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Molecular Characterization of Sixty Local Mango Germplasm of Chapainawabganj

Md. Mostafezur Rahman[1], Md. Golam Rabbani[1], Farid Ahmed[2*], Md. Zahurul Islam[3], Mohammad Joyel Sarkar[2]

[1]Department of Horticulture, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2]Horticulture Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh
[3]University of Rajshahi, Institute of Biological Science, Rajshahi, Bangladesh
Email: mostafizru910@gmail.com, drmgrabbani@gmail.com, *farid@bina.gov.bd, zahurul95@gmail.com, sarkarjewel11@gmail.com

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Abstract

The experiment was conducted at Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute (BARI) and the genotypes were collected from Chapainawabganj, the most mango variability rich district in Bangladesh. The molecular characters of mango germplasm were assessed by using six simple sequence repeat (SSR) markers. Polymerase chain reaction (PCR) amplification of the DNA isolated from 60 mango germplasm with 6 SSR primers was performed. The sizes of the alleles detected ranged from 112 to 221 bp. SSRs exhibited moderate values of polymorphic information content (PIC) range of 0.9405 to 0.6501. Genetic distances (D) between varieties were computed from combined data of the 6 primers, ranging from 0.5000 to 1.0000. Moderate degree of genetic diversity was obtained where the highest level of gene diversity value was noted 0.9433 in loci MIGA179 and the lowest level of gene diversity value was computed 0.6683 in loci MIGA253 with a mean diversity of 0.8842. The dendrogram generated from the unweighed pair group arithmetic average (UPGMA) cluster analysis broadly placed 60 mango cultivars into ten major clusters. The cluster size varied from 1 to 12 and cluster-VI was the largest cluster comprising of 9 cultivars. The tendency of clustering among mango cultivars revealed that they have strong affinity towards further breeding programme.

Keywords

Mango Germplasm, SSR, PCR, Gene Diversity, PIC

1. Introduction

Mango (*Mangifera indica* L.) is one of the most important fruit crops of the

Anacardiaceae family. It is widely accepted throughout the world for its excellent flavour, attractive colour and delicious taste. It is the most preferred fruit in Asia along with Bangladesh. It has been cultivated in different countries of the world from times immemorial. Records suggested that it has been in cultivation in the Indian subcontinent since 4000 years ago [1]. The fruit is believed to have originated in the Eastern India, Burma or in the Malayan region [2]. It has got a unique consumer preference due to its high nutritional value, pleasant taste, attractive appearance etc. among the 70 different kinds of fruits grown in Bangladesh [3]. In Bangladesh, Chapainawabganj, Rajshahi, Dinajpur, Satkhira and Kustia are the principal mango producing districts. Among the districts, Chapainawabganj has the highest variation of mango in terms of plant morphology and fruit shape, size and taste. According to BBS 2019 (2020), out of 1,219,450 metric tons from 95.30 thousand hectares of land about 197,080 metric tons of mango was produced in Chapainawabganj, the second most mango producing districts of Bangladesh. It is ranked the number one in terms of both area and production in the country [4].

Characterization of fruits is very essential for evaluation and conservation of genetic diversity. In recent years, different molecular systems such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) have been developed and applied to a range of crop species including cereals [5]. Molecular markers provide a way to measure true genetic variability in the absence of environmental influences [6]. SSR markers are very much important particularly for variety selection for breeding purpose, for hybridization and evaluation of the progenies and for conservation of diverse gene pool. Hence, 60 local mango germplasm from Chapainawabganj district of Bangladesh was gathered for their molecular characterization using SSR markers.

2. Materials and Methods

Plant materials

Among the 64 districts of Bangladesh, Chpainawabganj has the maximum diversity of mango. Sixty local germplasm of mango (*Mangifera indica* L.) were selected from Chapainawabganj. Approximately 50 g of recently matured leaves (15 - 20 days old) were collected and washed using distilled water. The leaves were then air dried prior to storage in sealed plastic bags at −20˚C.

