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Assessment of genetic diversity using morphological and molecular markers in traditional cultivars of Mango (*Mangifera indica* L.)

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Mango (*Mangifera indica* L.) is an economically important tropical and subtropical fruit crop consumed worldwide. Alloploidy, nature of cross-pollination and a wide range of predominant agro-ecologies of the country have contributed large genetic diversity of mango in India. The present study assessed 16 traditional mango cultivars to get a unique insight on cultivars' diversity through deploying integration of both morphological and molecular markers. The cultivars were appraised for consecutive two years under the aegis of All India Coordinated Research Project on Fruits, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Gayeshpur, West Bengal regarding observation on 26 morphological and fruit quality parameters followed by assessing diversity at molecular level through deploying 20 SSR makers. Presence of adequate genetic variability was reflected for all the tested traits. Principal Component Analysis (PCA) ascertained seven PCs towards contribution of more than 84.25% genetic diversity harbored by the tested cultivars. Out of 20 SSRs, 8 microsatellites were amplified and produced 27 putative alleles in 16 cultivars. Genetic divergence through multivariate analysis, as well as through UPGMA dendrograms, classified 16 mango cultivars into five major clusters, though, the cluster composition was different. The dendrogram affirmed that the highest similarity (88%) was observed in between Ranipasand and Gulab Khas. Sharing of common gene pool coupled with exertion of similar selection pressure during domestication as well as selection of cultivars in this region exhibited similar tradition.

Keywords: Diversity, Mango, Quality traits, SSR markers, Variability

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Mango (*Mangifera indica* L.), a key member of the Anacardiaceae family, is known as the "king of fruits" and is regarded as the world's superior and prized fruit crop in tropical and subtropical climates[1]. Originating in the Indian subcontinent, this fruit crop further distributed to other ecogeographical areas with 1000 varieties identified worldwide[2]. India is the richest source of mango germplasm accessions and acknowledged as the top mango producer in the world, having an area of 2258.1 thousand ha, 21822.3 thousand MT productions and productivity of 9.7 MT/ha[3]. Allopolyploidy, out-crossing along with unrestrained gene flow and agro-ecological diversity of this country resulted wide genetic variability. Additionally, mango breeding encouraged hybridization and recombination in recent decades and created enormous genetic diversity in the gene pool[4]. However, many traditional Indian mango cultivars have malformation, alternate bearing habit, poor fruit quality, and low yield potential. It is therefore a prime requisite to decipher the genetic diversity existing in the gene pool and consequently to protect both promising and endangered species to widen the genetic base[5,6].

Assessing genetic diversity among the cultivars is an integral part of breeding programme towards identifying the superior diverse parents for getting better transgressive segregants[7]. Information on the genetic distance among the cultivars will also facilitate to avoid duplication, thus clearing the ambiguity in the nomenclature especially in case of crops like mango, expanding the genetic base of the major collections and ultimately help to preserve the valuable diversity. Characterization of mango germplasm through morphological markers has some difficulties as these markers alone do not provide adequate information to understand genetic diversity because of low penetrance and heritability as well as paucity in number. This kind of problem becomes more magnified in perennial fruit

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trees like mango because of their long juvenile period, poor and unreliable information about the cultivars and duplicity within the local cultivars due to different dialectal names. The discovery of molecular markers simplified the assessment of diversity to find out the genome's distinctive features with less laborious and quicker way. In recent decades, different kinds of molecular markers have been used for cultivar identification in mango, such as AFLP[8], ISSR[4,9-10], SCoT[11], RAPD[12,13] and SSR[14-16] towards testing clonal fidelity and for prediction of genetic relationships among the cultivars. Among these, microsatellite markers (SSR) are more propitious than various other markers as these are co-dominant, more polymorphic, easily transferable, highly abundant and simple to examine. So, keeping pace with the background, the present study was outlined with the following objectives of appraisal of genetic diversity of the traditional mango cultivars considering yield attributing and qualitative traits along with molecular markers.

Materials and Methods

Plant materials and experimental layout

The present study was conducted in the mango orchard of All India Coordinated Research Project on fruits at the Regional Research Station, Gayeshpur (Lat: 22.95 N; Long: 88.49 E and Altitude: 9.7 m), Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India during 2017 and 2018. The observations of different qualitative fruit and tree characters were recorded as per descriptor list[17,18] and DUS (Distinctness, Uniformity and Stability) guidelines[19]. Based on the descriptors, finally among the variable cultivars, 16 mango cultivars of Indian origin mostly collected from the state of West Bengal, Uttar Pradesh and Bihar were considered for further study (Table 1 and Fig. 1). The age of the plants of each cultivar was approximately 30 years. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Proper plant geometry was maintained with a spacing of 10 cm each between plant to plant and row to row.

