

Association analysis for pomological traits in mango (*Mangifera indica* L.) by genic-SSR markers

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Abstract

Key message A set of five genic-SSR markers ($p < 0.1$) are significantly associated ($r^2 = 19.92\%$) with six critical pomological traits. This information will facilitate marker-assisted genetic enhancement in mango.

Abstract India is considered the home of mango cultivation and is the highest contributor to the global mango production. However, India has low productivity because of the lack of understanding of the genomic complexity of regular bearing habit, fruit quality, and yield-related traits. In the present investigation, 17 critical pomological traits in mango were mapped through genome-wide association mapping using the generalized linear model (GLM) and mixed linear model (MLM) approaches. Significant phenotypic variability in the selected traits was observed in 60 mango varieties, which indicated the suitability of these varieties for association studies. Genotyping of the varieties was performed using 87 polymorphic genic-simple sequence repeats (SSR) markers. Population structure analysis performed using the genotyping data revealed three distinct sub-populations that corroborated with the geographical origin of the varieties.

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Subsequently, the association analysis performed using the GLM identified 23 genic-SSR markers associated with 13 different pomological traits. However, only five genic-SSR markers were associated with six different pomological traits in MLM approach. The A and C alleles of the MSSR 190 locus were significantly associated with fruit diameter, pulp: stone ratio and fruit weight with significant phenotypic variance, and the B allele of the MSSR 146 locus was associated with fruit length and fruit width in both the GLM and MLM approaches with a high amount of phenotypic variance. The ranges of phenotypic variation explained by the associated loci were 13.18–42.39 and 13.31–27.34% in the GLM and MLM approaches, respectively. Extensive phenotypic and genotypic characterization of mango varieties, population structure, and markers associated with critical traits provided in this study will facilitate marker-assisted improvement programs in mango.

Keywords Mango · Population structure · Marker–trait association · Genic-SSR markers · Yield

Abbreviations

AMOVA	Analysis of molecular variance
ABI	Alternate bearing index
SSR	Simple sequence repeat
GLM	Generalized linear model
MLM	Mixed linear model

Introduction

Mango (*Mangifera indica* L.) is the favorite fruit of India because of its sweetness, richness of taste, enormous variability, large production volume, and variety of end

usage (Singh 1960). Mango, belonging to family Anacardiaceae, and genus *Mangifera*, is an amphidiploid or allotetraploid tree ($2n = 40$). It has a small genome size of approximately 450 Mbp (Arumuganathan and Earle 1991). It originated in the northern foothills of India-Myanmar region. It includes 73 genera and approximately 830 species (Singh 1996; Yamanaka et al. 2006). India is the largest producer of mango in the world, with an annual production of 18 million tons from an area of 2.50 million hectares (NHB 2014), contributing approximately 56% of the total world production. Despite sustained research efforts for increasing the production and productivity over the past four decades, the productivity (t/ha) of mango orchards in India remains low at 7.20 with that of China 9.35, Thailand 8.20, Pakistan 10.62, Mexico 9.33 and Brazil 15.83 (Yadav and Pandey 2016). In addition, most of the produced fruit does not fulfill the quality requirement of consumer and meet export quality standards. Several factors are associated with low productivity and poor fruit quality. Among these factors, the alternate fruit-bearing habit of mango is of immense concern, because it causes considerable economic losses to growers because of poor yield or failure of the entire crop during the “off year” and low price during “on year” caused by a glut in the market (Monselise and Goldschmidt 1982). The sub-standard fruit quality [in terms of peel color, dwarfness, total soluble solids (TSS), ascorbic acid, fiberlessness, shape, size and pulp:stone ratio] for table and export purposes is another major problem in mango production. In the past, breeding approaches have been used by several researchers to improve yield and quality parameters such as alternate bearing (Roy 1953; Singh 1961; Luckwill 1970; Shamy and Eissa 1990; Davenport 2007; Ramírez et al. 2010; Nakagawa et al. 2012), fruit peel color (Sharma et al. 1983; Sharma 1987; Iyer and Subramanyam 1987; Pandey and Singh 1993, 1999; Pandey 1994; Prabhuram 1998) and fruit shape and size (Nayak 2010; Lavi et al. 1989). The main hurdles limiting the genetic studies and breeding programs of mango are a long juvenile period, large tree size, single-seededness, high fruit drop, sex ratio, self-incompatibility, and high requirement for cross-pollination (Nayak 2010). Therefore, efforts to enhance genomic resources and genetic understanding in mango are needed.

For genetic and genomics studies, microsatellite markers or simple sequence repeat (SSR) markers, have become the markers of choice because of their robustness, co-dominance, locus-specific nature, and abundance in genome. Consequently, they have been extensively used in genetic diversity analysis studies in mango (Surapaneni et al. 2013; Dillon et al. 2014; Begum et al. 2014; Gitahi et al. 2014; Ravishankar et al. 2015; Dinesh et al. 2015; Panwar et al. 2015). The SSR markers have been successfully used for genetic mapping, gene tagging, and

genetic diversity assessment in numerous fruit crops including mango (Dinesh et al. 2015; Liang et al. 2015; Chaluvadi et al. 2014; Li et al. 2014; Rana et al. 2015). Identification of linked markers underlying major economic traits, such as disease resistance or stress tolerance in mango is extremely necessary. Furthermore, regular bearing, fruit quality, and yield-associated traits are extremely essential in mango. Thus far, however, sufficient efforts have not been made towards the identification of genomic loci governing crucial phenotypic traits in mango. Genic-SSRs are a type of gene-specific marker derived from the transcribed regions of the genome. They specifically target the functional region of the genome. They have potential for linkage to loci that mainly contribute to phenotypes. Such an endeavor will facilitate marker-assisted selection (MAS) and planning future improvement programs by selecting parents with favorable alleles. A promising approach for plant breeders, association analysis eliminates the main drawback of classical linkage analysis, namely prolonged and expensive development of specific mapping populations. Furthermore, the association analysis approach has the potential to assess a large number of alleles with higher mapping resolution (Yu et al. 2006; Gupta et al. 2014) than other approaches.

The identification of genomic regions responsible for alternate bearing, fruit quality, and yield-related traits is the first step for the identification of candidate genes. At present, few studies on the identification of genomic loci for the selected traits, such as biennial bearing habit in different fruit crops in olive (Sadok et al. 2013; Yanik et al. 2013; Dündar et al. 2013), apple (Guitton et al. 2012), cranberry (Schlautman et al. 2015), and citrus (Garcia et al. 2000), are available. Genomic SSR markers have been used to dissect fruit quality and yield attributes in apple (Espley et al. 2007; Morimoto et al. 2013), raspberry (McCallum et al. 2010), pear (Quilot et al. 2004), pomegranate (HarelBeja et al. 2015), peach (Verde et al. 2002), apricot (Salazar et al. 2013), sweet cherry (Ganopoulos et al. 2011), and papaya (Blas et al. 2012).

Apart from the use of genic-markers, several analytical advances are available for the identification of loci governing traits. These advances include the stratification of population structure and statistical models such as generalized linear model (GLM) and mixed linear model (MLM) approaches. A unified, mixed-model approach for association mapping combined with a population structure analysis is a dependable and robust system for identifying reliable quantitative trait loci (QTL) (Yu and Buckler 2006; Zhao et al. 2007). This study focuses on analyzing the population structure of some varieties and identifying linked molecular markers for alternate bearing, fruit quality, and yield-related traits for mango. The proposed research work on association studies would considerably

facilitate the understanding of the genetic basis of the component traits involved in alternate bearing, fruit quality, yield, and its components will enable the development of functional markers for these economic traits and will accelerate the mango breeding programs.

Materials and methods

Selection of varieties and genomic DNA extraction

A total of 60 mango varieties were collected from the scientifically maintained mango germplasm block of Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India (Table 1), where uniform treatment was given to all the varieties based on scientific recommendations and operations, such as spraying, intercultural operation, water and nutrient scheduling, insect pest management, pruning or training, harvesting, and weeding. These mango varieties were collected from different regions of India. Except for a few recently bred hybrids, all other varieties represent land races, which originated as chance seedlings and have been conserved in situ by vegetative propagation. These varieties were selected for screening and evaluation of component traits to identify the loci governing alternate bearing, fruit quality, and yield. The young, light-green tender leaves (5 g) of each variety were used for DNA extraction using the CTAB method described by Murray and Thompson (1980) with minor modifications. To overcome the co-precipitation of polysaccharides, the DNA precipitation step included NaCl (5 M) treatment (Porebski et al. 1997), and polyvinyl pyrrolidone (PVP, 2%) was used for the removal of phenolic compounds. Purified DNA was quantified using nanodrop (Thermo Scientific, USA). The integrity was assessed using agarose (0.8%) gel electrophoresis.

