescence,

Figure 2. Surjapuri (more than 200 years old tree).

fruit and stone characters were recorded following IPGRI descriptors for mango (IPGRI, 2006). The photographs of the specific trait considered to be helpful for identification of the variety/cultivar were taken from each genotype at appropriate time of traits to compare the distinctness among them. Data related to distinctness in morphological traits were photographed on each of the 19 mango genotypes.

Studies on molecular characterization

Extraction of genomic DNA

Young and mature leaf samples were collected from the particular plants which were used for morphological characterization at the particular geographical locations and the research station from where the variety was released. The genomic DNA was isolated from leaf tissues following a standard protocol described by Uddin et al. (2014) with minor modifications. Essentially, the extraction buffer-1 composition was [0.4 M glucose, 20 mM ethylenediamine tetra acetic acid (EDTA; pH 8.0), 3% (w/v) polyvinyl pyrrolidone (PVP)-40 (molecular weight 40,000) and 0.2% (v/v) *β*-mercaptoethanol]. Preheated (65°C) solution-2 [2% cetyl trimethyl ammonium bromide (CTAB) (w/v), 100 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, and 0.15% (v/v) *β*- Mercaptoethanol] was added as extraction buffer-2, 0.15% (v/v) *β*-mercaptoethanol was added and the mixture was mixed gently and incubated at 65°C in a water bath for 1 h with intermittent shaking. DNA was precipitated with ice-cold and extra pure isopropyl alcohol and purification with absolute ethanol (Plus sodium acetate, 3 M) and 70% ethanol chronologically. DNA sample of each mango germplasm was dissolved in 50 µl of TE buffer. When the DNA pellet was totally dissolved in TE buffer, 4 µl RNase (10 mg/ml) was added to isolated DNA and incubated at 37°C for 1.5 h. Finally, DNA sample was stored at -20°C.

Quantification and optimization of DNA concentration

The presence of genomic DNA was confirmed on 1% agarose gel qualitatively. The gels were visualized under UV light and photographed using photo documentation system (UV Transilluminator, Uvitec, UK). All of the DNA samples were found to be in good quality in this study. The amount of genomic DNA was quantified using UV a spectrophotometer (Spectronic® GENESYS™ 10 Bio) at 260 nm. Using the absorbance reading obtained for DNA sample of each mango genotypes, the original DNA concentrations were determined.

Selection of microsatellite primers

Twenty five SSR primer pairs described previously in the literature (Schnell et al., 2005, Duval et al., 2005, Viruel et al., 2005, Kittipat, 2007; Wahdan et al., 2011) were used for microsatellite analysis in the present study. Among the 25 primers pairs, 21 except MiSHRS-18, mMiCIR022, mMiCIR029 and mMiCIR020 (Table 2) showed better responsiveness with clear and expected amplified product sizes.

PCR standardization and amplification

Microsatellites amplification was performed in 10-µL volume containing 5X Green GoTaq® Reaction Buffer (Promega, USA) 15 mM $MgCl_2$, 1.25 U *Taq* DNA polymerase (Thermo Scientific, USA), 0.4 mM each of the dNTPs (NEB, USA), 10 µM forward and reverse primers and 50 ng template DNA. The mixtures were prepared at 0°C and transferred to the thermal cycler. Amplification reactions of SSR loci were carried out in a Mastercycler® nexus Gradient thermal cycler (Eppendorf, Germany). The PCR profile included initial denaturation for 4 min at 94°C, followed by 32 cycles of denaturation at 94°C for 45 s, annealing at 46 to 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 8 min. After completion of cycling program, reactions were held at 10°C. For checking amplification, the amplified products were resolved using on 2% agarose gel containing Ethidium bromide in electrophoresis chamber. If the primer was shown good band resolution intensity, less smearing, amplifying the target genomic region of template DNA, the PCR protocol was considered to be correct.

Electrophoretic separation and visualization of PCR products

After standardization of PCR, amplified products of 19 mango genotypes against each primer were electrophoresed on a 5% denaturing polyacrylamide gel containing 19:1 acrylamide: bis-acrylamide, 10X TBE buffer, 10% APS and ultrapure Temed. Electrophoresis was done using the Triple Wide Mini-Vertical Electrophoresis System, MGV-202-33 (CBS Scientific, USA). The gel is run at 80 to 90V and 20°C temperature maintained by a cooling system (Julabo, Germany) upon loading of PCR products for a specified period of time depending on the size of amplified DNA fragment (usually 1 h for 100 bp). After completion of electrophoresis, the gel was stained with Ethidium bromide and the individual bands were scored for analysis.

Scoring and analysis of microsatellite data

SSR markers were scored as codominant, so homozygous and heterozygous genotypes could be distinguished in individual plants. The bands representing particular alleles at the microsatellite loci were scored manually and designated the bands as A, B, C, etc. from the top to the bottom of the gel. The genotypes of different individuals were hypothetically scored as AA, BB, CC, etc. for homozygous or as AB, AC, BC etc. for heterozygous. A single genotypic data matrix was constructed for all loci. Statistics of genetic variation (number of observed and effective alleles, Nei's gene diversity, Shannon's information index, heterozygosity and polymorphic) were calculated using allelic frequency estimates obtained from genotypic frequencies of SSR loci using the

Table 2. List of microsatellite primers used in this study.