Study Location

The experimental activities were performed in two locations; one from where the genetic materials were collected and the other in where the molecular characterization of those materials had been executed using SSR markers along with other experimental procedures. Geographically the studied mango germplasm were identified and collected between 24˚22' to 24˚57' North latitude and 87˚23' to 88˚23' East longitude. The molecular characterization of the genotypes was conducted at Plant Genetic Resources Centre, Bangladesh Agricultural Research

Institute (BARI), Gazipur, Bangladesh.

DNA extraction

DNA was isolated following the protocol of Doyle and Doyle (1987) with slight modifications [7]. Two solutions were used to extract DNA. The solution 1 consisted of 0.4 M glucose, 20 mM Methylenediamine tetra acetic acid (EDTA; pH 8.0), 3% (w/v) polyvinylpyrrolidone (PVP)-40 (molecular weight 40,000) and 0.2% (v/v) β-mercaptoethanol. The solution 2 consisted of 2% cetyltrimethyl 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. In both cases, β-mercaptoethanol was added just prior to use. In addition, chloroform: isoamayl alcohol (24:1), 70% alcohol, 100% alcohol, 3 M sodium acetate (pH 5.2) and Tris-EDTA buffer consisting of 10 mM Tris (pH 8.0), 1 mM EDTA (pH 8.0) and 0.1 µg/µl RNase A were also used. Frozen mango leaves were taken and midribs along with secondary veins were removed. About 0.2 - 0.3 g of mango leaves were weighed and homogenized with a pre-chilled mortar and pestle in liquid nitrogen. The leaves were ground to a fine powder and transferred into a 2 ml tube to which 800 µl of the solution 1 was added followed by 0.2% β-mercaptoethanol. The mixture was vortexed then centrifuged at 12,000 rpm for 10 min at 4˚C. The supernatant was discarded. The same procedure was repeated with 700 µl of the solution 1. To the pellet, 700 µl of preheated (65˚C) solution 2 was added as extraction buffer, 0.15% (v/v) β-mercaptoethanol was added and the mixture was mixed gently. The tubes, each containing a different leaf sample, were incubated at 65˚C in a water bath for 1 h with intermittent shaking. Centrifuge tubes were cooled to room temperature and an equal volume of chloroform: isoamayl alcohol (24:1) was added. The contents were mixed well by vortexing or shaking manually for 5 min then centrifuged at 12,500 rpm for 10 min at room temperature. The supernatant was transferred to a fresh 1.5 ml tube and this clean-up step was repeated until a clear supernatant was obtained. The upper aqueous phase was transferred into a new tube (1.5 ml) containing twice the volume of 100% ethanol and 1/10 (v/v) of sodium acetate, mixed gently and kept at −20˚C for 1 h. The tubes were centrifuged at 12,000 rpm for 15 min and the supernatant was discarded. The DNA pellet was washed twice with 70% ethanol and dried at room temperature. The dried pellet was resuspended in 200 µl 0.1xTE (Tris-EDTA (ethylenediamine tetra acetic acid)) and incubated at 37˚C for 30 min. An equal volume of chloroform was added, mixed and tubes were centrifuged at 12,000 rpm for 5 min. The upper aqueous phase was carefully collected and transferred into a new sterile 1.5 ml Eppendorf tube containing twice the volume of 100% ethanol, mixed gently and kept at −20˚C for 1 h. Tubes were centrifuged at 12,000 rpm for 15 min, the liquid was discarded and tubes were dried at room temperature. The final pellet was dissolved in 20 - 40 µl ddH_2O or TE buffer and kept at −20˚C indefinitely [8].

DNA amplification

DNA amplification was performed in an oil-free thermal cycler (Master Cycler

Table 1. List of 60 Mango germplasm.