Phenotypic evaluation

To assess the phenotypic variability, correlation, and heritability as detailed below, a total of ten randomly selected fruits were used for bio-metric observation in each variety and replication. Data of 17 major quantitative traits with three replications (a single tree was selected to represent one replication) were analyzed using a randomized block design suggested by Singh and Chaudhury (1977). Uniform cultural practices were applied to experimental materials. The fruits were harvested at the horticultural maturity stage and for uniform ripening, the mango fruits immersed in ethrel solution (700 ppm; 1.8 mL/L) prepared in lukewarm water (52 ± 1 °C) for 5 min. The addition of carbendazim (0.5 g/L) to this solution provided protection from fungal

diseases after harvest. For estimation of the alternate bearing index (ABI) and other traits, 5-year and 2-year average data, respectively, were considered and analyzed.

Fruit yield

Fruit yield was recorded by counting the number of fruits at the time of harvesting. The total yield in kg per tree was calculated by multiplying the number of fruits per tree with average fruit weight.

Alternate bearing index

A 5-year (2011, 2012, 2013, 2014, and 2015) yield per tree data were used and calculated as per formula given by Monselise and Goldschmidt (1982). $ABI = \{1/n - 1\} \{ |(a_2 - a_1)/(a_2 + a_1) + |(a_3 - a_2)/(a_3 + a_2) | \}$; where n is the number of years, and $a_1, a_2, \dots, a_{(n-1)}, a_n$ is the yield of corresponding years.

Peel color

The peel color of each variety was measured at ripening using a calibrated Hunter Lab UltraScan PRO colorimeter attached with an Easy Match QC software (Hunter Associate Laboratory Inc., Reston, USA), and the values were expressed as L^* (lightness; 0 = black, 100 = white), a^* ($-a$ = greenness, $+a$ = redness), and b^* ($-b$ = blueness, $+b$ = yellowness). The values of C for chromaticity (color intensity), and H for hue were calculated using the following equations.

$$C = [(a^*)^2 + (b^*)^2]^{1/2}$$

$$H = \arctan b^*/a^*$$

Fruit length

The fruit length was measured from the base of the fruit to the top of the groove at the calyx end using digital vernier calipers and expressed in millimeters.

Fruit diameter

The fruit diameter was measured at the maximum width of the fruit using digital vernier calipers and expressed in millimeters.

Estimation of fruit shape index

The fruit shape index (FSI) was calculated as the ratio of fruit length and diameter measured by vernier calipers.

The FSI value was determined as follows:

Table 1 List of mango varieties used as association mapping panel with their source and subpopulation (based on Fst value) in which they are grouped during population structure study

S. no.	Variety	Origin	Fst1	Fst2	Fst3	Population	S. No.	Variety	Origin	Fst1	Fst2	Fst3	Population
1	Alphan	Saharanpur, Uttar Pradesh	0.006	0.989	0.005	B	31	Pusa Shresth	I.A.R.I., New Delhi	0.86	0.099	0.041	A
2	Janardan Pasand	Kadiam, East Godavari, Andhra Pradesh	0.163	0.787	0.051	B	32	Pusa Arunima	I.A.R.I., New Delhi	0.93	0.017	0.053	A
3	Bhadauran	Saharanpur, Uttar Pradesh	0.008	0.987	0.005	B	33	Pusa Lalima	I.A.R.I., New Delhi	0.728	0.15	0.122	A
4	Dushehari	Sabour, Bihar	0.008	0.949	0.044	B	34	Amrapali	I.A.R.I., New Delhi	0.953	0.041	0.006	A
5	Gulab Khas	Sabour, Bihar	0.447	0.538	0.014	B	35	Ramkela	Saharanpur, Uttar Pradesh	0.958	0.029	0.013	A
6	Hardil Aziz	Saharanpur, Uttar Pradesh	0.103	0.891	0.005	B	36	Dushehari	Malhabad, Uttar Pradesh	0.552	0.257	0.191	A
7	Husnara	Sabour, Bihar	0.034	0.952	0.013	B	37	Pusa Peetamber	I.A.R.I., New Delhi	0.747	0.243	0.009	A
8	Hybrid-165	Delhi	0.068	0.926	0.007	B	38	Kala	Saharanpur, Uttar Pradesh	0.933	0.061	0.006	A
9	Xavier	Panji, Goa	0.007	0.989	0.004	B	39	Kaleped	Sangareddy, Telangana	0.489	0.492	0.019	B
10	Vanraj	Paria, Gujrat	0.006	0.991	0.003	B	40	Olour	Thrissur, Kerala	0.024	0.958	0.018	B
11	Extreema	Brazil	0.007	0.986	0.007	B	41	Gulab Jamun	Saharanpur, Uttar Pradesh	0.186	0.726	0.089	B
12	Machli	Lucknow, Uttar Pradesh	0.009	0.987	0.004	B	42	S.B. Alitbagh	Saharanpur, Uttar Pradesh	0.334	0.609	0.056	B
13	Mahmood Vikarabad	Lucknow, Uttar Pradesh	0.09	0.904	0.006	B	43	Kurrakan	Thrissur, Kerala	0.228	0.711	0.061	B
14	Mombasa	Saharanpur, Uttar Pradesh	0.023	0.969	0.008	B	44	Ferandin	Panji, Goa	0.16	0.822	0.019	B
15	Primor-de Amoreira	Brazil	0.022	0.969	0.009	B	45	Irwin	Miami, Florida	0.153	0.751	0.096	B
16	Safdar Pasand	Saharanpur, Uttar Pradesh	0.063	0.868	0.069	B	46	Chausa	Lucknow, Uttar Pradesh	0.477	0.414	0.109	A
17	Rautal	Saharanpur, Uttar Pradesh	0.071	0.855	0.074	B	47	Tommy Atkins	Miami, Florida	0.011	0.061	0.929	C
18	Sonatal	Muzaffarnagar Uttar Pradesh	0.037	0.957	0.006	B	48	Smith	Miami, Florida	0.017	0.27	0.713	C
19	Tephala	Lucknow, Uttar Pradesh	0.109	0.884	0.008	B	49	Langra	Lucknow, Uttar Pradesh	0.538	0.361	0.101	A
20	Anniesia Hyderabad	Sangareddy, Telangana	0.04	0.953	0.008	B	50	Bombay Green	Kirkee, Maharashtra	0.009	0.986	0.006	B
21	Neelum	Krishnagiri, Tamilnadu	0.03	0.96	0.01	B	51	Zardalu	Murshidabad, West Bengal	0.027	0.626	0.347	B
22	Nissar Pasand	Malhabad, Uttar Pradesh	0.017	0.977	0.006	B	52	Pusa Surya	Miami, Florida	0.387	0.008	0.605	C
23	Ratna	Rantnagiri	0.021	0.966	0.013	B	53	Edward	Miami, Florida	0.008	0.004	0.988	C
24	Alphanso	Rantnagiri	0.017	0.927	0.056	B	54	Saint Alexandrina	Brasilia	0.427	0.501	0.073	B

Table 1 continued

S. no.	Variety	Origin	S. No.	Variety	Origin	Fst1	Fst2	Fst3	Population	S. No.	Variety	Origin	Fst1	Fst2	Fst3	Population
25	Ametista	Brazil	55	Elard	Miami, Florida	0.007	0.989	0.004	B	55	Elard	Miami, Florida	0.041	0.349	0.61	C
26	Itruba	Brazil	56	Lucknow Safeda	Lucknow, Uttar Pradesh	0.054	0.937	0.009	B	56	Lucknow Safeda	Lucknow, Uttar Pradesh	0.534	0.015	0.451	A
27	Rosari	Brasilia	57	Sensation	Miami, Florida	0.047	0.923	0.03	B	57	Sensation	Miami, Florida	0.032	0.08	0.888	C
28	Totapari Red Small	Krishnagiri, Tamil Nadu	58	Khasulkhas	Saharanpur, Uttar Pradesh	0.028	0.952	0.021	B	58	Khasulkhas	Saharanpur, Uttar Pradesh	0.543	0.397	0.06	A
29	Mallika	I.A.R.I., New Delhi	59	Willard	Miami, Florida	0.799	0.192	0.009	A	59	Willard	Miami, Florida	0.072	0.501	0.427	B
30	Pusa Pratibha	I.A.R.I., New Delhi	60	Zill	Miami, Florida	0.464	0.218	0.318	A	60	Zill	Miami, Florida	0.069	0.909	0.023	B

Fst fixation index, I.A.R.I. Indian Agricultural Research Institute

$$\text{Fruit Shape Index (FSI)} = \frac{\text{Length of fruit}}{\text{Diameter of fruit}}$$

Pulp:stone ratio

This ratio was calculated by dividing the pulp weight by fresh stone weight.