Recording of observation

Observation was recorded considering 20 quantitative traits as well as 6 fruit quality parameters for diversity study. Tree height (cm) was measured from ground level to the top of the tree and classified as short (≤ 6.0), medium (6.1 – 9.0), tall (9.1 – 12.0) and very tall (> 12.0). Leaf and flower characters viz., blade and

Table 1 — Description regarding Mango cultivars used in the present study

Sl. No	Cultivar	Place of origin	Tree height	Leaf blade shape	Fruit Shape	Skin colour of ripen fruit	Type of embryony
1	Chatterjee	Hoogli, West Bengal	Medium	Elliptic	Oblong	Yellow	Mono embryony
2	Gulab Khas	Bihar	Tall	Lanceolate	Oblong	Green with red blush	Mono embryony
3	Ranipasand	Murshidabad, West Bengal	Tall	Elliptic	Roundish	Yellow	Mono embryony
4	Sarikhas	West Bengal	Tall	Elliptic	Oblong	Green with red blush	Mono embryony
5	Himsagar	Malda, West Bengal	Tall	Elliptic	Roundish	Greenish yellow	Mono embryony
6	Banganpalli	Andhra Pradesh	Tall	Elliptic	Roundish	Yellow	Mono embryony
7	Langra	Varanasi, Uttar Pradesh	Medium	Lanceolate	Oblong	Greenish yellow	Mono embryony
8	Fazli	Bihar	Very tall	Ovate	Oblong	Green	Mono embryony
9	Gopal Bhog	Malda, West Bengal	Tall	Obovate	Oblong	Green	Mono embryony
10	Lakhan Bhog	Malda, West Bengal	Medium	Lanceolate	Roundish	Green with red blush	Mono embryony
11	Kancha Mitha	Murshidabad, West Bengal	Tall	Oblong	Oblong	Yellow	Mono embryony
12	Kanchan Kosa	Malda, West Bengal	Very tall	Elliptic	Oblong	Green with red blush	Mono embryony
13	Kamala Bhog	Malda, West Bengal	Tall	Lanceolate	Roundish	Green with red blush	Mono embryony
14	Gopi Bhog	Murshidabad, West Bengal	Very tall	Lanceolate	Roundish	Yellow	Mono embryony
15	Madhu Chuski	Murshidabad, West Bengal	Tall	Lanceolate	Oblong	Greenish yellow	Mono embryony
16	Khota Lagga	Malda, West Bengal	Tall	Elliptic	Oblong	Greenish yellow	Mono embryony

Fig. 1 — Variability in fruit morphology of 16 mango cultivars

petiole length (cm) and width (cm), inflorescence length (cm) and width (cm), were measured considering an average of 10 mature leaves, petioles or inflorescences. Regarding various fruit descriptors, data was recorded considering 10 randomly selected fruits. TSS was recorded with the help of ERMA hand refractrometer and the average was worked out. Reducing sugars as well as total sugars were appraised using the Lane and Eyon method[20]. Non-reducing sugars in juice was measured by subtracting reducing sugars from total sugars. Titratable acidity was recorded in terms of percent citric acid[21]. Ascorbic acid content was measured by taking 10 mL of juice following standard protocol[20]. TSS: acid ratio was estimated by dividing TSS with the acidity.

DNA extraction and SSR analysis

Genomic DNA was isolated from 100 mg of fresh young leaf tissues collected only from 10-days old mango cultivars grown in the orchard. Genomic DNA was isolated using modified CTAB DNA extraction protocol[22] and the quality was checked in 1% agarose gel. In accordance with standard protocols, DNA purity and concentration were measured using a UV-vis spectrophotometer (Model: Beckman DU 650 model)[23]. SSR primers were used to analyze diversity using diluted genomic DNA at a concentration of 50 ng/µL after quantification. In the present study, 20 previously reported SSR primers were preferred for molecular diversity analysis[24]. A total volume of 25 µL was used for the PCR reaction using the PCR master mix kit. For PCR amplifications, Eppendorf flexid Thermal Cyclers were utilized. The temperatures used were: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing primer pairs at appropriate temperatures (49-53°C) for 45 seconds and subsequent polymerization at 72°C for 1 min. After that, the samples were extended at 72°C for 7 min before being held at 4°C for 5 min. On completion of PCR, the amplification products were stored in (-) 20°C freezer. The PCR amplified products were electrophoresed on a 1% agarose gel using a DNA ladder of 100 bp for determining the molecular size. Trans-illuminator imaging was used to visualize and capture banding patterns on the gel stained with Ethidium bromide.

Statistical analysis

The analysis of genetic divergence was done using Mahalonobis D^2 statistics[25]. The cultivars were grouped into different clusters or clades followed by aligning the inter and intra cluster distances. Principal component analysis (PCA) was contemplated according to the standard procedure[26]. Among mango cultivars, the SSR amplified alleles were detected as presence of the corresponding band (1) or absence of it (0) to determine genetic distance and cluster

analysis. Polymorphism information content (PIC) for each SSR marker was calculated to measure how informative the markers are by using the following formulae: $PIC=1-\sum Pi^2 - \sum\sum Pi^2\ Pj^2$, where 'i' represents the total number of identified alleles for each SSR marker and 'Pi' is the frequency of the i^{th} allele in the set of 16 mango cultivars deployed in the study and $j = i+1$[27]. To determine the genetic diversity among the studied cultivars, a binary data matrix was created and subjected to cluster analysis. The binary data was used to determine the Similarity Index as Jaccard's coefficient using SIMQUAL subroutine in SIMILARITY routine using Windostat Version 9.3.[28]. The genetic relatedness was determined by deploying the similarity matrix for computing dendogram using the Unweighted Pair Group Method with Arithmetic Means (UPGMA).

Results

Analysis of variance revealed presence of significantly higher amount of variability among the 16 mango cultivars for all the morpho-physiological characters studied, which validated the presence of adequate genetic variability (Table 2).