SL. No.	Name of germplasm	SL. No.	Name of germplasm
01	Fazli	31	Borogutti
02	ShurmaFazli	32	Sipiard
03	Khirsapat	33	SadaGutti
04	KhudiKhirsa	34	ChongaFazli
05	Gopalbhog	35	Nora
06	Langra	36	Modhuchoski
07	Bombai	37	Ranibhog
08	China Bombai	38	Kohitur
09	Kachamitha	39	Golapkhas
10	Ashwina	40	Dudsor
11	Kuapahari	41	Gutti-1
12	Kalua	42	Gutti-2
13	Kalibhog	43	Gutti-3
14	Boglagutti	44	Gutti-4
15	Batasa	45	Gutti-5
16	Mohonbhog	46	Gutti-6
17	Lokhonbhog	47	Gutti-7
18	Sindhuri	48	Gutti-8
19	Himsagor	49	Gutti-9
20	Dalvanga	50	Gutti-10
21	Lokhna	51	Gutti-11
22	Fonia	52	Gutti-12
23	Kaiadip	53	Gutti-13
24	Narikali	54	Gutti-14
25	Brindaboni	55	Gutti-15
26	Shamlota	56	Gutti-16
27	Darika	57	Gutti-17
28	KalamSindhuri	58	Gutti-18
29	Misrikanto	59	Gutti-19
30	Miakhaoa	60	Gutti-20

Gradient, Eppendorf). The reaction mix was preheated for 5 min at 94˚C for 1 cycle, followed by 40 cycles of denaturation at 94˚C for 45 sec, annealing at 51˚C for 1 min and elongation at 72˚C for 1 min. After the last cycle, a final step of 8 min at 72˚C was added to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4˚C [9].

Table 2. Sequence of six set of SSR primers used in the study.

SL. NO.	Sequence name	Primer sequence
01	MiSHRS-18-F	AAA CGA GGA AAC AGA GCA C
	MiSHRS-18-R	CAA GTA CCT GCT GCA ACT AG
02	mMiCIRO14-F	GAG GAA CAT AAA GAT GGT G
	mMiCIRO14-R	GAC AAG ATA AAC AAC TGG AA
03	mMiCIRO18-F	CCT CAA TCT CAC TCA ACA
	mMiCIRO18-R	ACC CCA CAA TCA AAC TAC
04	mMiCIRO22-F	TGT CTA CCA TCA AGT TCG
	mMiCIRO22-R	GCT GTT GTT GCT TTA CTG
05	MIGA253-FP	CATGAGAGAGAGAGAGAGAGA
	MIGA253-RP	AAAGGAAAGGCAGGGAAATG
06	MIGA179-FP	CCTGAGAGAGAGAGAGAGA
	MIGA179-RP	GAGAGAGAGAGAGAGGTGG

SSR data analysis

The summary statistics including the number of alleles per locus, major allele frequency, genetic diversity, polymorphism information content (PIC) values and classical Fst values were determined using POWER MARKER version 3.23 [10], a genetic analysis software. Molecular weights for microsatellite products, in base-pairs, were estimated with AlphaEase4C software. The individual fragments were assigned as alleles of the appropriate microsatellite loci. Allele molecular weight data were also used to determine the genetic distance for phylogeny reconstruction based on the neighbor joining method [11] as implemented in POWER MARKER with the tree viewed using TREEVIEW [12].

3. Results and Discussion

The microsatellite enriched DNA fingerprint was constructed using the standard procedures. In this study, 60 germplasm of mango were analyzed using 6 primer pairs (**Table 1** and **Table 2**). Amplified microsatellite loci were analyzed for polymorphism using Polyacrylamide Gel Electrophoresis (PAGE) and the results revealed that all the primer pairs detected polymorphism among the mango germplasm analyzed (**Figure 1**). The microsatellite loci were also multi-allelic (15 to 27 alleles per locus with a mean of 21.83 alleles per locus in the present study) and the alleles were co-dominant suggesting their relative superiority in detecting DNA polymorphism over some other markers. The detailed result which was obtained after analyzing fingerprinting data is briefly presented below:

Using 6 SSR markers, a total of 131 alleles were detected among the 60-mango germplasm (**Table 3**). The average number of alleles per locus was 21.83, with a range of 15 (mMiCIRO18, MIGA253) to as many as 27 (MiSHRS-18, MIGA179). Duval *et al.* (2005) observed 4 to 14 alleles with a mean value of 7.3 per locus and a total of 140 alleles of which 121 are specific to *Mangifera indica* [13]. Comparing microsatellite markers with the different repeat motifs, those with the high

number of MIGA179 repeats had the highest genetic diversity 0.9355; while the lower number of genetic diversity 0.6714 was noticed in MIGA253 repeats. The major allele is defined as the allele with the highest frequency and is also known as the most common allele at each locus. The frequency of the most common allele at each locus ranged from 11% (MIGA179) to 51% (MIGA253) with a mean frequency of 20.56. The size of the different major alleles at different locus ranged from 0 bp (mMiCIRO14) to 212 bp (MiSHRS-18) (Table 3).

According to Nei's (1973), the highest level of gene diversity value (0.9433) was noted in loci MIGA179 and the lowest level of gene diversity value (0.6683) was observed in loci MIGA253 with a mean diversity of 0.8842 (Table 4) [14]. It was examined that markers detecting the lower number of alleles exhibited lower gene diversity than those detected higher number of alleles also revealed higher gene diversity. This result has got support from the previous work done by Herrera *et al.* (2008), who reported that the gene diversity at each SSR locus was significantly correlated with the number of alleles detected, the number of repeat motif and with the allele size range [15]. As a measure of the informativeness of microsatellites, PIC values ranged from the lowest value of 0.6501 in MIGA253

Table 3. Data on number of alleles, major allele and allele size ranges found among 60 mango germplasm for 6 SSR primer.

Locus	No. of alleles	Major allele		Allele size (bp)	
		Size (bp)	Frequency (%)	Ranges	difference
MiSHRS-18	27	212	15	170-221	51
mMiCIRO14	21	0	13	154-179	25
mMiCIRO18	15	151	15	121-155	34
mMiCIRO22	26	150	13	112-161	49
MIGA253	15	0	55	131-164	33
MIGA179	27	0	11	122-164	42
Mean	**21.83**		**20.56**		

Table 4. Data on sample size, gene diversity (GD) and polymorphism information content (PIC) found among 64 mango germplasm for 6 SSR primers.

Locus	Sample size	Gene diversity	PIC
MiSHRS-18	60	0.9339	0.9304
mMiCIRO14	60	0.9283	0.9239
mMiCIRO18	60	0.8972	0.8884
mMiCIRO22	60	0.9339	0.9302
MIGA253	60	0.6683	0.6501
MIGA179	60	0.9433	0.9405
Mean	**60**	**0.8842**	**0.8773**

