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Research Paper

Genetic diversity and stability analysis of the improved cultivars of guava and their related species

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ABSTRACT

Guava (*Psidium guajava* L.) is an important fruit crop in the tropical and sub-tropical world. The morphological, biochemical, and molecular (using 56 microsatellite markers) characterization was carried out on 18 guava genotypes and related species (*P. guineense*) during 2018–2022. The present study exhibited a wider range of variability: fruit weight (61.5–68.4 g); fruit diameter (4.20–7.91 cm); total soluble solids (8.50–13.40 °Brix); titrable acidity (0.20–0.64%); reducing sugar (2.55–7.00 mg glucose /g); ascorbic acid (76.4–196.3 mg/100 g); total phenol (125.9–305.4 mg GAE/100 g pulp); and total flavonoid content (172.30–948.5 mg QE/100 g pulp). The antioxidant activities (FRAP and DPPH) showed a favorable relationship with ascorbic acid, total phenol, total flavonoid, and total flavonol content. Red-fleshed genotypes were found superior for all the quality parameters over white-fleshed genotypes. The molecular analysis generated 213 alleles from 56 markers, with 2–9 alleles per locus (mean = 3.80). Apart from the genetic diversity, the improved cultivars were also distinguished by a set of markers: RCGH-4 (mPGCIR-184 and mPGCIR-194); RCGH-1 (mPGCIR-108 and mPGCIR-243); RCG-11 (mPGCIR-206 and mPGCIR-325); and RCGH-7 (mPGCIR-16 and mPGCIR-19). The cluster analysis indicated that *P. guineense* was the most diverse of the cultivated species, and all the red flesh genotypes were close to each other. Furthermore, RCG-11 had a lower seed content (58.89 per 100 g pulp), while RCGH-1 and RCGH-4 were stable for fruit weight and seed number, which can be promoted for commercial production and future crop improvement programs.

1. Introduction

Guava (*Psidium guajava* L.) is an important fruit crop belonging to the Myrtaceae family and grown widely in tropical and sub-tropical regions of the world. The crop is native to tropical America and was introduced to India by the Portuguese in the early 17th century (Menzel, 1985). Today, it is grown in the majority of the fruit-growing regions of India due to its prolific bearer, highly remunerative, and wider adoptability (Rymbai and Reddy, 2010a). India ranks first in global production with an area of 359 thousand hectares and 5.96 million metric tons of production (Statista, 2023). The eastern Himalayas of India are considered part of the mega-center of biodiversity (Indo-Burma), which is mainly due to diverse terrain and topography as well as climatic conditions, and the region is broadly classified as having a humid subtropical climate (Rymbai et al., 2016). Guava is an important component of nutritional security due to its easy availability and richness in nutritional values. Hence, it is also known as the poor man's apple and "Apple of Tropics" (Bihari et al., 2009; Rymbai et al., 2010b). The fruits are rich sources of vitamin C, pectin, and minerals like calcium, phosphorus, and iron. The fruit also contains a substantial quantity of vitamin A, pantothenic acid, riboflavin, thiamin, and niacin. The fruits of the pink flesh cultivar particularly have a fair amount of beta-carotene, anthocyanin, and lycopene content (Kherwar and Usha, 2016), which contribute substantially to its high antioxidant content that prevents degenerative

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diseases (Khoo et al., 2019). In addition to fruits, the other parts of the plant are also traditionally used in the folk medicine of several civilizations (Gutiérrez et al., 2008). The leaves have been extensively used for the treatment of diarrhea, bacterial infection, pain, and inflammation (Ojewole, 2006), and the bark extract for the treatment of diabetes (Oh et al., 2005). The essential oil isolated from the leaves also possesses anti-cancer properties (Manosroi, 2006). The crops also have great commercial value in the processing industry, such as in the preparation of jam, jelly, cheese, ketchup, ready-to-serve (RTS), nectar, powder, toffee, flakes, and butter paste for the domestic market as well as export (Choudhary et al., 2008).