Genetic Divergence through multivariate analysis: The data collected on quantitative characters (both morphological and fruit quality) for 16 cultivars of mango were subjected to multivariate analysis by using Mahalanobis D^2 statistic for quantitative assessment of genetic divergence. D^2 values were calculated for 120 possible pairs of combinations [n (n-1)/2] from means of 16 cultivars for 26 characters. Using the Tocher method, the tested cultivars were categorized into five diverse clades (Fig. 2) considering the principle that the mean D^2 values within the cluster should be less than the mean D^2 values between the clusters. Amid the five distinct clades, the largest was cluster III with 8 traditional cultivars of mango (Gulab Khas, Ranipasand, Sarikhas, Himsagar, Madhu Chuski, Khota Lagga, Kancha Mitha, Gopal Bhog) followed by cluster I consisting of 3 cultivars (Chatterjee, Langra, Lakhan Bhog), IV and V with two cultivars each (Fazli, Banganpalli and Kanchan Kosa, Gopi Bhog,

Table 2 — Analysis of Variance (ANOVA) for morphological and fruit quality parameters in Mango

Sl. No	Character	Mean sum of square		
		Replication	treatment	Error
1	Tree height (m)	0.02	19.05**	0.02
2	Leaf blade length (cm)	5.12	66.57**	2.83
3	Leaf blade width (cm)	0.57	6.08**	0.32
4	Petiole length (cm)	0.35	7.03**	0.61
5	Inflorescence length (cm)	0.09	243.63**	1.96
6	Inflorescence width (cm)	1.28	209.65**	1.70
7	Fruit length (cm)	0.26	18.10**	0.63
8	Fruit diameter (cm)	0.09	8.41**	0.08
9	Fruit weight (g)	421.11	45100.80**	661.05
10	Fruit skin thickness (mm)	0.00	0.17**	0.00
11	Pulp content	0.00	1.75**	0.00
12	Stone length (cm)	0.01	6.46**	0.03
13	Stone width (cm)	0.02	1.02**	0.02
14	Stone thickness (cm)	0.00	0.39**	0.01
15	Stone weight (g)	0.36	291.35**	1.64
16	Seed length (cm)	0.08	3.47**	0.19
17	Seed width (cm)	0.03	2.99**	0.02
18	Seed weight (g)	1.71	191.20**	0.81
19	TSS (^{O}Brix)	0.51	46.62**	0.49
20	Total sugars (%)	0.22	15.05**	0.80
21	Reducing sugars (%)	0.08	4.93**	0.06
22	Non-reducing sugars (%)	0.05	20.64**	0.84
23	Titratable acidity (%)	0.01	0.37**	0.02
24	TSS: acid ratio	232.10	14548.87**	787.89
25	Ascorbic acid (mg/100g)	0.99	753.91**	6.71
26	Yield/plant (q)	0.01	3.01**	0.02

** Significant at 1% level of significance * Significant at 5% level of significance

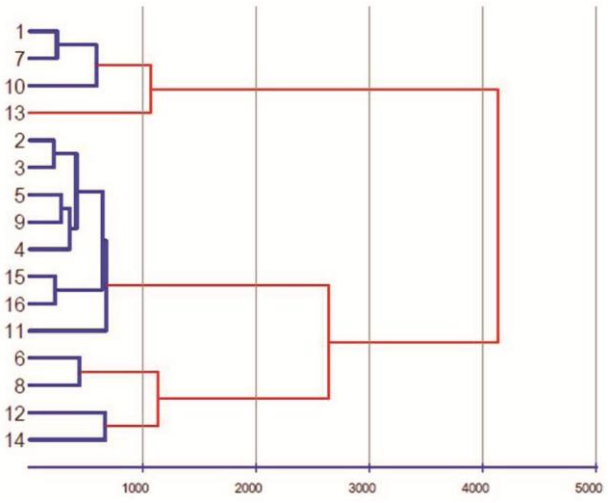

Fig. 2 — Dendrogram depicting the grouping of 16 mango cultivars. Numbers correspond to genotypes as listed in Table 1.

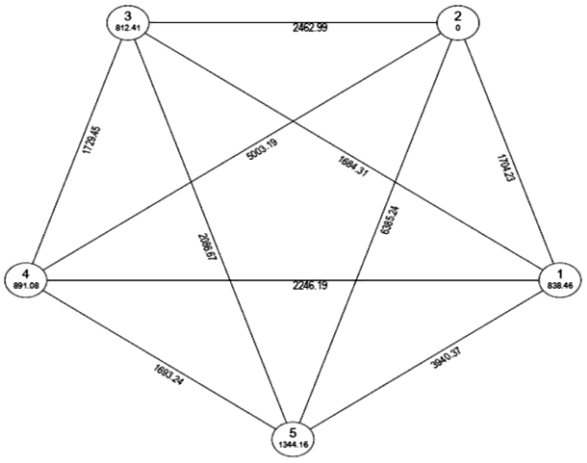

Fig. 3 — The Mahalanobis Euclidean Distance approach was used to determine the clustering arrangement and their mutual interaction for morphological and fruit biochemical characters of 16 mango cultivars.

respectively) and II cluster consisting of only one cultivar (Kamala Bhog). The average inter and intra-cluster distances among the five clusters are depicted in Figure 3. The D^2 values between clusters ranged from 1693.23 to 6385.24, indicating that the tested mango cultivars contains a high amount of genetic variation. The highest inter-cluster D^2 value was recorded between clusters II and V (6385.24) while the lowest inter-cluster D^2 value was recorded between clusters IV and V (1693.23). This suggested wide genetic diversity between these clusters. The result on character wise contribution towards total genetic divergence showed that pulp content contributed the maximum (36.67%) to the diversity followed by tree height (31.67%) (Fig. 4).