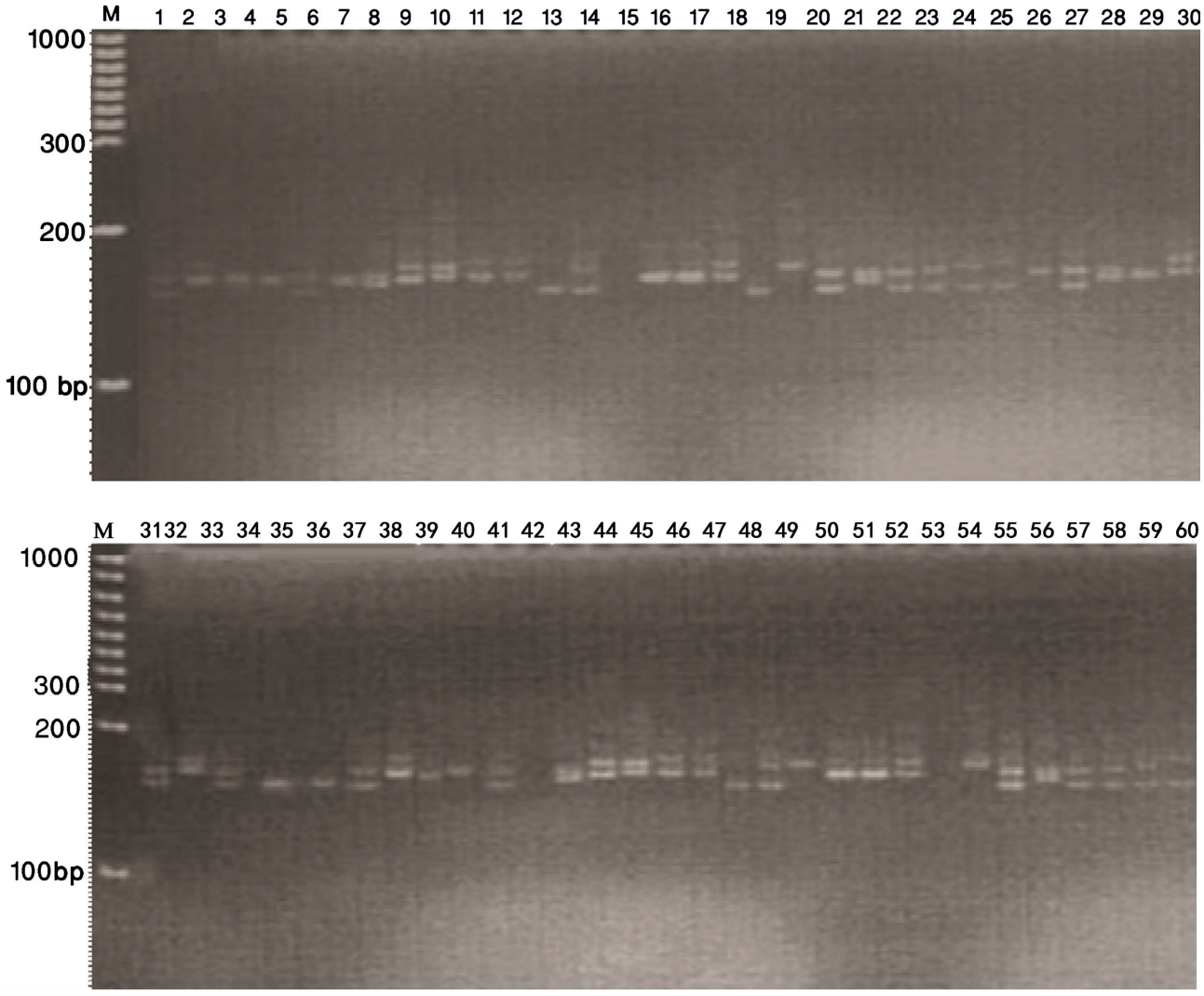

Figure 1. Microsatellite profile of 60 mango germplasm using mMiCIRO14, Molecular weight marker (1000 bp DNA ladder).

to the highest value of 0.9405 in MIGA179 and the average PIC value was computed 0.8773 (**Table 4**). PIC values also showed a significant and positive correlation with the number of alleles and allele size range for microsatellites evaluated in this study.

The genetic distance-based results seen in the unrooted neighbor-joining tree revealed two major groups in the studied 60 mango germplasm (**Figure 2**). Group-A and group-B again sub-divided in sub-cluster A-I, A-II, A-III and B-I, B-II, B-III. The largest group A-I included the germplasm Sadagoti, Goti-9, Goti-12, Goti-8, Goti-1, Modhochoski, Gopalvog, Goti-11, Goti-10, Goti-15, Ranivog, Nora, Goti-13, Goti-4, Goti-6 and Goti-5 (16 genotypes). The second largest group B-I contained the germplasm Goti-16, Kohitor, Kuapahari, Darika, Himsagor, Kaidip, Borogoti, Kalamsindhuri, Sipiard, Fonia, Misrikanti, Kalua, Bridaboni, Dalvanga and Shamlota (15 germplasm). The remaining germplasm Kachamithi, Sindhuri, Chainabombai, Lokhna are clustered in group A-II. The