Guava is mainly a self-pollinated crop, but cross pollination does occur (35–40% outcrossing), thus generating heterozygozity and an open-pollinated seedling population with adequate genetic variation for the selection of desirable types (Nakasone and Paul, 1998). As a consequence, several guava cultivars have evolved through seedling selection in India. The guava clones vary greatly with respect to their fruit quality and yield potential (Deshmukh et al., 2013). The crop is propagated commercially through vegetative means; there is huge potential for the exploitation of heterosis through hybrids, which have multiple desirable traits. To develop a hybrid with desirable traits, diverse parents are required. Genetic variability studies based on quantitative traits may indicate genetically divergent wild genotypes with suitable traits for crop improvement (Nogueira et al., 2012). The assessment of genetic parameters, *viz*., genotypic coefficient of variation (GCV), phenotypic co-efficient of variation (PCV), heritability, and genetic advance (GA), is a pre-requisite for effective selection of the genotypes and improvement in the base population. Multivariate analyses such as cluster analysis (CA) and principal component analysis (PCA) are effective in the identification and selection of divergent genotypes on the basis of the traits and their contribution to the divergence in guava (Nogueira et al., 2014; Yousaf et al., 2020; Mishra et al., 2022). The selection of divergent parents on the basis of morphological traits may not be a reliable method of distinguishing the genotypes that are closely related, considering the pleiotropic effects, epistatic interactions, environmental changes, and other factors. In recent years, quantitative traits, proteins (Patel et al., 2013), and molecular markers (Rai et al., 2010) have been employed for the analysis of the genetic diversity of the guava accessions. Similarly, different molecular markers have been used for the estimation of genetic diversity by random amplified polymorphic DNA (Dahiya et al., 2002; Chen et al., 2007; Feria-Romero et al., 2009); genetic characterization by amplified fragment length polymorphisms (Hernández-Delgado et al., 2007); sequence-related amplified polymorphisms, simple sequence repeat for identification of cultivars, diversity, and linkage mapping (Risterucci et al., 2005; Latha Kanupriya et al., 2011; Padmakar et al., 2015; Ma et al., 2020); and single nucleotide polymorphisms genotyping for pigments (Thakre et al., 2023).

Although the eastern Himalayas of India are known for being rich in genetic diversity and guava as a major component of nutritional security in the region, little study has been conducted so far utilizing these genetic resources. Furthermore, there are no commercial or popular cultivars released in the region, and the farmers are growing local genotypes that are poor in yield and quality, leading to a hindrance in the cultivation of this crop. In view of these, a guava improvement program was started in the late 1990s by the ICAR Research Complex for NEH Region, Umiam, Meghalaya, suitable for the mid-hill conditions of the eastern Himalayas, India. As a result, several superior varieties/ hybrids were evolved, potentially for commercial production, both for dessert and processing purposes. Therefore, the present investigation was undertaken to study the genetic variability and correlation among the guava genotypes for yield and quality traits, to characterize the guava genotypes using microsatellite markers, and to identify the potential guava genotypes with stable fruit traits.

2. Materials and methods

2.1. Experimental site

The experiment was conducted in ICAR Research Complex for NEH Region, Umiam, Meghalaya, during the years 2018–2022. The study area is situated at 25° 41′ N latitude, 91° 55′ E longitude, and 1010 m altitude above mean sea level. The experiment site is clay loam to sandy clay loam. Soil is acidic in nature with a pH of 4.9, P-deficient acid alfisols with an initial SOC of 1.77 %, exchangeable Al^{3+} (148.6), Ca^{2+} (240.5), Mg^{2+} (120), K^{+} (66.7), Bray's P2-P (1.2), and available B (0.9 $mg.kg^{-1}$).

2.2. Weather parameters

The climatic conditions at the experimental site are humid subtropical. The mean maximum temperature of 26.16 °C and the mean minimum temperature of 14.0 °C were recorded during the study period, with the maximum temperature recorded during the month of July (28.57 °C) and the minimum in January (5.68 °C). Total annual rainfall of 2273.8 mm, with more than 90 % falling during April to October; maximum rainfall was received during August (495.8 mm) and minimum during December (7.1 mm). Relative humidity (RH) ranged between 77.18–88.12 % and 46.00–75.70 % for maximum and minimum RH, respectively.