Total soluble solids

Total soluble solids ($^{\circ}$ Brix): the total soluble solids (TSS) content of the berry was determined with the help of hand refractometer at room temperature $26 \pm 2^{\circ}\text{C}$ (AOAC 1994).

Titrateable acidity

Titrateable acidity was determined using 10-g aliquots of strawberry fruits poured into 50 mL of distilled water and titrated against 0.1 N NaOH to an end-point of pH 8.1, where a light pink color developed. Titrateable acidity was expressed as the percentage of citric acid and was calculated using the method given in AOAC (1994).

TSS/acid

This ratio was calculated by dividing TSS and titrateable acidity values.

Ascorbic acid

Ascorbic acid content of fresh fruit was determined spectrophotometrically by metaphosphoric acid extraction of 2,6-dichlorophenol indophenol dye as described by Robinson and Stotz (1945) using a NanoDrop spectrophotometer (Model Thermo 8000, USA) at a wavelength of 500 nm. The results were expressed as mg ascorbic acid \times 100/g fresh weight.

SSR genotyping

A total of 100 new genic-SSR loci were designed and synthesized from the leaf transcriptome sequencing data of mango (*M. indica* L.) cv. Amrapali (Table S1) (Singh et al. 2014; Mahato et al. 2015). The selected markers were chosen randomly from different contigs of mango leaf transcriptome sequences (unpublished data) hypothesizing their genome-wide distribution because although mango genome sequencing is in progress, no reliable high-density linkage map is available for this crop. Both PCR reaction master mix composition and temperature conditions were empirically standardized for newly synthesized genic-SSR

markers. Each 10 μL reaction mixture contained 3 μL genomic DNA (10 ng/ μL), 1.0 μL 10 \times PCR buffer (10 mM Tris HCl, 50 mM KCl, pH 8.3), 0.5 μL dNTP mix (0.2 mM each of dATP, dGTP, dTTP, and dCTP), 0.5 μL (10 pmol) of each of the two primers, 0.15 μL *Taq* DNA polymerase (5 U/ μL) (Vivantis Technologies, USA), and 4.35 μL MilliQ water. The PCR reaction was carried out as follows: 1 cycle of 3 min at 94 $^{\circ}\text{C}$ for initial denaturation, then 36 cycles with a denaturation step at 94 $^{\circ}\text{C}$ for 30 s, an annealing step for 1 min at the respective annealing temperatures of each primer in the range of 48.3–53 $^{\circ}\text{C}$, an initial extension at 72 $^{\circ}\text{C}$ for 1 min, and a last cycle for final extension at 72 $^{\circ}\text{C}$ for 10 min. A 3% metaphor agarose gel was used to resolve the amplified PCR products. Amplified results were confirmed by three replicate assays. Nonspecific fragments (errors due to stuttering) and null alleles amplified by genic-SSR markers were analyzed using Microchecker 2.2.3 (van Oosterhout et al. 2004). The clear and reproducible alleles amplified by each genic-SSR marker were scored based on their fragment size (bp).

Statistical analysis

Variations in 17 pomological traits studied among 60 mango varieties were observed in three independent replicates (consisting one tree in each replication) for two consequent years (2013 and 2014), except for ABI, which was calculated on the basis of 5-year yield data (2011–2015) under field conditions. The data were analyzed using the ANOVA procedure of SAS 9.2 (SAS Institute, Cary, NC, USA) to derive their summary statistics including mean, range, standard deviation, variance, and coefficient of variation. Furthermore, the Shapiro–Wilk normality test, measuring the data distribution of each trait, was carried out using the univariate procedure in the SAS 9.2 software. The positive and negative correlations among the 17 pomological traits in 60 varieties were measured using Pearson correlation coefficient at 1 and 5% levels of significance in SAS 9.2. Genotypic (σ_g^2) and phenotypic variances (σ_{ph}^2) were obtained from the analysis of variance table according to Comstock and Robinson (1952) using the following equations: (where r : replication, s : season MS1: mean square for variety, MS2: mean square for cultivar \times season).

$$\sigma_g^2 = \frac{\text{MS1} - \text{MS2}}{r \times s}$$

$$\sigma_{ph}^2 = \frac{\text{MS1}}{r \times s}$$

Broad-sense heritability was estimated as per standard methods described by Lush (1949), Johnson et al. (1955) and Hanson et al. (1956).

$$\%h_b^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

where, h_b^2 is the heritability, σ_g^2 is the genotypic variance, σ_p^2 is the phenotypic variance.

In the current study, 100 novel genic-SSRs were selected and used for genotyping. Among those markers, 87 polymorphic markers were used for further population structure and marker–trait analysis. The remaining 13 genic-SSRs were monomorphic and were not included in the analysis.

For population structure, we assumed an admixed model with a uniform prior probability and independent allele frequency of the number of populations, K . All the runs with 100,000 MCMC replicates after a burn-in of 100,000 replicates were conducted for $K = 2$ –10. Five independent runs were performed for each value of K to generate our estimate of the true number of sub-populations (Pritchard et al. 2000). The ‘Structure harvester’ program (<http://taylor0.biology.ucla.edu>) was used to determine the final K value ($K = 3$ was optimum for this analysis), based on both the $\text{Ln}P$ (D) and Evanno’s ΔK (Evanno et al. 2005; Earl et al. 2012). Markers exhibiting a probability value (p value) less than 0.01 thresholds were considered significantly associated with the particular phenotypic trait. The association of each alleles of the locus with the trait of interest was tested using two approaches, namely the GLM and MLM (Yu et al. 2006) approaches, wherein the population structure and kinship in the model was implemented using the TASSEL v2.0.1 software (Bradbury et al. 2007). The MLM model exhibited the least variation in observed p values from expected p values in the quartile–quartile plot compared with the variation of the Q (population structure) or K (kinship) model only.

Results

Phenotypic evaluation

Experimental field data were distributed normally, except for traits such as L^* , a^* , fruit length, fruit width, and FSI (Table S2; Fig. S1). Significant differences among individuals were observed through ANOVA for all measured traits (Table S3; Table 2). Among the traits analyzed, the ABI varied from 0.03 to 0.93 (average of 0.42 ± 0.03); however, the pulp:stone ratio ranged from 0.45 to 9.37 (3.28 ± 0.23) (Table 3). The yield range was 13.50–88.00 and the fruit weight varied in the range 71.62–327.50 (average 30.84 ± 1.79 kg) and 163.16 ± 8.04 g), respectively. The a^* , pulp:stone ratio, ABI, and fruit weight exhibited greater CVs, 1.11, 0.53, 0.63, and 0.38,

Table 2 Mean square and analysis of variance for different phenotypic traits over two years