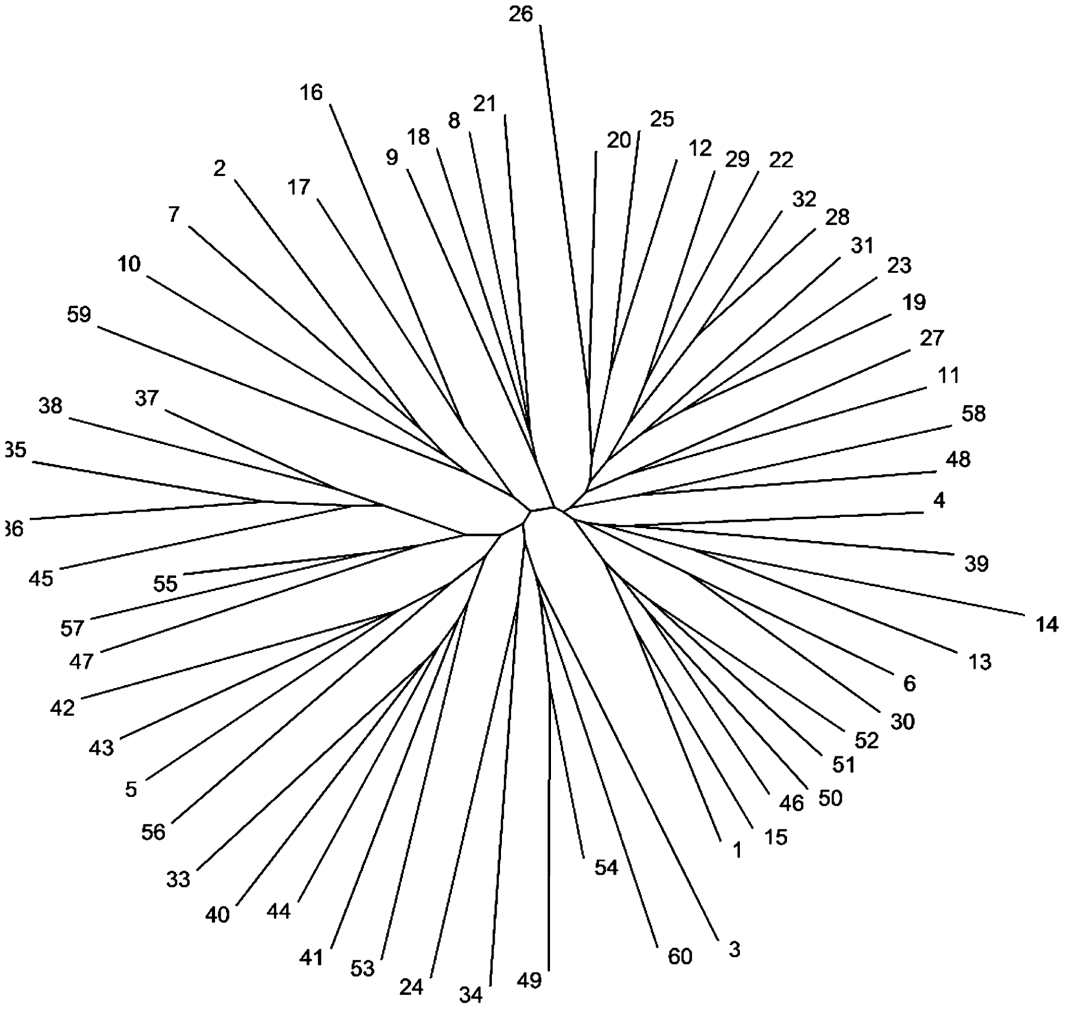

Figure 2. An unrooted neighbor-joining tree showing the genetic relationships among 60 mango germplasm based on SSR primers.

germplasm in the same group showed their distance from the other groups and their similarity within the same group. Srivastava *et al.* (2007) used an NJ tree based on the cumulative data from all methods correlated well with the parentage of the mango hybrids and the grouping of cultivars on a regional basis [16].