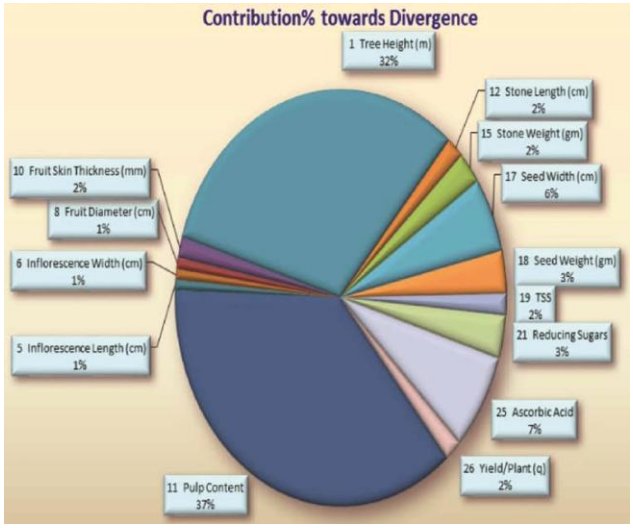

Fig. 4 — Different traits' relative contributions to genetic divergence in mango cultivars.

The variance among mango cultivars was judged by PCA with an objective to curtail down the numbers of the observations that have been considered during characterization into few principal components considering their independentness. A total of 84.25% of variability amongst the tested mango cultivars could be explained by the principal components (PCs) with having Eigen values >1 (Table 3). According to PCA results, Eigen value and variance percentage were highest in PC-I i.e., 6.17 and 23.73, respectively, followed by PC-II (4.18) with variance percentage of 16.07. In case of PC-III the Eigen value was 3.30 with variance percentage of 14.05. The weights specifying the contribution of distinct characters to the respective PCs were indicated by the character loading values of the PCs. Moreover, the loading signs (+ / -) denote the contribution direction, similar to regression coefficients. The maximum contributing variables viz., tree height; fruit length, diameter and weight; stone length, width and weight; seed length; titratable acidity; ascorbic acid and yield/plant substantially loaded in PC I and thus represented highest contribution towards variability (Table 4). The tested mango cultivars were grouped into three clusters according to a two-dimensional scatter plotting diagram (Fig. 5) generated using component score 1 on the X axis and component score 2 on the Y axis.

Genetic divergence study at molecular level

Out of 20 SSR markers, 8 SSRs were amplified and produced putative 27 alleles in 16 cultivars. The total number of alleles ranged from two to four, with an average of three alleles per locus (3.38). The 8 SSR

Table 3 — Eigen values and percentage of variation for Principal Components (PCs) of morphological and fruit biochemical Parameters in 16 Mango cultivars

PCs	Eigen value (Root)	Variation extracted in percentage	Cumulative variation explained
PC I	6.17	23.73	23.73
PC II	4.18	16.08	39.81
PC III	3.31	12.72	52.53
PC IV	2.53	9.73	62.26
PC V	2.14	8.22	70.48
PC VI	1.90	7.32	77.81
PC VII	1.68	6.44	84.25

Table 4 — The loading of principal components for morphological and fruit bio-chemical parameters in mango cultivars

S. No	Character	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
1	Tree height (m)	0.677	0.012	0.418	-0.092	-0.014	-0.204	-0.262
2	Leaf blade length (cm)	-0.102	-0.470	0.648	0.515	0.165	0.169	-0.004
3	Leaf blade width (cm)	0.409	-0.478	0.287	0.484	0.451	0.096	-0.075
4	Petiole length (cm)	-0.319	-0.329	0.325	0.393	0.377	0.223	0.283
5	Inflorescence length (cm)	0.114	0.459	-0.376	0.375	0.411	0.214	0.258
6	Inflorescence width (cm)	0.302	0.483	-0.245	0.276	0.494	0.317	0.074
7	Fruit length (cm)	0.736	-0.303	0.416	0.193	0.125	-0.065	-0.094
8	Fruit diameter (cm)	0.745	-0.033	-0.209	-0.218	0.052	-0.275	0.351
9	Fruit weight (g)	0.860	-0.320	-0.060	0.056	-0.220	0.190	0.204
10	Fruit skin thickness (mm)	0.266	0.468	-0.082	0.386	0.010	-0.076	-0.385
11	Pulp content (%)	0.314	-0.495	-0.509	0.262	-0.317	-0.315	0.008
12	Stone length (cm)	0.725	-0.404	0.086	-0.338	0.102	0.103	-0.353
13	Stone width (cm)	0.619	-0.376	-0.394	0.030	0.284	-0.321	0.105
14	Stone thickness (cm)	0.318	0.438	0.093	-0.324	0.241	0.206	0.530
15	Stone weight (g)	0.490	0.212	0.488	-0.359	-0.165	0.395	0.164
16	Seed length (cm)	0.678	0.110	0.282	-0.494	0.019	0.156	-0.360
17	Seed width (cm)	0.128	0.117	0.501	0.459	-0.110	-0.411	-0.129
18	Seed weight (g)	0.233	0.300	-0.176	-0.416	0.654	-0.176	-0.134
19	TSS (^{O}Brix))	-0.275	0.637	0.138	0.172	-0.444	0.170	-0.055
20	Total sugars (%)	0.285	0.777	0.437	0.128	0.017	-0.063	-0.088
21	Reducing sugars (%)	0.130	0.044	-0.346	0.117	-0.029	0.723	-0.514
22	Non-reducing sugars (%)	0.185	0.648	0.544	0.052	0.030	-0.406	0.175
23	Titratable acidity (%)	-0.589	-0.382	0.450	-0.406	0.108	0.010	0.139
24	TSS: acid ratio	0.435	0.488	-0.433	0.289	-0.090	-0.224	-0.091
25	Ascorbic acid mg/100 g)	0.599	0.069	0.150	0.210	-0.511	0.254	0.351
26	Yield/plant (q)	0.661	-0.206	-0.170	0.117	-0.296	0.191	0.253