Source of variation	df	L*	a*	b*	C	H	FW	FL	FD	FW	FSI	TSS	A	T/A	AA	P:S	Y
Year 2013																	
Variety	59	148.0**	202.6**	278.6**	274.3**	0.0**3	11646.5**	958.7**	343.5**	254.6**	0.34**	20.6**	0.03**	75.6**	9.09**	1643.5**	608.0**
Replication	179	0.590	0.06	0.053	0.56	0.001	6.5375	11.80	1.99	0.06	0.001	0.089	0.0001	52.60	0.001	0.154	0.149
Error	118	1.12	0.034	0.73	1.05	0.001	12.53	3.62	1.22	1.36	0.0001	0.138	0.0001	1.54	0.004	2.57	0.260
Source of variation	df	L*	a*	b* <td>C</td> <td>H</td> <td>FW</td> <td>FL</td> <td>FD</td> <td>FWi</td> <td>FSI</td> <td>TSS</td> <td>A</td> <td>T/A</td> <td>AA</td> <td>P:S</td> <td>Y</td>	C	H	FW	FL	FD	FWi	FSI	TSS	A	T/A	AA	P:S	Y
Year 2014																	
Variety	59	123.7**	332.2**	250.7**	296.5**	0.066	11903.11**	1534.86**	736.9**	754.1**	0.31**	21.67**	0.02**	74.9**	9.06**	1622.3**	662.1**
Replication	179	0.014	0.008	0.666	0.487	0.004	71.62*	0.620	0.524	2.85	0.001	0.135	0.0001	51.95	0.011	0.142	0.993
Error	118	1.23	0.059	0.847	0.715	0.027	11.98	3.94	2.11	1.93	0.001	0.129	0.0001	1.71	0.006	2.28	0.672

df degrees of freedom, L lightness, a extends from green (-a) to red (+a), b blue (-b) to yellow (+b) redness, C chroma, H: hue, FW fruit weight (g), FL fruit length (mm), FD fruit diameter (mm), FWi fruit width (mm), FSI fruit shape index, TSS total soluble solids (°B), A acidity (%), T/A TSS:acidity, P:S pulp:stone, Y yield (kg/tree)

* Significant at 0.05 and ** significant at 0.01 level of probability

respectively, and a minimum in hue value (0.07). The broad-sense heritability ranged from 75.47 (TSS) to 93.23% (fruit weight) and all the selected traits had an H^2 higher than 75%, suggesting that all these traits are stably inherited (Table 3) (Fig. 1).

The correlations among the yield and quality traits are presented in Table 4. The b^* value was found positively and significantly correlated with L^* and a^* . Similarly, the significantly positive correlation was also found between chromaticity, L^* , a^* , and b^* . The yield exhibited a positive and significant correlation with the fruit weight, fruit diameter, and fruit width. The fruit length had a significant and positive correlation with fruit weight, however, the fruit diameter exhibited a positive and significant association with fruit weight and fruit width, whereas a negative and significant association with fruit length. The FSI showed a significantly positive correlation with the fruit length, and a significantly negative association with fruit diameter. In the case of sugar:acid ratio, a highly positive significant correlation was observed with the TSS, however, a negative significantly correlation was found with titratable acidity. The ascorbic acid exhibited a positive significant correlation with titratable acidity and had exhibited highly negative significant correlation with yield per tree. These wide phenotypic trait variations among 60 mango varieties indicated that the constituted association panel was suitable for association mapping for selected traits.

Population structure analysis

Of the 100 genome-wide genic-SSR loci, 87 loci were found polymorphic and 13 loci were monomorphic in nature and these polymorphic markers were further used in determining the population structure and marker-trait association analysis (Table S4). The Bayesian model-based program STRUCTURE 2.2 was used to infer the population structure. Admixture model-based simulations were carried out by varying K from 2 to 10 with ten runs for each K , using all 60 varieties which revealed evident knees at $K = 3$. The average $\text{Ln}P(D)$ (log-likelihood) value increased continuously with the increase in K from 2 to 10. However, its most apparent inflection was obtained at one of the best replicates of $K = 3$. The results of population numbers (K) were further confirmed using ΔK estimation. A sharp peak with the maximum value of ΔK was obtained at $K = 3$, thereby confirming the classification of 60 mangoes varieties into three distinct population groups (Fig. 2). Using this approach, 60 varieties were assigned to the corresponding A–C subpopulations, representing 23.33% (14), 66.66% (40), and 10.00% (6) of the varieties used for analysis (Table 1). The three sub-populations (A–C) had fixation index (Fst) equal to 0.15, 0.22, and 0.16,

Table 3 Summary statistics of 17 pomological traits evaluated in 60 mango varieties

Variable	Minimum	Maximum	Mean ± SE	Std dev	CV	Variance	Skewness	Kurtosis	H ² (broad sense) (%)	p value of normality test
L*	42.23	71.68	54.97 ± 0.85	6.61	0.12	43.74	0.49	-0.12	90.30	0.272
a*	-10.07	28.54	7.51 ± 1.08	8.37	1.11	70.06	-0.25	-0.35	85.67	0.141
b*	16.89	56.67	42.97 ± 1.21	9.41	0.22	88.48	-0.73	0.01	84.95	0.009
Chroma	18.08	57.65	44.40 ± 9.43	9.43	0.21	88.89	-0.73	0.14	83.98	0.008
Hue	0.95	1.57	1.50 ± 0.10	0.10	0.07	0.01	-3.49	15.72	85.04	0.001
Fruit weight	72.61	327.50	163.16 ± 8.04	62.29	0.38	3879.86	0.73	-0.13	93.23	0.008
Fruit length	52.14	138.39	90.99 ± 2.51	19.47	0.21	378.94	0.37	-0.17	81.06	0.313
Fruit diameter	40.40	94.53	62.48 ± 1.55	12.01	0.19	144.15	0.80	0.28	81.23	0.012
Fruit width	35.13	89.04	57.49 ± 1.42	11.00	0.19	120.98	0.66	0.15	90.40	0.054
Fruit shape index	0.72	2.54	1.49 ± 0.04	0.33	0.22	0.11	0.24	1.46	83.45	0.210
TSS	12.50	21.75	17.32 ± 0.34	2.62	0.15	6.87	0.01	-1.09	75.47	0.021
Acidity	0.16	0.51	0.28 ± 0.01	0.09	0.31	0.01	0.72	-0.28	86.56	0.003
TSS/acidity	34.91	110.46	67.85 ± 2.83	21.93	0.32	480.74	0.26	-1.14	84.28	0.010
Ascorbic acid	22.67	46.72	32.32 ± 0.75	5.84	0.18	34.09	0.43	-0.67	85.34	0.036
Pulp:stone	0.45	9.37	3.28 ± 0.23	1.75	0.53	3.06	1.04	1.78	82.05	0.005
Yield (kg)/tree	13.50	88.00	30.84 ± 1.79	13.84	0.45	191.57	1.58	3.88	84.23	0.001
ABI	0.03	0.93	0.42 ± 0.03	0.27	0.63	0.07	0.06	-0.97	82.94	0.001

ABI alternate bearing index, TSS total soluble solids, CV coefficient of variation, Std dev standard deviation, p probability

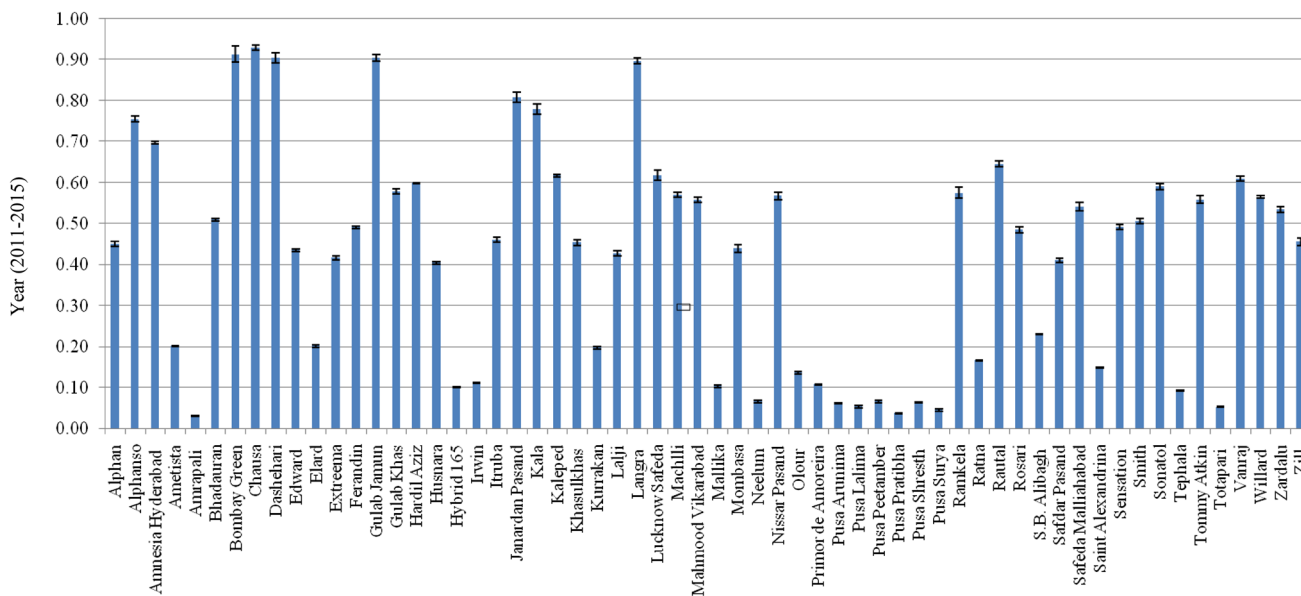


Fig. 1 Trends of alternate bearing index over 5-year period (2011–2015) among 60 mango varieties. Vertical bar represents mean ± standard error

respectively, supporting the existence of moderate population structures. The overall Fst value estimated within the subpopulations was 0.176 (Table 5).