2.3. Plant materials

A total of 18 genotypes, including six hybrids/ selections (RCG-4, RCG-3, RCG-2, RCGH-1 (Megha Wonder), RCGH-4 (Megha Magenta), RCGH-7 (Megha Supreme), RCGH-10, and RCG-11 (Megha Seedless), developed by the ICAR Research Complex for NEH Region, Umiam, India, and other 12 popular landrace genotypes (L-49, Allahabad Safeda, RCGS-1, Apple Color, Mizo Purple, RCG-1, Lalit, Allahabad Surkha, Local Pink, and *P. guineense* (Sw.)) were used for the study. The details of the genotypes and their pedigree are presented in supplementary Table 1.

The guava genotypes were maintained on hill slopes at a spacing of 6 x 6 m and 8–10 year old trees in Horticulture Farm, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India. The recommended package of practices was followed throughout the crop season. The observations for fruits and quality attributes were taken for the 17 genotypes, excluding the non-edible, wild species of guava, *P. guineense* (Sw.). The experiment was laid out in randomized complete block design (RCBD) with three replications. The observations were recorded in five plants for each replication.

2.4. Determination of physical parameters

About thirty mature fruits randomly selected from all the direction of the canopy were harvested for determining the physical parameters. The fruit weight (g) was measured using weighing balance, fruit length (top-bottom) and diameter (maximum diameter) was measured using digital vernier calliper (in cm). The seeds were separated manually and counted to obtain number of seeds per fruit.

2.5. Determination of biochemical attributes

Biochemical parameters such as total soluble solids (TSS) were determined using hand-held refractometer (HI 96,801) and titratable acidity and ascorbic acid, were analyzed according to Rangana (1997). Reducing sugar was determined according to Miller (1959) and expressed as mg glucose/g. Moisture content of the fruits was determined gravimetrically by weighing fresh fruit sample before and after drying in hot air oven (thermostatically controlled, Model–IC7).

2.6. Determination of antioxidant activity

Five grams of pulp from each fruit were ground and added to 50 mL of aqueous methanol at ambient temperature. Incubate for 1 hour at room temperature with continuous magnetic stirring at 200 rpm. Centrifuge at 1000 g for 20 min. Collect the supernatant and store it at −20 °C until analysis. The extract was used for the estimation of antioxidants, including total phenolic content (TPC) according to Singleton and Rossi (1965), total flavonoids content (TFC, Zhishen et al., 1999), total flavonols (Miliauskas et al., 2004), DPPH free radical scavenging activity (Blois, 1958), and FRAP assay (Wetchakul et al., 2019).

2.7. Molecular analysis

2.7.1. DNA extraction

The total gDNA was extracted from fresh young leaves (2 g) of newly emerged shoots as described by Saghai-Maroof et al. (1984) with the addition of Polyvinylpyrrolidone (1 %) in the extraction buffer. The sample was then ground to a fine powder using liquid nitrogen. Nanodrop™ 1000 Spectrophotometer (Thermo Scientific, USA) was used for DNA quantification.

2.7.2. PCR and gel electrophoresis

PCR reactions were carried out in a Thermal Cycler (Veriti, Applied Biosystem, USA). Each 20 ml reaction mixture contained 1X reaction buffer (10 mM Tris–HCl, pH 8.3 and 50 mM KCl), 2.5 mM $MgCl_2$, 1 U of Taq DNA polymerase; 200 mM each of dATP, dTTP, dCTP, and dGTP (all reagents from Thermo Fisher Scientific), 0.6 mM of primer, and approximately 25 ng of template DNA. A total of 56 polymorphic expressed sequence tag-Simple Sequence Repeat (EST-SSR) primers reported earlier (Risterucci et al., 2005) were screened and selected for the analysis. The PCR amplification conditions were as follows: an initial extended step of denaturation at 94 °C for 5 min., followed by 30 cycles of denaturation at 94 °C for 45 s, primer annealing at 50 °C for 60 s., and primer elongation at 72 °C for 60 s, followed by an extended elongation step at 72 °C for 10 min. Reaction products were mixed with 2 µl of 6X loading dye (Thermo Fisher Scientific) and spun briefly in a microfuge before loading. The amplification products were electrophoresed on 3.0 % agarose gel at 60 Vs. Gels were stained with ethidium bromide and documented using a Chemidoc™ (BioRad, California, USA).