primers amplified alleles across the 16 cultivars with varying degrees of polymorphism. A high level of polymorphism was observed with EF592182 primer (4 alleles per locus). Further, null alleles were also observed among the mango cultivars with SSR primers. The PIC was calculated according to the data matrix generated using SSR markers (Table 5). The highest PIC was recorded by the marker EF592182 (0.69) followed by EF592195 (0.68) and EF592211 (0.67) (Fig. 6), while it was found to be the lowest for the marker MiIIHR18 (0.36). The high PIC value of these markers indicated that the primers were highly informative. For each pairwise comparison among the 16 cultivars, the banding pattern of SSR markers scored as binary data was used to compute similarity index values. The Jaccard's pair wise similarity coefficients were deployed to establish genetic relatedness among the tested mango cultivars, which revealed a moderate level of genetic diversity among the cultivars. The Jaccard's similarity coefficient values varied from 0.03 (between Kanchan Kosa and Chatterjee, Kanchan Kosa and Sarikhas) to 0.88 (between Ranipasand and Gulab Khas). The dendrogram generated from the cluster analysis of UPGMA broadly classified the 16 mango cultivars into five major clusters as it was based on morphological markers (Fig. 7). However, the cluster composition was different in comparison to the cluster

constructed based on morphological traits. Gulab Khas, Ranipasand and Chatterjee were the three most varied cultivars in Cluster 'A.' Cluster ‘B' consisted of 2 cultivars (Madhu Chuski and Khota Lagga), Cluster ‘C' consisted of 5 cultivars (Sarikhas, Banganpalli, Langra, Fazli and Himsagar), Cluster ‘D' had four (Gopal Bhog, Lakhan Bhog, Gopi Bhog and Kancha Mitha) and cluster ‘E’ with two cultivars (Kanchan Kosa and Kamala Bhog). With an 88% similarity, Ranipasand and Gulab Khas were found to be the most comparable cultivars.

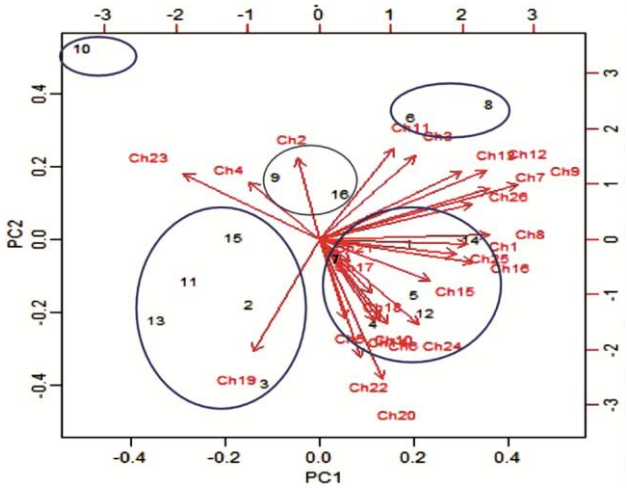

Fig. 5 — PCR scatter diagram illustrating distribution of various groups formed from 16 cultivars of mango.

Discussion

Genetic diversity indicates the presence of heritable variation within the gene pool of a crop species. The tested mango cultivars were grouped into five distinct clusters based on morphological and fruit quality traits in the present study. Creation of different individual clusters might occur because of the prevention of genetic flux due to geographical barriers or the intensity of combined artificial and natural selection that preferred superior acclimatized allelic combinations, which was further responsible for creation of genetic variation. The tested mango cultivars with large-sized fruit can be used as donors

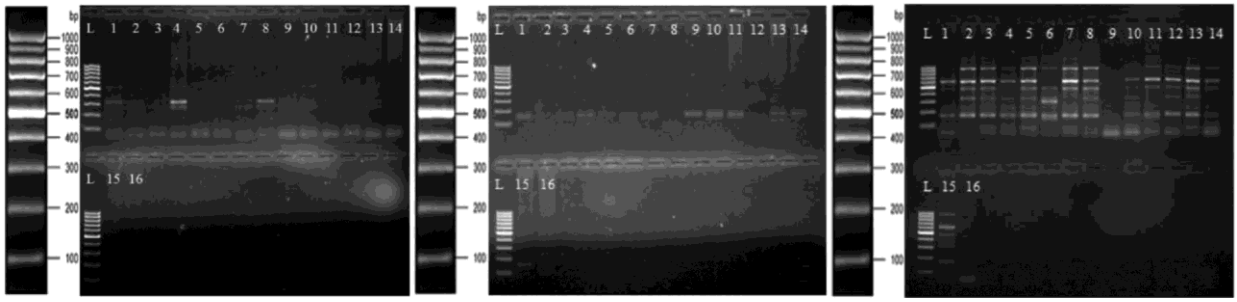

Fig. 6 — SSR gel profiles of 16 mango cultivars. SSR gel picture of 16 mango cultivars created by primer EF592182. b. SSR gel picture of 16 mango cultivars created by primer EF592195. SSR gel picture of 16 mango cultivars created by primer EF592211. Numbers correspond to cultivars as listed in Table 1.