S/N	Locus	Forward primer	Reverse primer	Annealing temperature	Expected size (bp)	Reference
1	MiSHRS-18	AAACGAGGAAACAGAGCA C	CAAGTACCTGCTGCAACTAG	50	90-111	Schnell et al. (2005)
2	mMiCIR014	GAGGA CATAAAGATGGTG	GACAAGATAAACAAC TGGAA	51	190-196	Duval et al. (2005)
3	mMiCIR018	CCTCAATCTCACTCAACA	ACCCCACAATCAAACTAC	51	216-244	Duval et al. (2005)
4	mMiCIR022	TGTCTACCATCAAGTTCG	GCTGTTGTTGCTTTACTG	51	148-190	Duval et al. (2005)
5	mMiCIR029	GCGTGTCAATCTAGTGG	GCTTTGGTAAAAGGATAAG	51	190-196	Duval et al. (2005)
6	mMiCIR032	TCATTGCTGTCCCTTTTC	ATCGCTCAAACAATCC	51	176–204	Duval et al. (2005)
7	MiSHRS-1	TAACAGCTTTGCTTGCCTCC	TCCGCCGATAAACATCAGACA	50	191-207	Schnell et al. (2005)
8	MiSHRS-4	CCACGAATATCAACTGCTGCC	TCTGACACTGCTCTTCCACC	57	121-131	Schnell et al. (2005)
9	MiSHRS-32	TTGATGCAACTTTCTGCC	ATGTGATTGTTAGAATGAACTT	53	200-224	Schnell et al. (2005)
10	MiSHRS-48	TTTACCAAGCTAGGGTCA	CACTCTTAAACTATTCAACCA	57	201-226	Schnell et al. (2005)
11	MIAC-4	CGTCATCCTTTACAGCGAACT	CATCTTTGATCATCCGAAAC	51	93-112	Kittipat (2007) and Wahdan et al. (2011)
12	MIAC-6	CGCTCTGTGAGAATCAAATGGT	GGACTCTTATTAGCCAATGGGAG	51	270-307	Kittipat (2007) and Wahdan et al. (2011)
13	MiSHRS-29	CAACTTGGCAACATAGAC	ATACAGGAATCCAGCTTC	46	174-182	Schnell et al. (2005)
14	MiSHRS-37	CTCGCATTTCTCGCAGTC	TCCCTCCATTTAACCCTCC	46	127-132	Schnell et al. (2005)
15	MiSHRS-39	GAACGAGAAATC GGGAAC	GCAGCCATTGAATACAGA G	53	348-369	Schnell et al. (2005)
16	mMiCIR009	AAAGATAAG ATTGGGAAGAG	CGTAAGAAGAGCAAAGGT	51	156-170	Duval et al. (2005)
17	mMiCIR020	GACTTGCAGTTTCCTTTT	TCAAGAACCCCATTTG	51	148-176	Duval et al. (2005)
18	mMiCIR025	ATCCCCAGTAGCTTTGT	TGAGAG TTGGCAGTGTT	51	210-244	Duval et al. (2005)
19	mMiCIR030	GCTCTTTCCTTGACCTT	TCAAAATCGTGTCATTTC	51	174-194	Duval et al. (2005)
20	MIAC-3	TAAGCTAAAAAG GTTATAG	CCATAGGTGAATGTAGAGAG	51	185-193	Kittipat (2007) and Wahdan et al. (2011)
21	MIAC-5	AATTATCCTATCCCTCGTATC	AGAAACATGATG TGAACC	51	117-124	Kittipat (2007) and Wahdan et al. (2011)
22	MIAC-11	GTGCGAGGAGAT ATCTGT	CTGGTTCTTCATTGTTGAGATG	53		Kittipat (2007) and Wahdan et al. (2011)
23	LMMA1	ATGGAGACTAGAATGTACAGAG	ATTAAATCTCGTCCACAAGT	55	202	Viruel et al., 2005
24	LMMA7	ATTTAACTCTTCAACTTTCAAC	AGATTTAGTTTTGATTATGGAG	55	212	Viruel et al., 2005
25	LMMA9	TTGCAACTGATAACAAATATAG	TTCACATGACAGATATACACTT	55	185	Viruel et al., 2005

computer program POPGENE (Version 1.31) (Yeh et al., 1999). In addition, Chi-square test (1:2:1) for Hardy-Weinberg equilibrium for each population was obtained for SSR alleles using this programme. The microsatellite data matrix was used to calculate Nei's distance (Nei, 1972), and to generate the corresponding matrix of genetic distance estimates among accessions and cluster analyses were performed on the genetic distance matrix by using UPGMA method to determine the relationships among accessions (dendrograms) using POPGENE (Version 1.31) (Yeh et al., 1999). The polymorphism information content (PIC) of the SSR used or gene diversity value was calculated as PIC= 1- Σf^2_{ij}; where fij is the frequency of the ith allele for the jth SSR locus (Anderson et al., 1993). PIC values provided an estimate of the discriminatory power of any locus by considering the number of alleles per locus and the relative frequencies of those alleles in the population. The software DNA FRAG version 3.03 was used to estimate allelic length (Nash, 1991).

RESULTS AND DISCUSSION

Historical background of GIs

During visit to Shibganj upazila under the district

of Chapainawabganj, Bangladesh, scientists located some very old trees of Fazli (>150 years) and took photographs as evidence. Mr. Md. Emajuddin is the owner of this tree. As the statement of the local people this cultivar was first collected from an old lady Fazli Bibi residing in this upazila. An English Collectorate, Ravenosh has given the name of the cultivar in honour of Fazli Bibi. Similarly, Md. Fariduddin, a man of 80 years old said that he has been seeing Ashwina variety in Bangladesh since his childhood. He also said that he has been informed about this variety from his grandfather late Md Abdul Motaleb. The variety is extremely late and has been commercially cultivating in Chapainawabganj and neighbouring districts of Bangladesh from more than 150 years. Khirsapat mango of Rajshahi region (Northern region of Bangladesh) bears some special quality in respect of taste and flavour, which it might gain from that geographical location. Since fruit pulp of this variety resembles Khirsa (Bengali terminology) hence it is called Khirsa variety of mango. Md. Fariduddin, a man of 80 years old said that he has been seeing this variety in Bangladesh since his childhood. He also said that he has informed about this variety from his grandfather late Md Abdul Motaleb. Scientists located a Langra mango tree having 32 m × 29 m canopy coverage at Namo Chakpara village under Shibganj upazila of Chapainawabganj district. The local people said that the tree is more than 150 years old. Langra mangoes of Chapainawabganj district especially of Shibganj have some special quality in respect of taste and flavour, which it might gain from that geographical location. The name of this variety was given according to a lame man (*synonym* Langra in Bangla). Md. Fariduddin, a man of 70 years old said that he has been informed about this variety from his grandfather and it has been cultivated in Chapainawabganj from generation to generation. Gopalbhog is a well-known mango variety to everybody and has been commercially cultivated in Chapainawabganj and neighbouring districts. Laxmanbhog (BARI Aam-2) is also a popular mango cultivar commercially cultivated in greater Rajshahi districts but in limited scale all over the country. As per opinion of local people including aged ones, they have been noticed the trees of the cultivar in this area since their childhood. Very old trees located in Chapainawabganj area also indicated its presence in this region from more than 100 years. The cultivar was released as BARI Aam-2 by BARI in 1996 from RHRS, Chapainawabganj, As per description of local people one earthenware vessel maker (Kumar) of Unchubalua village under Mithapukur upazilla of Rangpur district planted a seed of Maldia mango near the garbage of broken earthenware vessel (in Bangla which is termed as ‘haribhanga’) more than 100 years back. According to the statement it can be assumed that the cultivar was originated from chance seedling. Because of excellent taste, the variety got popularity, and people of nearby villages started collecting grafts of the variety, and spontaneously it was named as ‘Haribhanga’ according to its location. After the division of Indian sub-continent, that Kumar migrated to India, and one slaughter named Suhrab Kosai purchased his house. Late Nofol Uddin Paiker planted one graft of the variety in his homestead about 65 years ago, which is the oldest tree of the cultivar. The variety spread in other areas of the upazilla from this plant. Md. Abdul Mannan (71 years old villager) described the history of ‘Haribhanga mango’, which was supported by other villagers. It is evident from a very old tree that the Surjapuri cultivar evolved more than 200 years ago. Md. Nurul Islam son of late Sharif Uddin, Village- Harinmari Nayapara, Union- Harinmari, Upazila- Baliadangi, District- Thakurgaon is the owner of the tree. His fore father Kontu Mohammad (grandfather of his father) planted it. As per opinion of the local people and DAE officials, the variety was evolved from natural cross pollination, and is being cultivated in Baliadangi and Ranishankail Upazilas since several centuries back.