Furthermore, the genetic distances among these three sub-populations by Fst value were also measured which showed the variable level of genetic differentiation

Table 4 Pearson correlation coefficient among 17 pomological traits evaluated in 60 mango varieties

Trait	<i>L</i> *	<i>a</i> *	<i>b</i> *	<i>C</i>	<i>H</i>	<i>FW</i>	<i>FL</i>	<i>FD</i>	<i>FWi</i>	<i>FSI</i>	<i>TSS</i>	<i>A</i>	<i>T/A</i>	<i>AA</i>	<i>P:S</i>	<i>Y</i>	<i>ABI</i>
<i>L</i> *	1.000																
<i>a</i> *	0.127 ^{NS}	1.000															
<i>b</i> *	0.615 ^{**}	0.482 ^{**}	1.000														
<i>C</i>	0.572 ^{**}	0.608 ^{**}	0.987 ^{**}	1.000													
<i>H</i>	0.330 ^{**}	-0.571 ^{**}	0.264 ^{**}	0.083 ^{NS}	1.000												
<i>FW</i>	0.145 ^{NS}	0.112 ^{NS}	0.015 ^{NS}	0.038 ^{NS}	-0.139 ^{NS}	1.000											
<i>FL</i>	-0.039 ^{NS}	0.287 ^{**}	0.003 ^{NS}	0.061 ^{NS}	-0.324 ^{**}	0.469 ^{**}	1.000										
<i>FD</i>	0.136 ^{NS}	0.030 ^{NS}	-0.104 ^{NS}	-0.091 ^{NS}	-0.081 ^{NS}	0.630 ^{**}	0.327 ^{**}	1.000									
<i>FWi</i>	0.150 [*]	0.277 ^{**}	0.057 ^{NS}	0.110 ^{NS}	-0.286 ^{**}	0.663 ^{**}	0.592 ^{**}	0.768 ^{**}	1.000								
<i>FSI</i>	-0.092 ^{NS}	0.284 ^{**}	0.132 ^{NS}	0.177 [*]	-0.246 ^{**}	-0.021 ^{NS}	0.677 ^{**}	-0.455 ^{**}	-0.017 ^{NS}	1.000							
<i>TSS</i>	0.046 ^{NS}	0.071 ^{NS}	0.039 ^{NS}	0.041 ^{NS}	-0.013 ^{NS}	0.067 ^{NS}	0.067 ^{NS}	-0.209 ^{**}	-0.189 [*]	0.247 ^{**}	1.000						
<i>A</i>	-0.015 ^{NS}	0.036 ^{NS}	-0.063 ^{NS}	-0.023 ^{NS}	-0.230 ^{**}	0.188 [*]	0.215 ^{**}	0.075 ^{NS}	0.055 ^{NS}	0.104 ^{NS}	0.103 ^{NS}	1.000					
<i>T/A</i>	-0.002 ^{NS}	0.025 ^{NS}	0.063 ^{NS}	0.033 ^{NS}	0.169 [*]	-0.148 [*]	-0.175 [*]	-0.191 [*]	-0.152 [*]	0.022 ^{NS}	0.389 ^{**}	-0.844 ^{**}	1.000				
<i>AA</i>	0.168 [*]	0.035 ^{NS}	-0.051 ^{NS}	-0.024 ^{NS}	-0.150 [*]	0.088 ^{NS}	0.282 ^{**}	0.086 ^{NS}	0.172 [*]	0.190 [*]	-0.124 ^{NS}	0.165 [*]	-0.210 ^{**}	1.000			
<i>P:S</i>	0.029 ^{NS}	-0.278 ^{**}	0.082 ^{NS}	0.058 ^{NS}	0.122 ^{NS}	-0.208 ^{**}	-0.185 [*]	-0.390 ^{**}	-0.418 ^{**}	0.156 [*]	0.157 [*]	0.091 ^{NS}	-0.003 ^{NS}	0.108 ^{NS}	1.000		
<i>Y</i>	-0.187 [*]	0.179 [*]	-0.221 ^{**}	-0.159 [*]	-0.333 ^{**}	0.237 ^{**}	0.374 ^{**}	0.393 ^{**}	0.368 ^{**}	-0.016 ^{NS}	-0.107 ^{NS}	0.239 ^{**}	-0.260 ^{**}	0.139 ^{NS}	-0.367 ^{**}	1.000	
<i>ABI</i>	0.028	-0.279 ^{NS}	0.082 ^{NS}	0.057 ^{NS}	0.120 ^{NS}	-0.208 ^{NS}	-0.184 ^{NS}	-0.389 ^{NS}	-0.417 ^{NS}	0.155 ^{NS}	0.156 ^{NS}	0.092 ^{NS}	0.108 ^{NS}	0.161 ^{NS}	0.019 ^{NS}	-0.365 [*]	1.000

L lightness, *a* extends from green (-*a*) to red (+*a*), *b* blue (-*b*) to yellow (+*b*) redness, *C* chroma, *H* hue, *FW* fruit weight (g), *FL* fruit length (mm), *FD* fruit diameter (mm), *FWi* fruit width (mm), *FSI* fruit shape index, *TSS* total soluble solids (°B), *A* acidity (%), *T/A* TSS:acidity, *P:S* pulp:stone, *Y* yield (kg/tree), *ABI* alternate bearing index

* Significant at 0.05 and ** significant at 0.01 level of probability, ^{NS} non-significant

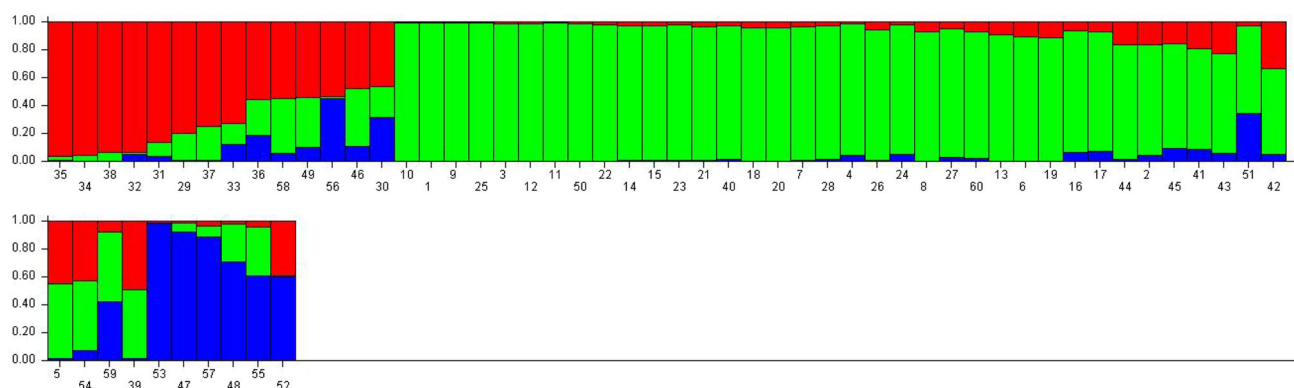


Fig. 2 Model based population structure plot for each variety with $K = 3$, using Structure software with 87 polymorphic genic-SSR loci. Color codes are as follows: Population A red, Population B green,

and Population C blue. The single vertical line represents an individual variety and different segments of each vertical line show extent of admixture in an individual (color figure online)

Table 5 Significant divergence between groups (sub-populations) and average distances (expected heterozygosity) between mango populations

Mean value of Fst within population		Heterozygosity (He)	Number of varieties
Fst_1	0.150	0.508	14
Fst_2	0.220	0.475	40
Fst_3	0.160	0.540	6

Fst fixation index as measure of genetic differentiation

between inferred populations. The F_{st} value ranged from 0.133 to 0.63 with an average 0.381, revealing smallest genetic distances between sub-pop B and C the largest between sub-population A and C (Table 6). There are mixed proportions of accessions with admixture with parental varieties in sub-populations defined by the structure, which might have grouped in different defined clusters. In the sub-population A, the percentage of mixed individuals was found to be 57.14%, likewise 25% for B and 50% for C having q K values of less than 80% for all three populations.