Table 5 — Polymorphic Information Content (PIC) and variation regarding Allelic number and size obtained using 8 SSRs in 16 Mango cultivars

Sl. No	Primer	Primer Sequence	Annealing temp.	No. of alleles	Allele size (bp)	PIC
1	EF592182	F:CCCCAACATTTCATAAACACA R:CCTCCTTACATGCCTCCTTG	49	4	280-320	0.69
2	EF592183	F:GTCGATGCCTGGAATGAAGT R:AAGCATCGAACAGCTCCAAT	50	4	210-260	0.65
3	EF592195	F:CTAACCATTCGGCATCCTCT R:TCTGTGATAGAATGGCAAAAGAA	51	4	120-160	0.68
4	EF592211	F:TTCTGTTAGTGGCGGTGTTG R:CACCTCCTCCTCCTCCTCTT	52	4	170-240	0.67
5	EF592216	F:TCTATAAGTGCCCCCTCACG R:ACTGCCACCGTGGAAAGTAG	52	4	210-270	0.54
6	MiIIHR18	F:TCTGACGTCACCTCCTTTCA R:ATACTCGTGCCTCGTCCTGT	51	2	130-160	0.36
7	MiIIHR34a	F:CTGAGTTTGGCAAGGGAGAG R:TTGATCCTTCACCACCATCA	51	2	230-250	0.37
8	MiIIHR36a	F:TCTATAAGTGCCCCCTCACG R:ACTGCCACCGTGGAAAGTAG	53	3	210-260	0.48

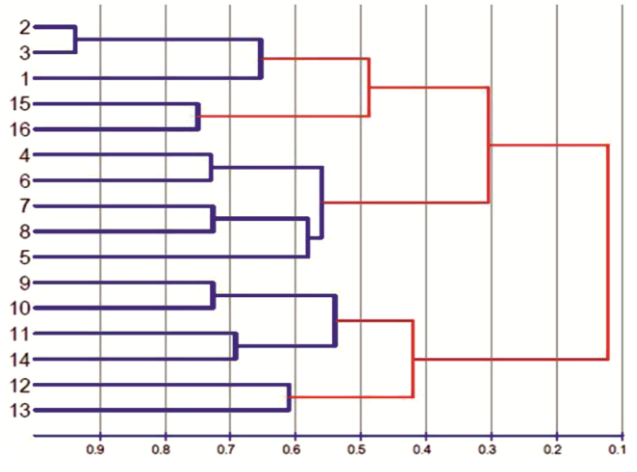

Fig. 7 — Genetic diversity analysis in 16 mango cultivars using SSR markers. Numbers correspond to genotypes as listed in Table 1

in hybridization programs for creation of enormous genetic variation in the subsequent segregating generations for implementing mango breeding for large fruit size. Earlier reports also corroborated with the present finding of clustering of large-sized mango cultivars into a single cluster[29-31]. Cluster-V (1344.23) had maximum intra-cluster D^2 value indicating the presence of wide genetic variation among the cultivars viz., (Kanchan Kosa, Gopi Bhog). Previous studies affirmed the presence of high inter-cluster distance within a cluster in mango[32,33]. Pulp content, tree height, ascorbic acid and seed width, contributed maximum towards diversity. Characters that contribute the most to explain diversity should be given major attention in the mango crop improvement programme. Similar trends of findings with regard to contribution of pulp content towards divergence were reported earlier in mango by Majumder *et al*.[29].

In PCA analysis, the Eigen value indicates the relative significance of each component towards estimating diversity of the variables where Eigen value of more than 1 should be considered ignoring the values less than 1[34]. The maximum contributing variables viz., tree height; fruit length, diameter and weight; stone length, width and weight; seed length; titratable acidity; ascorbic acid and yield/plant substantially loaded in PC I and thus represented highest contribution towards variability. In PCA, the relative contributions are more essential than the signs (indicative of direction) for evaluating the variance. Previous studies proved the superiority of utilizing microsatellite markers towards differentiating mango cultivars and determining genetic diversity[14,35,36]. The information obtained by using molecular markers like SSRs offers many benefits for identifying variation and for establishing diversity among the cultivars. The annealing temperatures and PCR conditions for these 20 SSRs were first standardized using a PCR with temperature gradient technique, which indicated that annealing temperatures of 49 to 53°C were optimum for obtaining scorable bands. Stuttering of bands was common with SSRs if annealing temperatures were not optimized. Out of 20 SSRs, 8 were amplified and produced 27 alleles in 16 cultivars. The total number of alleles ranged from two to four, with an average of three alleles per locus (3.38). In the previous reports, 5.5[37]; 6.96[38]; 5.78[39]; 3.47[40]; 2.70[41] alleles per locus in mango were reported. The PCR product size obtained by amplifying 8 SSRs varied from 120 to 320 bp which was comparable with the results generated by polymorphic bands ranging from 100 bp to 480 bp[40], 90 bp to 370 bp[41] and 130 bp to 245 bp[42] in mango.