Historical background of released variety and parental line

BARI Aam-1

The origin of this variety is in Chapainawabganj Sadar. It is clear that this variety evolved from chance seedling more than 100 years ago. Its old name is Satiar Kara. Bangladesh Agricultural Research Institute registered this variety as BARI Aam-1 in 1996 from National Seed Board of Bangladesh after a long-time evaluation at Regional Horticulture Research Station, Chapainawabganj. The variety possesses countrywide adaptability, and high export potentiality.

BARI Aam-3

The origin of this variety is in India. It is an Indian hybrid which is known as Amrapali. Bangladesh Agricultural Research Institute registered the variety for cultivation as BARI Aam-3 in 1996 from National Seed Board of Bangladesh after a long time evaluation at Regional Horticulture Research Station, Chapainawabganj and Fruit Research Station, Binodpur, Rajshahi. The variety is commercially cultivated all over the country.

BARI Aam-4

This is the only hybrid variety of Bangladesh. Scientist of RHRS, BARI, Chapainawabganj developed this variety in 1993 from a crossing between Ashwina (a commercial cultivar) and M-3896 (a Florida line). After evaluation, the hybrid was registered as BARI Aam-4 in 2003 from National Seed Board of Bangladesh. It is an outstanding variety. The variety possesses country wide adaptability, and at present commercially cultivated all over the

country. It is a late variety.

BARI Aam-5

The variety is developed from a chance seedling found in the office premises of Regional Agricultural Research Station, Jessore. After evaluation the hybrid was registered as BARI Aam-5 in 2010 from National Seed Board of Bangladesh. It is an early variety. The original mother tree of the variety is about 40 years old, and is still alive. It is an early coloured variety with less juice in the pulp and less sweetness having high export potentiality.

BARI Aam-6

The origin of this variety is in Chapainawabganj, Bangladesh. It was evolved from chance seedling about 100 years ago. Locally the cultivar is known as 'Bou Bholani'. Bangladesh Agricultural Research Institute organized a National Mango Show at RHRS, Chapainawabganj in 1993. 'Bou Bholani' won first prize in 'Other' group. RHRS scientists collected the cultivar, and evaluate for more than 15 years. Then it is registered as BARI Aam-6 in 2010 from National Seed Board of Bangladesh. Cultivation of this variety is limited at Chapainawabganj.

BARI Aam-7

The origin of this variety is in Chapainawabganj, Bangladesh. It was evolved from chance seedling 20 years ago. Bangladesh Agricultural Research Institute registered this variety as BARI Aam-7 in 2010 from National Seed Board of Bangladesh. Cultivation of this variety is limited at Chapainawabganj. It is a coloured variety.

BARI Aam-8

The origin of this variety is in Myanmar. It is a Burmese poly-embryonic variety, which is known as Ranguaichi. Bangladesh Agricultural Research Institute registered the variety for cultivation as BARI Aam-8 in 2010 from National Seed Board of Bangladesh after a long time evaluation at different Regions of the country (Chapainawabganj, Jessore, Chittagong, Rangamati Hill district, Khagrachari Hill district). The variety is commercially cultivated in hilly areas of the country. As it has wider adaptability, it can be grown commercially all over the country.

BARI Aam-9

The origin of this variety is in Chapainawabganj, Bangladesh. It was evolved from chance seedling more 100 years ago. Locally the cultivar is called Tikkafaras. Bangladesh Agricultural Research Institute registered this variety as BARI Aam-9 in 2011 from National Seed Board of Bangladesh after a long time evaluation at the Regional Horticulture Research Station, Chapainawabganj. Cultivation of this variety is limited at Chapainawabganj. It is sweet at green stage (Kanchamitha).

M-3896

The origin of this line is in Florida, USA. It was introduced to Bangladesh during the 1980s with the financial and technical support of FAO Mango Improvement Project. It is a coloured variety and showed very good performance in respect of yield and quality under Bangladesh condition. Because of high incidence of diseases, the line was not registered for cultivation in the country, and exploited as gene donor. It is the male parent of BARI Aam-4.

Studies on morphological traits

Nineteen varieties/cultivars were used for DUS (Distinctness, Uniformity and Stability) experiment to study the morphological traits and attempt to distinguish one from the other individual on the basis of traits as per IBPGR descriptor. According to the descriptor a total of 102 descriptor traits (15 plants, 20 leaf, 22 flower, 33 fruit, 10 stone and 5 seed descriptor) was used to characterize 19 mango genotypes. Nineteen are important among 102 traits for distinctness of the studied genotypes (Table 3). Among 19 mango genotypes, eight were distinct by two traits and 11 by only single character. The genotypes with respective distinct traits are given in Table 4. Fazli had the maximum fruit weight (780 g) and weak stalk attachment and these two traits distinguish this genotype from the others. Fruiting duration is an important trait which indicates the availability of this genotype. Considering this trait Ashwina was extremely late and its fruiting duration was recorded end of July to end of August which was an identifying character of this cultivar. Similarly, slightly prominent fruit beak discriminated Laxambough from all the genotypes. Pentamerous type flower was found in 16 genotypes while Haribhanga produced both pentamerous and tetramerous type flower which was considered as a unique trait for this cultivar identification. Fruit skin colour of ripe fruit is an important character and three genotypes viz. Surjapuri, BARI Aam-7 and M-3896 showed green with red blush, yellow with red blush and reddish yellow, respectively in ripening stage and these types of skin colour were distinct among the other 14 genotypes. Slightly prominent fruit sinus was a distinguishable character for M-3896. Considering colour of young leaf all the studied genotypes were

Table 3. Some distinct qualitative traits of 19 mango genotypes.