Association analysis

Marker–allele–trait association was performed for 17 pomological traits using GLM and MLM models using the TASSEL software. By performing genome scanning, a total of 23 genic-SSR markers were associated with 13 different pomological traits and contributed 13.18–42.39% of the phenotypic variation in the GLM approach. In combination, with all 23 markers explained an average of 21.20% of the trait variation (Table 7). However, only five SSR markers were associated with six different pomological traits and contributed 13.31–27.34% of the phenotypic variation (Table 8) in the MLM approach. In combination, all five markers explained an average of 19.92% of the trait variation.

In the GLM approach, a significant correlation was revealed by the B allele of MSSR 146, MSSR 153, MSSR

156, MSSR 173, and MSSR 130 markers for fruit width. The A/B allele of MSSR 186, A/B allele of MSSR 147, B allele of MSSR 156, B allele of MSSR 137, B allele of MSSR 179, and B allele of MSSR 146 were markers for fruit length. The C allele of MSSR 190, C allele of MSSR 189, B allele of MSSR 130, and B allele of MSSR 156 were markers for fruit diameter. The C allele of MSSR 155 and A allele of MSSR 187 were markers for acidity. The B allele of MSSR 160 for TSS. The B allele of MSSR 174, MSSR 178, and MSSR 146 were markers for ABI. The A allele of MSSR 173, A/B alleles of MSSR 102, A allele of MSSR 163, B allele of MSSR 146, A/C alleles of MSSR 190, and MSSR 189 were markers for fruit weight. The A allele of MSSR 190 and A/C alleles of MSSR 189 were markers for pulp:stone ratio. The A allele of MSSR 140, B allele of MSSR 179, and MSSR 174 were markers for yield/tree. The A/B allele of MSSR 106, and B allele of MSSR 146 were markers for hue. The B allele of MSSR 187, MSSR 117, and B/C allele of MSSR 155 were markers for TSS/acid ratio. The A/B allele of MSSR 137 was a marker for FSI (Table 7). Of the total 23 associated genic-SSR markers, 11 were found to be associated with more than one pomological trait. The MSSR 146 marker showed significant p value for multiple traits such as fruit width, ABI, fruit length, hue value, and fruit weight with considerable phenotypic variance. Similarly, the MSSR 190 was associated with the fruit diameter, pulp:stone ratio, and fruit weight. The MSSR 189 exhibited association with fruit diameter, pulp:stone ratio, and fruit weight; however,

Table 6 Genetic distances between different populations from structure analysis

Clusters	Pop A	Pop B	Pop C
Pop A	0		
Pop B	0.237	0	
Pop C	0.631	0.133	0

Pop population

MSSR 155 was associated with fruit length and TSS/acid ratio. Similarly, MSSR 156 was associated with fruit length and fruit width, MSSR 130 with fruit diameter and fruit width, and MSSR 179 with fruit length and yield per tree. Meanwhile, MSSR 173 was associated with fruit weight and fruit width, MSSR 137 with FSI and fruit length, MSSR 147 with fruit length and yield, and MSSR 187 with acidity and TSS/acidity. These markers can be regarded as multi-trait associations.

However, in the MLM approach, a total of five SSR loci with different alleles showed a significant association with six pomological traits. The B allele of MSSR 146 marker was significantly associated with two traits, namely fruit length and fruit width with 27 and 24% of the phenotypic variance, respectively. The B allele of MSSR 160 with TSS, A/B allele of MSSR 106 with hue, A allele of MSSR 140 with yield per tree, and B allele of MSSR 174 with ABI. Of five significantly associated markers, one (B allele of MSSR 146) was associated with more than one trait, including fruit length and fruit width with considerable phenotypic variance (Table 8). The Manhattan plots of MSSR 146, in which the genomic coordinates are displayed along the X-axis and the negative logarithm of the association p value for each genic-SSR are displayed on the Y-axis, clarify that each dot on the Manhattan plot depicts a genic-SSRs. The Manhattan plot displaying the significant association of MSSR 146 with fruit width in both the approaches is depicted in Figs. 3 and 4.

Discussion

Phenotypic evaluation

Association analysis is a powerful tool to establish allele-trait associations and provides estimates of phenotypic variations exhibited by each locus. In the current study, significant phenotypic variations were observed for some pomological traits. The higher coefficients of variation for a^* , pulp:stone, ABI, and fruit weight, suggests high potential for selection in a mango improvement program.

Selection applied to one character can affect other characters because of the genetic correlation between traits (Cheng et al. 2004). The high heritability was observed for fruit characteristics such as fruit weight (93.23%), L^* (90.30%), fruit width (90.40%), pulp:stone ratio (82.23%), fruit diameter (81.23%), and fruit length (81.06), thereby suggesting that selection for this character would be more effective because of a close correspondence between the genotype and phenotype (Singh 1991; Vasugi et al. 2013). The fruit weight and regular fruiting behavior are two determinative factors for high and consistent yield in mango. Because these traits are directly linked to each other, the major components of yield would lead to the selection of varieties with high fruit yield.

The alternate bearing trait showed highly significant negative correlation with yield per tree, which emphasized the hypothesis of the low number of fruit per tree is a sign of alternate bearing habit of varieties. In ber, Islam et al. (2010) and Saran et al. (2007) also reported similar findings among different pomological characteristics. The chromatic association (L^* , a^* , b^* , hue, and chroma) is critical for the consumer; mango fruits with a red blush on the yellow peel are mostly preferred. Except for a^* , all the color value exhibited a negative association with yield. The positively associated value of chromaticity parameter a^* , which indicates the high blush color affecting the cosmetic appearance. Mango fruits having a sufficient red blush on the peel are preferred in the international trade (Sethi et al. 2011). It is desirable to give due consideration to the pulp:stone ratio, while selecting mango cultivars for higher productivity. Higher pulp:stone ratio is desirable while selecting mango cultivars for pickle making. Among the quality traits, namely L^* , a^* , b^* , hue value, chromaticity, total soluble solids, titratable acidity, and ascorbic acid are the desirable attributes for fruit flavor, however, these traits do not affect the production. Mango cultivars having excellent flavor and taste may be selected without compromising total productivity of the crop. The major correlation analysis findings indicated that fruit weight, fruit diameter, stone length, and stone diameter were the major attributes contributing to fruit yield. Hence, selection of large-sized fruits will increase pulp recovery, which is suitable for the processing industry. Similarly, a highly positive correlations among fruit traits (fruit weight, fruit length, fruit diameter, stone weight, and stone diameter) were also reported by Majumder et al. (2013), Prasad (1987), Bhowmick and Banik (2008), Barholia and Yadav (2014), Karibasappa et al. (1999), Rai et al. (2001) and Yadav et al. (2003) for various mango cultivars. Knowledge about the correlation studies between various fruit quality, yield, and yield-related traits would certainly provide an idea, which could be utilized for the selection of desirable traits in future breeding programs and could

Table 7 Marker–trait association in 60 mango varieties using generalized linear model (GLM) approach