The dendrogram generated from the UPGMA clustering widely positioned 16 mango cultivars into 5 fundamental clusters in conformity of clustering with morphological and fruit quality traits though the composition of cluster was different at both morphological and molecular levels. Clustering through SSRs placed Gulab Khas, Ranipasand and Chatterjee within the same cluster (Cluster A). On contrary, Chatterjee was placed in the different cluster when clustering was done considering morphological traits. Likewise, Madhu Chuski and Khota Lagga was placed in cluster B through deploying molecular data though, they placed within the same cluster along with Gulab Khas and Ranipasand when clustering was done through utilizing morphological traits. The clustering of Sarikhas, Himsagar, Banganpalli, Langra, Fazli within the same cluster (cluster C) according to the UPGMA based on molecular data was not homogeneous as they placed in different clusters at morphological level. Though, Gopal Bhog and Kancha Mitha placed within the same cluster considering both morphological and molecular level, this was not true for Lakhan Bhog, Gopi Bhog, Kamala Bhog and Kanchan Kosa as their inclusion within the same cluster was changed. The dendrogram represented that Ranipasand and Gulab Khas had been the maximum comparable hybrids with 88% similarity index. The SSRs deployed in the present study generated multiple loci due to their non-specificity. The results of this study exhibited consistency with earlier reports of mango where

similar trend of SSR polymorphism (71 to 81.8%), the number as well as the size of the alleles were detected[42].

Conclusion

In summary, it can be ascertained that enough variability existed among the tested mango cultivars due to their cross-pollinating nature. SSR primers deployed in the present study exhibited valuable findings regarding evaluation of the relationship among the mango cultivars which showed a high level of polymorphism. However, no single SSR primer could distinguish all accessions independently. The clustering pattern detected in the present study will also be useful towards selection of diverse parents in future mango breeding programmes.

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Conflict of Interests

Authors declare that there is no conflict of interest.

Author's Contributions

DS and BG conceptualized the experiment. DS conducted the experiment and collected the data. DS did the statistical analysis under the supervision of AD. DS, AD and KP prepared the draft manuscript and AD and BG refined and finalized the manuscript.

References

1 Joshi R, Kundu M & Singh C P, Efficient tool for identification on different mango cultivars, *Env Ecol*, 31 (1) (2013) 385-388.

2 Rymbai H, Laxman R H, Dinesh M R, Sunoj V S J, Ravishankar K V, *et al*., Diversity in leaf morphology and physiological characteristics among mango (*Mangifera indica*) cultivars popular in different agro-climatic regions of India, *Sci Hort*, 176 (2014) 189-193.

3 Department of Agriculture Co-operation and Farmers Welfare, Horticulture Statistics at a Glance, Government of India Controller of Publication, India, 2018

4 Samant D, Singh A K, Srivastava A & Singh N K, Assessment of genetic diversity in mango using inter-simple sequence repeat markers, *Indian J Hort*, 67 (2010) 1-8.

5 Manjunathagowda D C, Anjanappa M, Jayaswall K, Venugopalan R, Kumar A, *et al*., Variability and genetic diversity among selfed lines (S1) of onion (*Allium cepa* L.), *Indian J Tradit Know*, 20 (2021) 563-568.

6 Rana M, Gupta S, Kumar N, Ranjan R, Sah R P, *et al*., Genetic architecture and population structure of oat landraces (*Avena sativa* L.) using molecular and morphological descriptors, *Indian J Tradit Know*, 18 (2019) 439-450.

7 Litz R E, Biotechnology and mango improvement, *Acta Hortic*, 645 (2004) 85–92.

8 Yamanaka N, Hasran M, Dong He Xu, Tsunematsu H, Idris S *et al*., Genetic relationship and diversity of four *Mangifera* species revealed through AFLP analysis, *Genet Reour Crop Evol*, 53 (2006) 949–954.

9 Rocha A, Carlos L, Saloma˜o C, Saloma˜o T M F, Cruz C D *et al*., Genetic diversity of 'Uba´' Mango tree using ISSR markers, *Mol Biotechnol*, 50 (2012) 108-113. DOI 10.1007/s12033-011-9419-1

10 Kumar K J, Sreekala A K, Manikandan K, Preetha T S & Padmesh P, Intravarietal diversity analysis of a Western Ghat *Mangifera indica* L. variety 'Kottoorkonam' using ISSR markers, *Int J Biotechnol Biochem*, 12 (1) (2016) 85-94.

11 Luo C, He X H, Chen H, Ou S J & Gao M P, Analysis of diversity and relationships among mango cultivars using Start Codon Targeted (SCoT) markers, *Biochem Syst Ecol*, 38 (6) (2010) 1176-84.

12 Pruthvish R & Chikkaswamy B K, Genetic diversity and relationships among mango varieties using RAPD molecular markers, *Int J Curr Microbiol Appl Sci*, 5 (1) (2016) 778-787.

13 Prativa L, Dinesh M R & Ravishankar K V, Validation of hybridity in mango (*Mangifera indica* L.) through markers, *Bioinfolet*, 13 (1b) (2016) 145-148.

14 Chiang Y C, Tsai C M, Chen Y K H, Lee S R, Chen C H, *et al*., Development and characterization of 20 new polymorphic microsatellite markers from *Mangifera indica* (Anacardiaceae), *Am J Bot*, 99 (2012) e117–e119.

15 Dillon N L, Bally I S E, Wright C L, Hucks L, Innes D J *et al*., Genetic diversity of the Australian National Mango Gene Bank, *Sci Hortic*, 150 (2013) 213-226.