Character	Fazli	Ashwina	Khirshapat	Langra	Gopalbough	Laxambough	Haribhanga	Surjapuri	Gourmoti	Ranipasanda
Leaf blade shape	Oblong	Oblong	Elliptic	Lanceolate	Elliptic	Oblong	Lanceolate	Elliptic	Lanceolate	Lanceolate
Leaf attitude in relation to branch	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect
Colour of young leaf (recorded on 5-10 days old leaf)	Reddish brown	Light brick red	Light brick red	Light green with brownish tinge	Reddish brown	Light brick red	Light brick red	Light green with brownish tinge	Light brick red	Reddish brown
Type of flower	PM	PM	PM	PM	PM	PM	Both PM and TT	PM	PM	PM
Fruiting duration	Mid-July to mid-August	End of July to end of August	Early June end of June	Mid June to mid-July	End of May to mid-June	Mid June to end of July	Mid June to end of July	End of June to end of July	Mid-August to mid-September	Mid June to end June
Fruit length (cm)	15.5	13.8	9.4	9.9	9.3	10.1	9.2	8.18	10.1	6.5
Fruit weight (g)	780	610	340	350	255	263	196	121	435	125
Fruit shape	Elliptic	Elliptic	Roundish	Elliptic	Oblong	Elliptic	Obovoid	Obovoid	Elliptic	Oblong
Fruit stalk attachment	Weak	Strong	Strong	Medium	Intermediate	Intermediate	Strong	Intermediate	Intermediate	Strong
Fruit neck prominence	Absent	Absent	Absent	Absent	Slightly prominent	Intermediate	Absent	Absent	Absent	Absent
Slope of ventral shoulder	Ending in a long curve	Ending in a long curve	Slopping abruptly	Ending in a long curve	Slopping abruptly	Slopping abruptly	Rising and then rounded	Rising the rounded	Slopping abruptly	Rising and then rounded
Fruit beak type	Perceptible	Perceptible	Perceptible	Perceptible	Perceptible	Slightly prominent	Pointed	Pointed	Pointed	Perceptible
Fruit stalk insertion	Vertical	Vertical	Vertical	Vertical	Vertical	Perceptible	Oblique	Vertical	Oblique	Vertical
Fruit sinus type	Absent	Absent	Absent	Absent	Shallow	Vertical	Shallow	Absent	Shallow	Absent
Skin colour of ripe fruit	Greenish yellow	Green	Yellowish green	Yellowish green	Yellowish green	Yellow	Yellowish green	Green with red blush	Yellowish green	Greenish yellow
Pulp colour of ripe fruit	Golden yellow	Golden yellow	Yellow orange	Golden yellow	Golden yellow	Golden yellow	Orange	Yellow	Orange	Yellow
Stone length (cm)	12.5	11.1	7.7	8.2	7.1	8.0	7.35	5.5	7.9	5.4
Type of embryo	ME	ME	ME	ME	ME	ME	ME	ME	ME	ME
Pulp TSS (%)	21	19	24	22	23	17	22	-	23	21

Character	M-3896	BARI Aam-1	BARI Aam -3	BARI Aam -4	BARI Aam -5	BARI Aam -6	BARI Aam -7	BARI Aam-8	BARI Aam-9
Leaf blade shape	Elliptic	Oblong	Oblong	Oblong	Lanceolate	Elliptic	Elliptic	Oblong	Elliptic
Leaf attitude in relation to branch	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Horizontal	Semi-erect	Semi-erect	Semi-drooping	Semi-erect
Colour of young leaf (recorded on 5-10 days old leaf)	Tinge	Green with red patches	Reddish brown	Light brick red	Light brick red	Light Brick red	Light brick red	Reddish brown	Reddish brown
Type of flower	PM	PM	PM	PM	PM	PM	PM	PM	PM
Fruiting duration	March to June	March to June	March to July	March to July	June	March to June	March to August	March to July	March to May
Fruit length (cm)	10.2	7.9	10.5	9.1	9.0	9.9	9.4	10.7	12.5
Fruit weight (g)	300	205	215	648	233	280	290	226	166

Table 3. Contd.

Fruit shape	Roundish	Roundish	Oblong	Roundish	Obovoid	Oblong	Roundish	Oblong	Elliptic
Fruit stalk attachment	Intermediate	Strong	Intermediate	Strong	Strong	Strong	Medium	Intermediate	Intermediate
Fruit neck prominence	Absent	Absent	Intermediate	Strong	Absent	Absent	Medium	Slightly prominent	Intermediate
Slope of ventral shoulder	Slopping abruptly	Ending in a long curve	Rising and then rounded	Slopping abruptly	Rising and then rounded	Ending in a long curve	Rising and then rounded	Ending in a long curve	Ending in a long curve
Fruit beak type	Perceptible	Perceptible	Perceptible	Absent	Perceptible	Perceptible	Absent	Prominent	Absent
Fruit stalk insertion	Vertical	Vertical	Oblique	Perceptible	Vertical	Vertical	Absent	Oblique	Vertical
Fruit sinus type	Slightly prominent	Absent	Shallow	Vertical	Shallow	Absent	Vertical	Shallow	Absent
Skin colour of ripe fruit	Reddish yellow	Yellow	Greenish yellow	Yellowish green	Yellow	Yellowish green	Yellow with red blush	Greenish yellow	Green
Pulp colour of ripe fruit	Yellow	Golden yellow	Dark orange	Light orange	Yellow	Yellowish orange	Yellow	Orange	White
Stone length (cm)	7.8	5.6	-	7.8	7.95	7.9	7.5	9.1	10.5
Type of embryo	ME	ME	ME	ME	ME	ME	ME	PE	ME
Pulp TSS (%)	23	22	26	24	19	22	21	21	11.2

ME: Monoembryony; PE: Polyembryony; PM: Pentamerous; TT: Tetramerous.