2.8. Data analysis

The replicated (3 of each parameter) data for biochemical and antioxidant attributes were analysed using R studio (Version 4.3.1) software. The genetic parameters such as phenotypic and genotypic variances of the genotypes were determined according to Burton and Devane (1952), heritability (Hanson et al., 1956), genetic advance (Johnson et al., 1955), and the correlation coefficient (genotypic and phenotypic) and path coefficient were estimated according to Dewey and Lu (1959). The possible relationship between antioxidant compounds and antioxidant activity was analysed through the Pearson correlation coefficient using R software (R version 4.3.1). The mean data of the three years (2018–22) were subjected to stability analysis using the additive main effects and multiplicative interaction (AMMI) based stability parameter was measured as AMMI stability value (ASV) as described by Purchase (2020 using the "metan" package (v. 1.16.0) (Olivoto and Lucio 2020) in R version 4.2.1 (http:// www.r-project.org/).

For molecular data, the molecular weights of bands (amplicons) were estimated by using a 50 bp DNA ladder, and the homology of bands (amplicons) was based on the distance of migration in the gel. The generated bands were scored based on the absence (0) and presence (1) of alleles. Only reproducible SSR amplicons obtained from each entry were resolved as a band on the gel system, and the data sets were used to calculate the major allele frequency and the polymorphism information content (PIC) for each locus using Power Marker software. GenAlEx v.6.5 software was used to analyze molecular variance (AMOVA), calculate pair-wise Nei's genetic distance, and identify private alleles. Cluster analysis (hierarchical clustering) was carried out based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method using DARwin v. 6.0.21 software. Principal coordinate analysis (PCoA) based on allele frequency was done using XLSTAT software.

3. Results

3.1. Genetic variability for fruits and quality attributes

The results of the fruits and quality attributes have shown wider variability among the accessions of the guava genotypes (Table 1). The average fruit weight ranges from 79.10 g (RCG-4) −148.80 g (RCGH-4), fruit length 5.33 (Mizo Purple) - 6.71 cm (Local Pink), fruit diameter 4.52 cm (RCG-4) - 6.75 cm (RCGH-10), number of seeds per 100 g pulp 58.89 (RCG-11) −492.78 (RCGS-1). Similarly, the wider variability was observed in quality attributes, like acidity ranges from 0.20 (RCG-3) - 0.64(RCGS-1), TSS 8.50 (Mizo Purple)−13.40 (Allahabad Surkha), reducing sugar 2.55 mg glucose/g (Mizo Purple) - 7.0 mg glucose/g (Local Pink), vitamin C 76.36 mg/100 g (RCG-1) −196.36 mg/100 g (L-49), TPC content 125.90 mg GAE /100 g (RCG-1) – 304.01 mg GAE /100 g (RCGH-7), flavonoids 172.30 mg QE/ 100 g (RCG-2) - 948.48 mg QE/ 100 g (Local Pink), Flavonols 13.16 mg QE /100 g (RCG-4) - 40.45 mg QE /100 g (Mizo Purple) and antioxidant activity such as DPPH (IC_{50}) 36.2 µg/ml (Standard ascorbic acid 11.60 µg/ml; RCGH-7) −71.3 µg/ml (RCG-3) and FRAP 2.56 mM $FeSO_4E$/g (RCGH-4) - 5.21 mM $FeSO_4E$/g (RCGH-1).

The genotypic coefficient of variation (GCV) contributed significantly to the phenotypic coefficient of variation (PCV). Except for TSS, high estimates of GCV (> 15 %) and PCV were recorded for all the fruits and quality traits. However, all the traits have shown high heritability (>60 %) and genetic advance (GA) as percent of the mean (> 20 %). Moreover, among the traits, higher heritability and GA were observed for flavonoid contents and the number of seeds/100 g pulp.