Pair-wise comparisons of Nei's (1973) Genetic distance (D) between varieties have been examined. Genetic distance (D) between varieties was computed from combined data of the 6 primers, ranging from 0.5000 to 1.0000 [14]. Comparatively higher genetic distance (1.000) was observed between a large number of germplasm pairs. Among them Fazli vs Gopalvog, Fazli vs Boglagoti, Fazli vs Mohonvog, Fazli vs Narikeli, Fazli vs Shamlota, Fazli vs Goti-1, Fazli vs Goti-2, Fazli vs Goti-4, Fazli vs Goti-6, Fazli vs Goti-8, Fazli vs Goti-9, Fazli vs Goti-11, Fazli vs Goti-12, Fazli vs Goti-13, Fazli vs Goti-15, Fazli vs Goti-17, Fazli vs Sadagoti, Kalua vs Mohonvog, Kalivog vs Mohonvog, Kalivog vs Shorma Fazli, Batasa vs Mohonvog, Batasa vs Golapkhas and many other germplasm pairs.

The higher genetic distance indicates that genetically they are diverse com-

pared to the lower genetic distance value. This value is an indication of their genetic dissimilarity. Variety pair with a higher value is more dissimilar than a pair with a lower value. The lowest genetic distance (0.5000) was found in Fazli vs Batasa, Kalua vs Brindaboni, Himsagor vs Kaidip, Dalvanga vs Sipiard, Kaiadip vs Kalam Shindhuri, Kaiadip vs Boro Goti, Himsagor vs Goti-5 germplasm pair indicating that they are genetically much closer to each other.

Dendrogram based on the Nei's genetic distance calculated from the 131 SSR alleles generated from the 60 mango germplasm (Figure 3). All 60 mango germplasm