classified into five groups and among these three were comprised of more than one genotype. As for instance, five genotypes (Fazli, Gopalbough, BARI Aam-3, 8 and 9) exhibited reddish brown, eight genotypes (Ashwina, Khirshapat, Laxmanbhog, Haribhanga, BARI Aam-4, 5, 6 and 7) light brick red, two genotypes (Langra and Surjapuri) light green with brownish tinge while the rest two groups contained only one genotype where green with red patches and tinge colour young leaf was observed in BARI Aam-1 and M-3896, respectively which was dissimilar with another one. Again, leaf attitude in relation to branch separated another two genotypes. All the genotypes except BARI Aam-5 and 8 exposed semi-erect leaf attitudes in relation to branch whereas two unique traits, that is, horizontal and semi-drooping attitude were recorded in respect of these two genotypes. In addition, poly-embryonic type has given to BARI Aam-8 for its discrepancy in comparison to another one. BARI Aam-9 showed green pulp colour in ripe fruit, which was divergent from the other genotypes. However, sometimes single character is not sufficient to differentiate one genotype. Therefore, it is effective to combine more than one character to distinguish completely one genotype from others. Such as, light green with brownish tinge colour was found in young leaf of Khirshapat and Surjapuri. When another trait viz. fruit shape or pulp colour of ripe fruit was considered with young leaf colour then this combination of two traits could easily distinguish Khirshapat from Surjapuri. Similarly, fruit shape and pulp colour of ripe fruit showed distinctness for BARI Aam-6. On the other hand, perceptible fruit stalk was observed in BARI Aam-3 and Laxmanbhog, while oblique type was common in BARI Aam-4 and Haribhanga. Hence, BARI Aam-3 and 4 could be identified from other genotypes when another trait like fruit beak type combined with fruit stalk. Again, colour of young leaf and fruit shape showed distinctness for Langra whereas Gopalbhog was unique for fruit neck prominence and fruit beak type. Gourmoti showed distinctness from the other genotypes based on fruit shape and pulp colour of ripe fruit whereas skin and pulp colour of ripe fruit showed uniqueness for Ranipasand (Figure 3).

Studies on molecular traits

Twenty five (25) SSR primers were used for generating banding profile. Out of which 21 primers (Table 2) were selected in the analysis for their reproducible and polymorphic DNA amplification patterns among genotypes. Two typical SSR profiles are shown in Figure 4. Analysis of the variability parameters for the 21 SSRs in the 19 mango genotypes are shown in Table 5. A total of 80 alleles with an average number of 3.81 alleles per locus were found in the present study. The number of alleles detected

Table 4. Distinctness of mango genotypes based on single and two qualitative traits.

	Varieties distinct from 17	In respect of traits	No. of genotypes
Distinction of genotypes through single trait	Fazli	Fruit weight or Fruit stalk attachment	11
	Ashwina	Fruiting duration	
	Laxmanbhog	Fruit beak type	
	Haribhanga	Type of flower	
	Surjapuri	Skin colour of ripe fruit	
	BARI Aam-1	Colour of young leaf	
	BARI Aam-5	Leaf attitude in relation to branch	
	BARI Aam-7	Skin colour of ripe fruit	
	BARI Aam-8	Type of embryo or Fruit beak type or Leaf attitude in relation to branch	
	BARI Aam-9	Pulp colour of ripe fruit	
	M-3896	Colour of young leaf or Fruit sinus type or Skin colour of ripe fruit	
Distinction of genotypes through double traits	Khirshapat	Fruit shape and Pulp colour of ripe fruit	08
	Langra	Colour of young leaf and Fruit shape	
	Gopalbhog	Fruit neck prominence and Fruit beak type	
	Gourmoti	Fruit shape and Pulp colour of ripe fruit	
	Ranipasanda	Skin and Pulp colour of ripe fruit	
	BARI Aam-3	Fruit stalk insertion and Fruit beak type	
	BARI Aam-4 (Hybrid)	Fruit stalk insertion and Fruit beak type	
	BARI Aam-6	Fruit shape and Pulp colour of ripe fruit	

varied form 2 (‘MiSHRS-39’ and ‘MIAC-11’) to 6 (‘MIAC-6’ and ‘MIAC-11’). The allele size ranged from 99 (MIAC-4) to 366 bp (MiSHRS-39). Earlier, Schnell et al. (2005) and Wahdan et al. (2011) reported similar values of SSR polymorphism, number of alleles and allele size in mango cultivars. In the present study, most of the SSR primers detected multiple loci, which can be attributed to the allopolyploid nature of mango (Mukherjee, 1950).

The PIC value provides an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at a locus but also the relative frequencies of these alleles (Huda et al., 2019). All studied SSRs were polymorphic among mango genotypes and informative for describing their genotypic variation (that is, PIC values different from zero). PIC values ranged from 0.349 to 0.781 (Table 5), with a mean PIC of 0.602. Thirteen of these SSRs were very informative (PIC>0.6), with the highest PIC value recorded for mMiCIR014 (0.781) and followed by mMiCIR032 (0.776), MIAC-5 (0.731) and LMMA9 (0.691) which were higher than the average PIC value reported by Wahdan et al. (2011) and Kumar et al. (2013). High PIC values were observed might be due to use of di-nucleotide repeats and also due to genotypic differences (Molla el al., 2010). Indeed, the very informative markers are extremely useful for genetic studies and determination of the level of polymorphism on a specific marker locus (Sundaram et al., 2007).

According to the banding patterns obtained with 21 selected primer pairs, one or two bands were present in each genotype; the amplification pattern seems to indicate the detection of a single locus. Mango has been described as allopolyploid (Mukherjee, 1997) and these results suggest a complete depolarization in this species. The genotypes studied were considered homozygous and heterozygous when one or two fragments were present per locus, respectively (Callen et al., 1993). Consequently, the average observed heterozygosity in each SSR locus considering all studied genotypes were 0.584. Similarly, higher level of heterozygosity (0.587) also found in 19 genotypes considering all SSR locus under study (Table 5) and the 17 out of 19 genotypes showed heterozygosity higher than 0.50 (Table 6). The great heterozygosity can be attributed to the mating system of this species that is normally out cross pollination with some self-pollination. The higher level of heterozygosity observed in the present study has also been reported by Shiran et al. (2007) and Wahdan et al. (2011), probably due to the greater diversity of genotypes used in the present study. For the 19 mango genotypes, a total of 80 alleles were detected using the 21 loci, ranging from 25 for genotype ‘BARI Aam-5’ to 37 for ‘Haribhanga’ (Table 6). The average values for observed number of alleles per genotype were 1.540. The analysis of allelic pattern showed that the number of polymorphic loci within accessions ranged from 4 (19.05) in BARI Aam-5 to 16 (76.19) in Haribhanga and Gopalbhog. The mean Shannon’s information index (I) was 0.409, and ranged from 0.132 in BARI Aam-5 to 0.555 in Gopalbhog (Table 6).