3.2. Correlation for quality attributes with antioxidant activity

Among the traits, TSS was significant ($p < 0.005$) and positively correlated with the AA, FVC and RS content (Fig. 1). Ascorbic acid content was significant and positively correlated with TFLC (0.57), TPC (0.33) and TSS content (0.48). TFC content was positively correlated with TFC (0.73), TPC (0.40), Ascorbic acid (0.35) content, and acidity (0.35). The antioxidant activity DPPH was significant and negatively correlated with TPC (−0.87), TFC (−0.29), and TFLC (−0.27), and ascorbic acid content (−0.17) and antioxidant activity FRAP (−0.87). However, antioxidant activity FRAP was significant and positively correlated with TPC (0.80), ascorbic acid content (0.32), and flavonoid content (0.29).

3.3. Principal component analysis

The results of PCA analysis also revealed the presence of variability for different traits. The first five components had extracted Eigen value of >1 and contributed 84.66 % of the total variation (Table 2, Supplementary Figure 1). Principal component 1(PC1) contributed 29.22 % of the total variability. The variation on PC1 was positively attributed by important traits like DPPH (0.35) and number of seeds/100 g pulp (0.19). PC2 contributed for 17.24% to the total variability and was depicted mainly by economic traits FW, FD, and ascorbic acid content. About 15.16% of the total variability was contributed by PC3 (main attributing traits were DPPH, FW, FD, TA and TSS) and 12.50 % by PC4 (mainly ascribed to TA and number of seeds). Moreover, some of the traits was found to be contributed to more than one component; PC (1–3) by DPPH, PC (2&3) by fruit weight and diameter, and PC (2–4) by

Table 1
Analysis of genetic parameters for fruits and quality attributes in guava genotypes.

Traits	Mean	Range	PCV (%)	GCV (%)	h^2	GA	GAM
Fruit weight(g)	122.08	79.10–148.8	23.21	22.19	91.46	51.78	43.79
Fruit length (cm)	5.89	5.33–6.71	38.53	28.11	53.22	2.57	42.30
Fruit diameter (cm)	6.04	4.52–6.75	38.85	30.28	60.76	3.08	48.69
Number of seeds/fruits	268.09	58.89–492.78	40.84	38.77	90.12	204.82	75.94
Acidity (%)	0.41	0.20 - 0.64	30.21	28.5	88.98	0.23	55.46
Total soluble solids (°Brix)	10.85	8.50 –13.40	11.7	11.3	93.4	2.45	22.54
Reducing Sugar (mg glucose/g)	4.12	2.55 - 7.00	23.27	22.97	97.45	1.92	46.77
Vitamin C (mg/100 g)	131.7	76.36 –196.36	20.11	19.09	90.12	49.23	37.38
Total phenolic content (mg GAE /100 g)	191.97	125.90 - 305.40	24.96	23.31	87.16	86.17	44.89
Total flavonoids content (mg QE/100 g)	323.14	172.30 - 688.0	42.32	42.19	99.37	280.36	86.76
Total flavanol content (mg QE/ 100 g)	23.0	13.16–40.45	24.38	24.14	98.03	11.28	49.39
DPPH IC_{50} value (µg/ mL)	51.9	36.2–71.3	19.85	19.60	97.46	2.07	39.91
FRAP (mM $FeSO_4E$/g)	4.06	2.56 - 5.21	21.46	21.04	96.13	1.73	42.56

PCV, phenotypic coefficient of variation; GCV, genotypic coefficient of variation; h^2, heritability; GA, genetic advance; GAM, genetic advance as percentage of mean; DPPH, 1, 1-diphenyl-2- picrylhydrazyl; FRAP, ferric reducing antioxidant power.