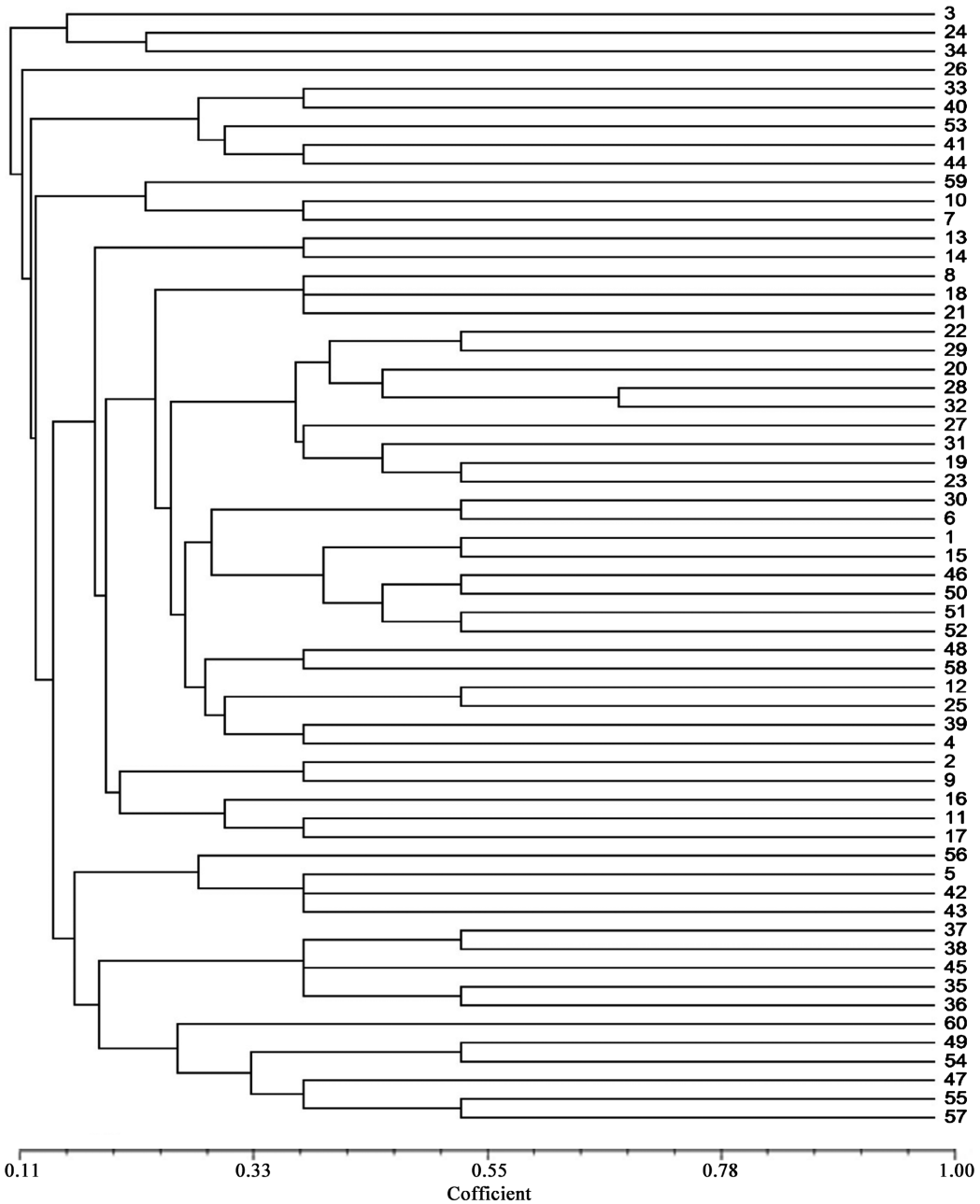

Figure 3. Dendrogram for 60 mango germplasm derived from a UPGMA cluster analysis using the Dice similarity coefficient.

could be easily distinguished. The Unweighed Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis lead to the grouping of the 60 germplasm into twelve major clusters.

These are Cluster-I which included Khirsapat, Narikeli, Goti-2 forming the sub-cluster I and Narikeli, Goti-2 in the sub-cluster II of local mango germplasm. The germplasm Goti-1, Goti-8, Sadagoti, Goti-9 and Goti-12 formed cluster-II. In cluster-III, Golapkhas, Ashina and Bombai are gathered and cluster-IV amassed Kalivog and Batasa. In cluster-V, the germplasm Chinabombai, Sindhuri and Lokhna were picked. In cluster-VI, Fonia, Mistikanto, Dalvanga, Kalamshindhuri and Sipiard formed the sub-cluster I and Darika, Borogoti, Himsagor, Kaiadp made the sub-cluster II. Cluster-VII included Miakhaoa and Lokhna forming the sub-cluster I and Fazli, Batasa, Goti-14, Goti-18, Goti-19, Goti-20 germplasm constructing the sub-cluster II. Cluster-VIII gathered the germplasm Goti-16 and Kohitor in the sub-cluster I and Kalua, Brindaboni, Goti-7 and Khodikhirsa germplasm in the sub-cluster II.

In cluster-IX, Shormafazli and Kachamithi formed sub-cluster I and Mohonvog, Kuapahari and Lokhonvog grouped in sub-cluster II. Cluster-X comprised of Modhochoski (sub-cluster I) and Gopalvog, Goti-10 and Goti-11 (sub-cluster II). In cluster-XI, Goti-5, Goti-6 formed sub-cluster I and Goti-13, Goti-3, Goti-4 made sub-cluster II. Cluster-XII involved Dodsor (sub-cluster I) and Goti-17, Chongafazli, Goti-15, Nora and Ranivog (sub-cluster II).

4. Conclusion

From this study, the dendrogram exhibited that the genotypes that were derivatives of the genetically similar type formed cluster together. The germplasm in the same group exhibited wider variations from the other groups and their similarity within the same group. This variability can be used for the selection of superior germplasm for cultivation at the farmer's level as well as the future breeding programme of mango in Bangladesh. Further collection of mango germplasm should be continued for getting more variability in respect of desired traits.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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