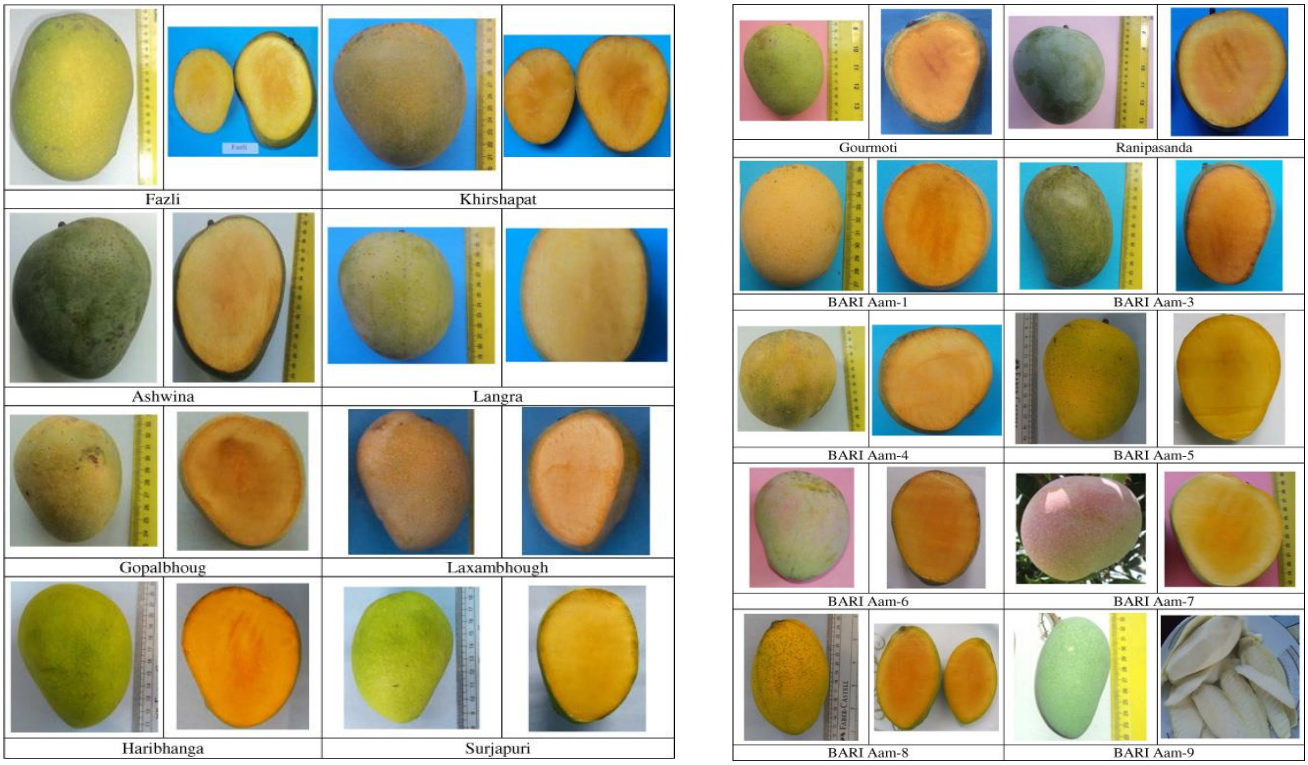

Figure 3. Differences among mango genotypes of Bangladesh in respect of fruit descriptor.

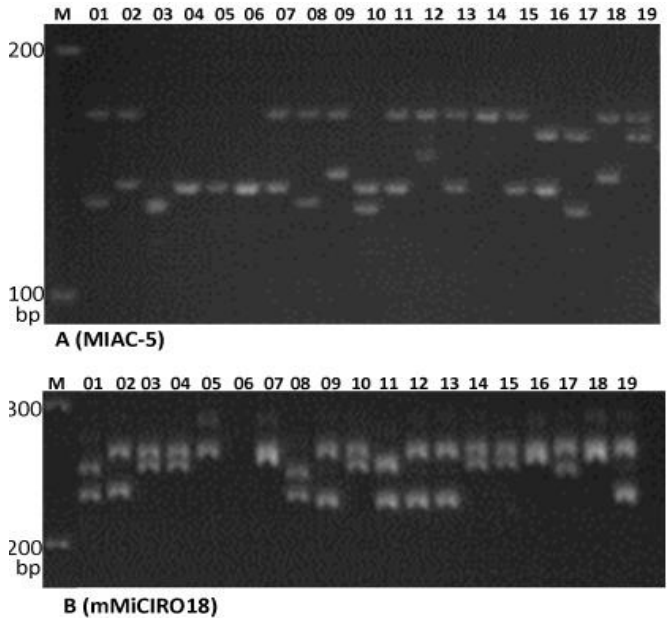

Figure 4. Microsatellite profiles of 19 mango genotypes at locus MIAC-5 (A) and mMiCIRO18 (B); M: Molecular wt. marker (100 bp DNA ladder); Lane 01, BARI Aam-1; Lane 02, Laxambough, Lane 03, BARI Aam-3; Lane 04, BARI Aam-4; Lane 05, BARI Aam-5; Lane 06, BARI Aam-6, Lane 07, BARI Aam-7; Lane 08, BARI Aam-8; Lane 09, BARI Aam-9; Lane 10, M-3896; Lane 11, Haribhanga; Lane 12, Surjapuri; Lane 13, Fazli; Lane 14, Gourmoti; Lane 15, Ashwina; Lane 16, Khirsapat; Lane 17, Gopalbhog; Lane 18, Langra; Lane 19, Ranipasanda.

Table 5. Variability of simple sequence repeat marker used for mango genotypes genetic analysis.

Locus	No. of allele	Allele sizes (bp)	Major allele frequency	Observed heterozygosity	Expected heterozygosity	PIC
mMiCIR014	5	154, 159, 163, 168, 174	0.290	0.737	0.802	0.781
mMiCIR018	5	215, 226, 234, 246, 256	0.528	0.778	0.679	0.660
mMiCIR032	5	158, 180, 189, 198, 204	0.290	0.684	0.797	0.776
MiSHRS-1	5	192, 200, 204, 211, 228	0.421	0.579	0.679	0.661
MiSHRS-4	3	131, 134, 139	0.684	0.158	0.494	0.481
MiSHRS-32	4	198, 209, 217, 224	0.684	0.579	0.485	0.472
MiSHRS-48	4	207, 214, 230, 240	0.528	0.278	0.649	0.631
MIAC-4	3	99, 105, 110	0.447	0.737	0.647	0.630
MIAC-6	6	244, 258, 271, 284, 298,312	0.472	0.667	0.725	0.705
MiSHRS-29	3	174, 179, 187	0.588	0.118	0.585	0.568
MiSHRS-37	3	138, 142, 146	0.588	0.647	0.522	0.507
MiSHRS-39	2	337, 366	0.588	0.000	0.499	0.484
mMiCIR009	3	158, 165, 179	0.444	0.722	0.652	0.634
mMiCIR025	3	214, 231, 254	0.474	0.947	0.649	0.632
mMiCIR030	4	183, 195, 202, 217	0.447	0.790	0.690	0.349
MIAC-3	3	183, 199, 209	0.658	0.368	0.479	0.467
MIAC-5	6	133, 137, 143, 150, 157, 167	0.342	0.737	0.751	0.731
MIAC-11	2	118, 125	0.526	0.842	0.512	0.499
LMMA1	4	201, 207, 219, 230	0.474	0.579	0.693	0.675
LMMA7	3	212, 220, 230	0.500	0.632	0.625	0.608
LMMA9	4	184, 190, 195, 201	0.421	0.684	0.710	0.691
Mean	**3.81**	**-**	**0.495**	**0.584**	**0.636**	**0.602**

** Nei's (1973) expected heterozygosity.

Table 6. Statistic of genetic variation for 19 mango genotypes as measured by 21 SSR loci.