16 Azmat M A, Khan A A, Khan I A, Rajwana I A, Cheema H M N *et al*., Morphological characterization and SSR based DNA fingerprinting of elite commercial mango cultivars, *Pakistan J Agril Sci*, 53 (2) (2016) 321-330.

17 IPGRI, Descriptors for mango (*Mangifera indica* L.). International Plant Genetic Resources Institute, Rome, Italy, 1989, 21-26.

18 IPGRI, Descriptors for mango (*Mangifera indica* L.). International Plant Genetic Resources Institute, Rome, Italy, 2006, 45-46

19 PPV, FRA, Guidelines for the conduct of tests for distinctness, uniformity, stability of mango (*Mangifera indica* L.), protection of plant varieties and farmers' right authority, Ministry of Agriculture, Govt of India, New Delhi, India, 2008, 17-19.

20 AOAC, Association of Official Analytical Chemists, Official methods of analysis, Washington D.C, 1965, 75-78.

21 Ranganna S, *Handbook of analysis and quality control for fruits and vegetable products*, (Tata Mc Graw Hill Publishing Company Limited, New Delhi), 1986, 45-51.

22 Doyle J J & Doyle J L, A rapid DNA isolation procedure for small quantities of fresh leaf tissue, *Phytochem Bull*, 19 (1987) 11 - 15.

23 Sambrook J, Fritsch E F & Maniatis T, *Molecular cloning: A laboratory manual*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), 1989, 50-54.

24 Ravishankar K V, Reddy B H, Anand L & Dinesh M R, Development of new microsatellite markers from mango (*Mangifera indica*) and cross-species amplification, *Am J Bot*, (2011) 96-99.

25 Mahalanobis P C, On the generalized distance in Statistics, *Proc Natl Acad Sci India*, 2 (1936) 49-55.

26 Banfield C F, Principal Component Analysis for Genstat, *J Statistics Comput Simul*, 6 (1978) 211-222.

27 Sehgal D, Rajpal V R, Raina S N, Sasanuma T & Sasakuma T, Assaying polymorphism at DNA level for genetic diversity diagnostics of the safflower world germplasm resources, *Genetica*, 135 (2009) 457-470.

28 Jaccard P, Nouvelle recherches sur La distribution florale, *Bull Soc Vaud Sci Nat*, 44 (1908) 223 - 270.

29 Majumder D A N, Hassan L, Rahim M A & Kabir M A, Genetic diversity in mango (*Mangifera indica* L.) through multivariate analysis, *Bangladesh J Agril Res*, 38(2) (2013) 343-353.

30 Galal O A, Galal H A & Aboulila A A, Genetic variability and molecular characterization of some local and imported mango cultivars in Egypt, *Egypt J Genet Cytol*, 46 (2017) 121-138.

31 Barholia A K & Sangeeta Y, Divergence for fruit characters in mango (*Mangifera indica* L.), *African J Agril Sci Technol*, 2 (2) (2014) 65-67.

32 Rajan S, Yadava L P, Kumar R & Sexena S K, Genetic divergence in mango varieties and possible use in breeding, *Indian J Hort*, 66 (1) (2009) 7-12.

33 Anuradha B, Aditya J P, Singh G, Gupta A, Agarwal P K *et al*., Assessment of genetic diversity in indigenous and exotic collections of black soybean (Glycine max (L.) Merrill.), *SABRAO J Breed Genet*, 43 (1) (2011) 81-90.

34 Duval M F, Bunel J, Sitbon C & Risterucc A M, Development of microsatellite markers for mango (*Mangifera indica* L.), *Mol Ecol*, 5 (4) (2005) 824-826.

35 Ravishankar K V, Reddy B H, Anand L & Dinesh M R, Development of new microsatellite markers from mango (*Mangifera indica*) and cross-species amplification, *Am J Bot*, 98 (4) (2011) 96-99.

36 Viruel M A, Escribano P, Barbieri M, Ferri M & Hormaza J I, Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L.) with microsatellites, *Mol Breeding*, 15 (2005) 383-393.

37 Schnell R J, Brown J S, Olano C T, Meerow A W, Campbell R J, *et al*., Mango genetic diversity analysis and pedigree influences for Florida cultivars using microsatellite markers, *J American Soc Hort Sci*, 131 (2) (2006) 214-224.

38 Singh S & Bhat K V, Molecular characterization and analysis of geographical differentiation of Indian mango (*Mangifera indica* L.) germplasm, *Acta Hortic*, 839 (12) (2009) 599-606.

39 Anshuman S, Singh A K & Singh S K, S S R markers reveal genetic diversity in closely related mango hybrids, *Indian J Hortic*, 69 (3) (2012) 299-305.

40 Kumar M, Ponnuswami V, Nagarajan P, Jeyakumar P & Senthil N, Molecular characterization of ten mango cultivars using simple sequences repeat (SSR) markers, *Afr J Biotechnol*, 12 (47) (2013) 6568-6573.

41 Begum H, Reddy M T, Malathi S, Reddy B P, Arcahk S, *et al*., Molecular analysis for genetic distinctiveness and relationships of indigenous landraces with popular cultivars of mango (*Mangifera indica* L.) In Andhra Pradesh, India, *Asian Aust J Plant Sci Biotechnol*, 6 (1) (2012) 24-37.

42 Begum H, Reddy M T, Malathi S, Reddy B P, Arcahk S, *et al*., Molecular analysis of intracultivar polymorphism of 'Panchadarakalasa' mango by microsatellite markers, *Jordan J Biol Sci*, 6 (2) (2013) 127-136.