S. no.	Trait	Marker ID	Major contributing allele (s)	Allele-wise average phenotypic value	<i>p</i> value	<i>r</i> ²	S. No.	Trait	Marker ID	Major contributing allele (s)	Allele-wise average phenotypic value	<i>p</i> value	<i>r</i> ²
1	Fruit width	MSSR 146	B	A = 48.36 B = 88.45	1.19E−04	0.3193	21	Pulp:stone	MSSR 189	A/C	A = 5.39 B = 4.11 C = 5.36	0.0076	0.1983
2	Fruit length	MSSR 186	A/B	A = 96.22 B = 94.14	8.86E−04	0.4239	22	Fruit width	MSSR 156	B	A = 49.12 B = 68.45	0.0077	0.1972
3	Fruit diameter	MSSR 190	C	A = 33.58 B = 46.69 C = 71.78	0.0014	0.2510	23	Fruit length	MSSR 146	B	A = 79.23 B = 98.38	0.0082	0.2142
4	Fruit diameter	MSSR 189	C	A = 38.35 B = 43.69 C = 72.47	0.0014	0.2686	24	Fruit weight	MSSR 102	A/B	A = 185.36 B = 187.45	0.0102	0.2408
5	Acidity	MSSR 155	C	A = 0.22 B = 0.21 C = 0.38	0.0014	0.2410	25	Fruit weight	MSSR 163	A	A = 193.31 B = 161.22	0.0105	0.1742
6	Fruit length	MSSR 147	A/B	A = 99.12 B = 97.98	0.0015	0.3408	26	Yield(kg)/tree	MSSR 152	A/B	A = 33.56 B = 50.89	0.0107	0.2497
7	TSS	MSSR 160	B	A = 14.14 B = 18.25	0.0026	0.2285	27	TSS/acidity	MSSR 187	B	A = 53.23 B = 86.25	0.0119	0.1544
8	Fruit length	MSSR 156	B	A = 75.45 B = 94.65	0.0033	0.2756	28	Hue	MSSR 146	B	A = 1.11 B = 1.65	0.0122	0.1712
9	Acidity	MSSR 187	A	A = 0.39 B = 0.25	0.0039	0.1983	29	Fruit width	MSSR 173	B	A = 43.65 B = 66.47	0.0132	0.1764
10	Fruit length	MSSR 137	B	A = 78.36 B = 92.25	0.0050	0.2527	30	Fruit weight	MSSR 146	B	A = 153.26 B = 186.38	0.0136	0.1652
11	ABI	MSSR 174	B	A = 0.24 B = 0.38	0.0059	0.1948	31	Fruit weight	MSSR 190	A/C	A = 185.23 B = 126.24 C = 184.25	0.0137	0.1535
12	Fruit weight	MSSR 173	A	A = 186.58 B = 156.47	0.0061	0.2043	32	TSS/acidity	MSSR 155	B/C	A = 35.22 B = 82.23 C = 84.14	0.0137	0.1535
13	Fruit diameter	MSSR 130	B	A = 104.36 B = 168.78	0.0064	0.1931	33	TSS/acidity	MSSR 117	B	A = 53.22 B = 86.23	0.0144	0.1781
14	Fruit length	MSSR 179	B	A = 77.78 B = 91.99	0.0065	0.1870	34	FSI	MSSR 137	A/B	A = 74.45 B = 76.36	0.0145	0.1930

Table 7 continued

S. no.	Trait	Marker ID	Major contributing allele (s)	Allele-wise average phenotypic value	<i>p</i> value	<i>r</i> ²	S. No.	Trait	Marker ID	Major contributing allele (s)	Allele-wise average phenotypic value	<i>p</i> value	<i>r</i> ²
15	Pulp:stone	MSSR 190	A	A = 4.12 B = 3.11	0.0066	0.1930	35	Fruit weight	MSSR 189	A/C	A = 136.12 B = 116.23 C = 134.45	0.0146	0.1649
16	Fruit width	MSSR 153	B	A = 40.11 B = 58.25	0.0067	0.2588	36	Yield (kg)/tree	MSSR 179	B	A = 39.23 B = 56.47	0.0152	0.1481
17	ABI	MSSR 178	B	A = 0.30 B = 0.42	0.0071	0.2104	37	Fruit diameter	MSSR 156	B	A = 49.25 B = 68.36	0.0159	0.1318
18	ABI	MSSR 146	B	A = 0.33 B = 0.44	0.0072	0.2228	38	Fruit width	MSSR 130	B	A = 40.36 B = 67.25	0.0167	0.1549
19	Yield(kg)/tree	MSSR 140	A	A = 45.12 B = 35.36	0.0073	0.1946	39	Yield (kg)/tree	MSSR 147	B	A = 42.36 B = 47.36	0.0180	0.2074
20	Hue	MSSR 106	A/B	A = 1.83 B = 1.81	0.0074	0.1833							

ABI alternate bearing index, TSS total soluble solids, FSI fruit shape index, A allele, B allele, A/B A and B allele, C C allele, A/C A and C allele, *p* probability

enable possible the selection of all correlated traits concurrently by evaluating only one trait during the selection (León et al. 2004). These wider phenotypic trait variations among 60 mango varieties indicated that the constituted association panel was suitable for association mapping for selected traits.

Population structure analysis

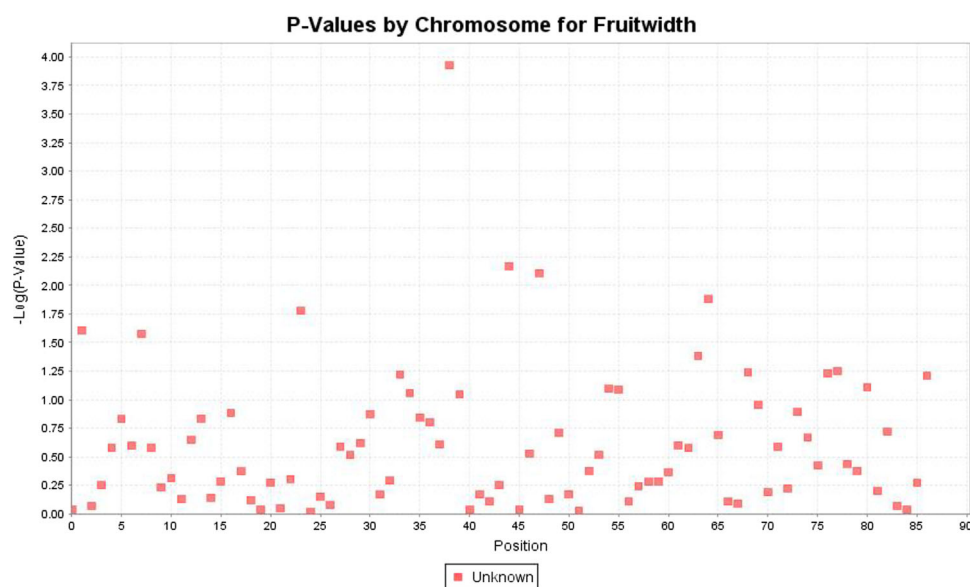
Usually, mango has a moderate genetic base with moderate to high variability. However, because of the inclusion of different varieties, including native and exotic collection of germplasm and improved genotypes, a wide variability was observed in this study. A wider variation in the morphological traits was also detected in mango by Majumder et al. (2013), Gitahi et al. (2014), Begum et al. (2014), and Kheshin et al. (2016) also reported considerable variations in the composition of the mango population. Population stratification contributes to false positive results (Cardon and Bell 2001; Yu and Buckler 2006). The population structure with respect to geographical origin contributes toward pseudo-associations (Maskri et al. 2012). Therefore, adequate methods need to be implemented to control the effects of population structure to avoid the high rate of Type-I error (Agrama et al. 2007). In this study, a Bayesian model-based population structure analysis indicated the occurrence of three sub-populations largely according to the major geographic regions of their origin or genetic makeup.

The varieties having prefix Pusa (Pusa Surya, Pusa Arunima, Pusa Shresth, Pusa Lalima, Pusa Peetamber) grouped with Mallika and Amrapali and the most popular varieties of Northern India. These varieties were bred and collected from the same geographical locality, namely New Delhi, India and shared common parents, which indicates their genetic closeness. In addition, to other popular varieties from Uttar Pradesh (India) as Langra, Chausa, and Lucknow Safeda were grouped together indicating geographically and genetically closeness. In fact, the Indian mango gene pool comprises primarily the germplasm collected and bred in North India, and hence, such results are obvious. The overlapping of structures by a number of varieties indicated that such varieties showed common ancestry. Similarly West Indian, East Indian, South Indian, and Brazilian varieties clustered together, which indicates that these varieties probably had a complex breeding history with inter, intra-crossing, introgression, and out crossing among germplasms from diverse spatial and genetic backgrounds, followed by strong selection pressure for desirable traits. The varieties belonging to Florida, USA, clustered together the genetic relatedness among selected varieties was expected because they were related to each other by their pedigree. “Haden”, a

Table 8 Marker–trait association in 60 mango varieties using mixed linear model (MLM) approach

Trait	Marker ID	Major contributing allele (s)	Allele-wise average phenotypic value	<i>p</i> value	<i>r</i> ²
Fruit length	MSSR 146	B	<i>A</i> = 74.24 <i>B</i> = 97.25	0.0081	0.2734
Fruit width	MSSR 146	B	<i>A</i> = 50.25 <i>B</i> = 65.83	0.0088	0.2468
TSS	MSSR 160	B	<i>A</i> = 120.36 <i>B</i> = 153.24	0.0102	0.1983
Hue	MSSR 106	A/B	<i>A</i> = 16.69 <i>B</i> = 16.36	0.0151	0.1705
Yield(kg)/tree	MSSR 140	A	<i>A</i> = 56.23 <i>B</i> = 42.32	0.0183	0.1731
ABI	MSSR 174	B	<i>A</i> = 123.58 <i>B</i> = 173.65	0.0191	0.1331