Plant designation	Ta	Na	I	Obs_Ho	Obs_He	Nei	NPL	% PL
BARI Aam-1	32	1.524	0.363	0.524	0.524	0.262	11	52.38
BARI Aam-2	32	1.524	0.363	0.476	0.524	0.262	11	52.38
BARI Aam-3	33	1.571	0.396	0.427	0.571	0.286	12	57.14
BARI Aam-4	33	1.571	0.396	0.427	0.571	0.286	12	57.14
BARI Aam-5	25	1.191	0.132	0.810	0.191	0.095	4	19.05
BARI Aam-6	32	1.650	0.451	0.350	0.650	0.325	13	61.9
BARI Aam-7	30	1.500	0.347	0.500	0.500	0.250	10	47.62
BARI Aam-8	34	1.619	0.429	0.381	0.619	0.310	13	61.9
BARI Aam-9	32	1.160	0.416	0.400	0.600	0.300	12	57.14
M-3896	34	1.169	0.429	0.381	0.619	0.310	13	61.9
Haribhanga	37	1.762	0.528	0.238	0.762	0.381	16	76.19
Surjapuri	33	1.650	0.451	0.350	0.650	0.325	13	61.9
Fazli	32	1.684	0.474	0.316	0.684	0.342	13	61.9
Gourmoti	30	1.476	0.330	0.524	0.476	0.238	10	47.62
Ashwina	31	1.550	0.381	0.450	0.550	0.275	11	52.38
Khirsapat	34	1.700	0.485	0.300	0.700	0.350	14	66.67
Gopalbhog	36	1.800	0.555	0.200	0.800	0.400	16	76.19
Langra	33	1.500	0.381	0.450	0.500	0.275	11	52.38
Ranipasanda	35	1.667	0.462	0.333	0.667	0.333	14	67.67
Mean	**32.53**	**1.540**	**0.409**	**0.412**	**0.587**	**0.295**	**12.053**	**57.45**

Ta: Total number of alleles detected with the 21 SSR for each genotypes, **Na**: Observed number of alleles, **I**: Shannon's information index, **Obs_Ho**: Observed homozygosity, **Obs_He**: Observed heterozygosity, **Nei**: Nei's (1973) expected heterozygosity, **NPL**: Number of polymorphic loci and **% PL**: Percentage of polymorphic loci.

Table 7. Fingerprinting key showing band pattern as generated using SSR marker profiles.

S/N	Genotype	Distinguishing primer with band position (bp)
1	BARI Aam-1	MMiCIR018 (234, 215), MMiCIR032 (180), MiSHRS-4 (134), MIAC-11 (125)
2	BARI Aam-2	MMiCIR014 (159)
3	BARI Aam-3	MMiCIR032 (204), MiSHRS-1 (211, 200), MIAC-6 (284, 244), MIAC-3 (199, 183), MIAC-5 (133)
4	BARI Aam-4	MMiCIR014 (154)
5	BARI Aam-5	LMMA7 (230)
6	BARI Aam-6	MMiCIR014 (174, 154), MMiCIR032 (206, 180)
7	BARI Aam-7	MiSHRS-1 (228, 211)
8	BARI Aam-8	MMiCIR018 (236, 226), MiSHRS-48 (214), MIAC-6 (312, 284), LMMA1 (201), LMMA9 (184)
9	BARI Aam-9	MMiCIR032 (198,180)
10	M-3896	MiSHRS-1 (211, 204), MiSHRS-32 (217, 198), mMCIR030 (195), MIAC-5 (137, 133), LMMA7 (230, 220), LMMA9 (195, 184)
11	Haribhanga	MMiCIR018 (246, 215), LMMA1 (219, 207)
12	Surjapuri	MIAC-5 (167, 150)
13	Fazli	MMiCIR014 (174, 163), MMiCIR018 (256, 215), MMiCIR032 (204, 189)
14	Gourmoti	MMiCIR014 (168, 154), MMiCIR032 (198, 189), MiSHRS-37 (142), MIAC-5 (167), LMMA1 (230, 201)
15	Ashwina	mMiCIR025 (231)
16	Khirsapat	MIAC-5 (157, 137)
17	Gopalbhog	MMiCIR018 (256, 234), MiSHRS-37 (146, 142), MIAC-5 (157, 133)
18	Langra	MMiCIR032 (204, 198), MIAC-6 (312, 298)
19	Ranipasanda	MiSHRS-1 (204), MIAC-4 (110, 99)

Allele sizing technologies are well established and can be readily used to size microsatellite alleles from any organism (Song et al., 1999). SSR genotypic data from a number of loci have the potential to provide unique allelic profiles or DNA fingerprints for precisely establishing genotypic identity. Distinguishing SSR marker band with their positions are shown in Table 7. The band patterns corresponding to individual genotype may help to recognize the genotype in question. When one primer would not distinguish individual variety from others, another primer should be considered and sometimes combination of more than one primer should be taken into account. Thus, additional primer or set of primers might be needed to test to identify all expected varieties. All the 19 mango genotypes were discriminated successfully by the 21 SSR markers. Among 80 alleles detected, 48 were specific to studied mango genotypes. From a total of 80 scorable alleles, 59 were polymorphic bands and 21 monomorphic. These results indicate that 48 out of the 80 (50%) fragments are considered putative genotypes specific markers for these genotypes. Most of the unique band patterns used for genotype identification was found at locus MIAC-11. Locus MIAC-11 alone discriminated seven genotypes (BARI Aam-3, M-3896, Surjapuri, Gourmoti, Khirsapat, Gopalbhog and Ranipasand). One specific allele was detected in the genotypes BARI Aam-1 (180 bp/mMiCIR032, 134 bp/MiSHRS-4, 125bp/MIAC-11), BARI Aam-2 (159 bp/mMiCIR014), BARI Aam-3 (204 bp/ mMiCIR032, 133 bp/MIAC-5), BARI Aam-4 (154 bp/mMiCIR014), BARI Aam-5 (230 bp/LMMA7), BARI Aam-8 (214 bp/MiSHRS-48, 201 bp/LMMA1, 184 bp/LMMA9), M-3896 (195 bp/mMCIR030), Gourmoti (138 bp/MiSHRS-37, 167 bp/MIAC-5), Ashwina (231 bp/mMiCIR025 and Khirsapat (207 bp/MiSHRS-48) (Table 7). Besides, BARI Aam-7, BARI Aam-9, Haribhanga, Surjapuri showed unique band with the primer MiSHRS-1 (228/211 bp), mMiCIR032 (198/180 bp), mMiCIR018 (246/215 bp), MIAC-5 (167/133 bp), respectively. Only two genotypes BARI Aam-6 and Fazli could be easily identified in combination of the primer mMiCIR014 and mMiCIR032, in which all primers showed heterozygous condition. Results of the present study represent one of the first attempts to find out a small set of microsatellite makers to discriminate mango genotypes of Bangladesh providing meaningful data that can be enlarged by additional mango genotypes and new microsatellite markers.

The analysis of molecular data showed different levels of genetic diversity among ten mango genotypes determined based on the Nei's (1972) genetic distance. The genotypes presented genetic distances between 0.260 and 1.557, which reflects the high genetic variability of the collection of mango genotypes studied (Figure 5). Based upon more recent genetic analysis involving microsatellite marker, it is now estimated that monoembryonic cultivar descended from polyembryonic cultivars (Schnell et al., 2005, Viruel et al., 2005). Mango accessions showed genetic differences based on geographical origins and their known history (Viruel et al., 2005, Pandit et al., 2007, Duval et al., 2009, Hirano et al.,

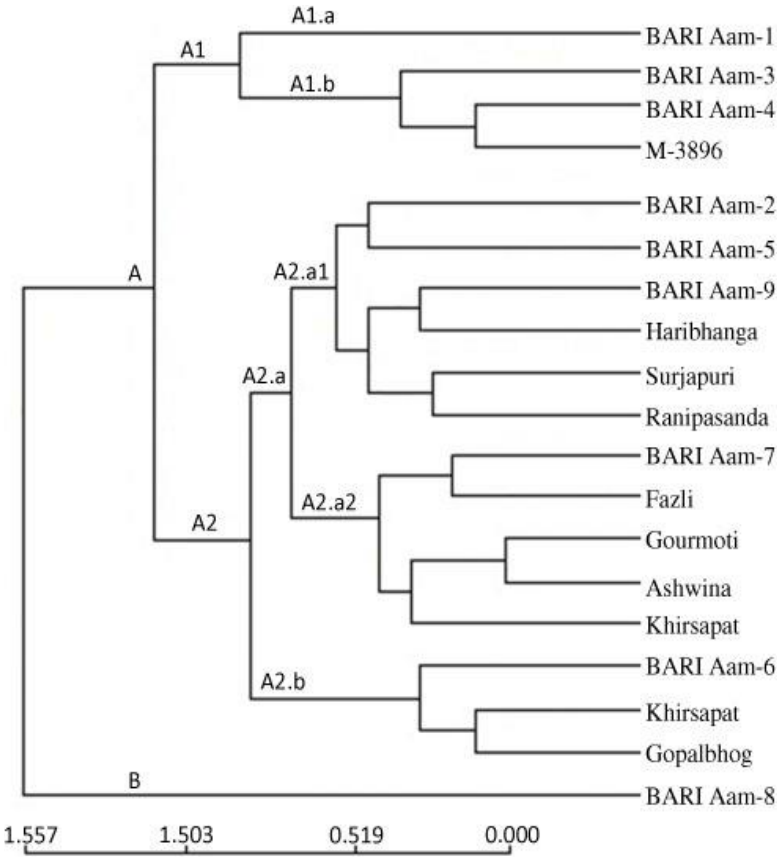

Figure 5. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation between 19 mango genotypes according to microsatellite analysis.

2010). On the whole, earlier studies clearly the differentiation of mango accessions regardless the marker system use to fingerprint based on type of embryo (mono- or poly-embryonic), geographical origins, or genetic status (cultivars, landraces, species). In this study, highest genetic distance value (1.557, 1.033, 1.261 and 1.149) were observed between the genotypes BARI Aam-8 vs BARI Aam-5, BARI Aam-2 and BARI Aam-1 (Figure 5). As in type of embryo BARI Aam-8 possess poly-embryonic and others are mono-embryonic. On the other hand, the minimal genetic distance (0.260) was observed between Gourmoti vs Ashwina, both two originated from similar location and popular as late variety.

The dendrogram generated from the unweighted pair group arithmetic average (UPGMA) cluster analysis broadly placed 19 mango genotypes into two major groups "A" and "B" in which only one poly-embryonic genotype namely BARI Aam-8 congregated in a distinct group "B" and other 18 mono-embryonic genotypes clustered in group "A" (Figure 5). Nevertheless, cluster "A" formed two sub-clusters "A1" and "A2". Sub-cluster "A1" subsequently separated into another two sub-clusters "A1.a", "A1.b" respectively. Similarly, based on their genetic distances different genotypes grouped into different sub-cluster. Upon subsequent separation, the highest genetic dissimilarity coefficient was observed between BARI Aam-5 vs BARI Aam-8 (GD=1.557) which is assembled in sub-cluster "A2.a1" and also formed a distinct group "B" respectively. Subgroup "A2.a2" gathered 5 genotypes in which genotypes Gourmoti and Ashwina comprised sharp similarity (GD = 0.260). The genotypes had a distinct status in the dendrogram, because there might have effect of morphological traits and geographical sources. As for instance, BARI Aam-8, BARI Aam-5, BARI Aam-2 and BARI Aam-1 are different based on their morphological features like, fruit shape, shape of fruit apex, fruit cavity, embryo type and so on (Hossain et al., 2014). Moreover, these varieties have been released from different location and also different year.

Conclusion

Historical background of geographical indications of

mango as described by aged people of their most concentrate areas of cultivation indicated that the cultivars have been originated naturally in those areas. The cultivars possess high commercial value and are being cultivated widely around their areas of origin. GIs possess some special character, which they might gain from their habitat. Characterization of mango genotypes on the basis of DNA fingerprinting data in combination with morphological traits has become an efficient tool to link phenotypic and genotypic variation. Morphological traits and SSRs have been shown to be highly efficient for genotype identification and provide a positive assessment to the ability of SSR marker to produce distinctive DNA profiles of 19 mango genotypes. The results of the present study could be applied as baseline information to maintain the appropriate identity and the construction of a database of all mango cultivars grown in Bangladesh and in broad sense, to protect the mango germplasm of Bangladesh.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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