ABI alternate bearing index, TSS total soluble solids, A allele, B B allele, A/B A and B allele, *p* probability

Fig. 3 Manhattan plots for fruit width trait using GLM

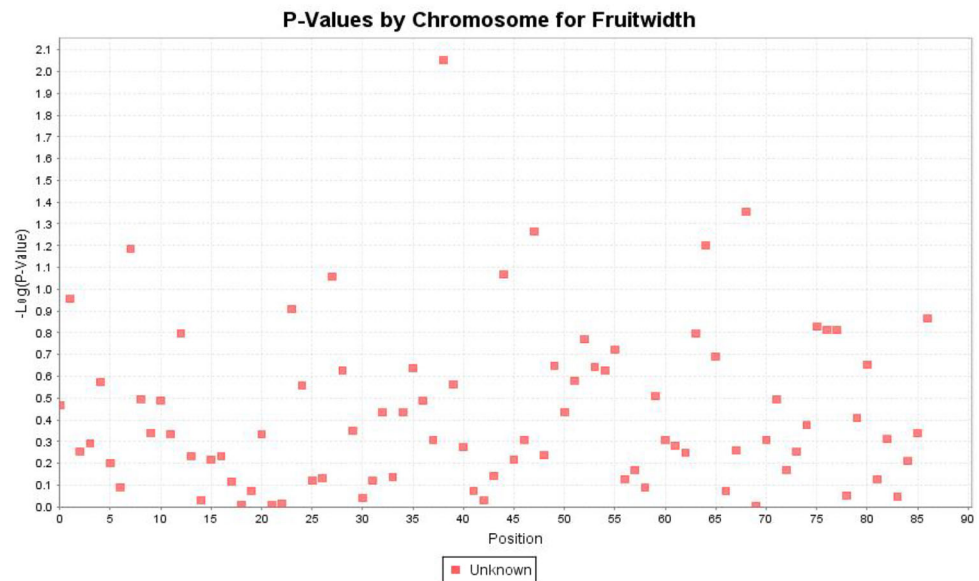
seedling of Indian variety Mulgoba gave rise to several Floridian mango varieties like “Tommy Atkins”, “Edward”, and “Eldon”. Florida (USA) served as a secondary geographical diversity center for several varieties as Pusa Surya (Eldon), Sensation, Smith, Elard, and Edward (Lopez-Velenzuela et al. 1997) and these varieties were grouped together owing to their common geographical origin (Schnell et al. 1995; Olano et al. 2005). Separation based on their geographical location was evident among the selected mango varieties. However, some of the varieties could not be differentiated according to geographical patterns and hence admixtures in the populations can be attributed primarily due to cross-pollination, gene flow, particularly orchards situated in close proximity, chance seedling, and exclusive vegetative propagation. The

information generated in this study population structure and ancestral background would facilitate germplasm conservation and management strategy and selection of suitable parents in mango breeding programs.

Marker–trait association analysis

In general, the GLM approach detects higher numbers of significant marker–trait associations than does the MLM approach (Neumann et al. 2011), however, GLM-based analysis considers only the *Q* matrix generated during the population structure analysis, whereas the MLM approach simultaneously accounts for both, population structure as well as kinship statistics (genetic relatedness among individuals). Hence, the MLM approaches are more reliable.

Fig. 4 Manhattan plots for fruit width trait using MLM



Only 22.27% of the markers associated with different traits in the GLM approach also exhibited significant marker–trait association in the MLM. Furthermore, the extent of phenotypic variation in the MLM was comparatively less than that in the GLM, which proves that it was a more stringent approach for eliminating the spurious associations than was GLM. Studies conducted in rapeseed, Arabidopsis, bread wheat, and soybean (Cai et al. 2014; Yu et al. 2006; Zhao et al. 2007; Kumar et al. 2014) demonstrated the effectiveness of the MLM approach over the GLM. Because population structure can cause spurious associations (Kang et al. 2008) the MLM approach, incorporates kinship in the association analysis allowing an improved control of type I and type II error rates over GLM due to relatedness and population structure (Yu et al. 2006; Sa 2016).

In the current study, we observed that alleles of various loci were significantly associated with multiple traits. A typical example was the B allele of the MSSR 146 locus, which was highly associated with fruit length and fruit width in the GLM and MLM approaches with a high amount of phenotypic variance and fruit weight and ABI in the GLM approach only. Because fruit length, fruit width, fruit weight, and ABI are significantly correlated with fruit yield per tree, the associated alleles of marker MSSR 146 may be used to select fruit yield per tree. Meanwhile, B allele of MSSR 160 was significantly associated with TSS and the A and B allele of MSSR 106 was associated with hue in both the GLM and MLM approaches. The plants that bear fruits and have higher TSS and hue values are expected to provide higher fruit quality with an attractive peel color and can meet the international standards of export of mango. Similarly, the A allele of MSSR 140 and

the B allele of MSSR 174 were associated with yield per tree and bearing habit. The similar type of association analysis for SSR and ISSR markers in sweet cherry cultivars revealed 114 SSR alleles apart from the three ISSR markers correlated with fruit harvest time and soluble solids and four ISSR markers with fruit skin color (Ganopoulos et al. 2011). In olive, significant associations were determined between five SSR markers and oleic, stearic, linolenic acids, and linoleic of olive oil (Ipek et al. 2015), similarly using the MLM approach, a total of 16 significant associations for chemical traits and 12 for physical traits were identified in almond (Font i Forcada et al. 2015). In peach (Cao et al. 2012), estimated the genome-wide marker–trait associations for ten complex traits, such as red pigment in the pulp, pulp color around the stone, flesh texture, pulp adhesion, pulp firmness, fruit weight, ripening time, chilling, flowering time, requirement, and fruit development period. In this study, among the particular alleles of a total of 23 associated genic-SSR markers, 11 were found to be associated with more than one pomological traits in GLM, and the single allele of one locus in MLM with two traits indicates the precise evaluation of traits.

The alleles of different genic-SSR loci, which exhibit overlapping association with diverse traits, signify the biological correlation among them. Therefore, it was presumed that the loci governing fruit length, fruit width, fruit weight, alternate bearing habit, and yield per tree are probably located closest to each other on the same chromosome. In this investigation locus, the A and C alleles of MSSR 190 were significantly associated with fruit diameter, pulp:stone ratio, and fruit weight with significant phenotypic variance in the GLM and the B allele of MSSR

146 was found to be associated with the fruit length and fruit width in the MLM approaches with high phenotypic variance. Therefore, the MSSR 190 and MSSR 146 markers can be used in breeding programs for yield improvement in mango. In the Manhattan plots, MSSR 146 exhibited the strongest associations by exhibiting the smallest p values (e.g., SSR 146 for fruit width) and the highest negative logarithms value in both the approaches. These markers were considered for correlating pomological traits that have not been reported. Alternatively, they confirmed the suspected correlation with pomological traits, but these correlations require further investigation.

Conclusion

Considering the perennial nature of mango and its inherent genetic hurdles, the alleles that are significantly associated with five genic-SSR markers will prove extremely useful to identify promising candidate loci governing complex traits such as alternate bearing, fruit quality, and yield. This association analysis may be sufficient for marker-assisted selection and mining alleles related to major traits in mango germplasm collection, which will further facilitate early identification of desired cultivars while simultaneously saving significant time and resources. Knowledge regarding these associated loci would make valuable contributions to mango breeding programs particularly in the absence of genetic information such as linkage map and QTL. The identification of new SSR markers for effective MAS and fine resolution mapping of the genes or QTL underlying a trait.

Author contribution statement SL, AKS, SKS, and NKS proposed, initiated, and led the project, interpreted scientific information, and participated in manuscript preparation. BPS and NS participated in DNA extraction, genotyping, statistical analysis, and manuscript preparation. MS maintained and provided the mango varieties and participated in manuscript preparation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Data archiving statement The authors declare that all the work described in this manuscript followed the standard Tree-structure and function journal policy. All the primers used in the current study have

been derived from leaf RNA-seq of mango variety Amrapali, and to date, no information pertain to this manuscript is submitted to any public databases.

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