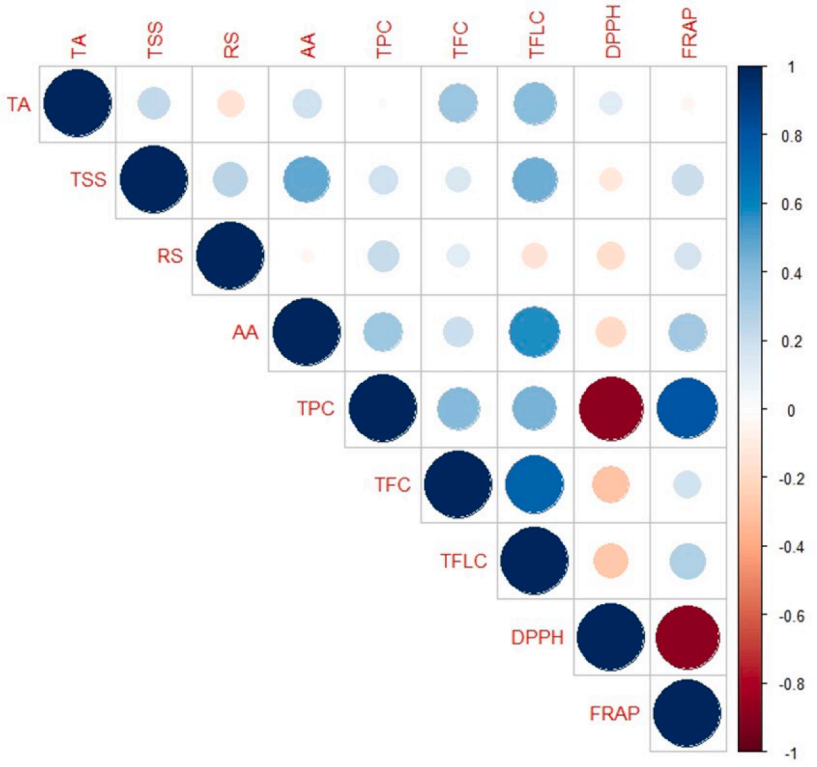

Fig. 1. Correlation among the quality attributes of guava genotypes. (A) titratable acidity (TA, mg/100 g), (B) total soluble solid (TSS, ^{0}B), (C) reducing sugar (RS, percentage), (D) ascorbic acid content (AA, mg/100 g fw); (B) total flavonoids content (TFC, mg QE/g); (C) total flavonol content (TFLC, mg QE/g); (D) total phenol content (TPC, mg GAE/g); (E) DPPH antioxidants capacity (DDPH, IC50 value mg/mL); (F) FRAP antioxidants capacity (FRAP, mg AAE/g) content.

titratable acidity and total soluble solids.

3.4. Diversity based on fruits and quality traits

Based on fruits and quality traits, guava genotypes were grouped into three major clusters (Table 3). Cluster-I was comprised of 4 local genotypes (RCG-1, RCG-2, RCG-3 and RCG-4), while, group-II was comprised of 7 genotypes and of which 4 were red fleshed genotypes (RCGH-4, RCGS-1, Allahabad Surkha and Lalit) and one of red peel (Apple Color) were found close to each other. The pale-yellow pulp cultivar RCGH-1 was found closer to L-49 in group-III (Fig. 2). Based on cluster mean value genotypes of cluster-I were comprised of local cultivars were found rich in maximum number of seeds (353.25) with smaller fruit size. However, genotypes of cluster-II were found superior for quality traits like TSS (10.71 °Brix), reducing sugar (4.24 mg glucose/g), ascorbic acid content (141.45 mg/100 g), TPC (240.65 mg GAE /100 g), flavonoid (430.32 mg QE/100 g), TFLC (28.66 mg QE /100 g) and antioxidant activity DPPH (42.4 µg/ml, IC_{50}) and FRAP (4.90 mg $FeSO_4E$/g) with minimum number of seeds (190.06/ 100 g pulp). However, the genotypes of cluster-III were found superior for the fruit weight and diameter.

3.5. Genetic diversity based on molecular markers

3.5.1. Allelic diversity

Molecular analysis of all the samples was carried out using 56 SSR markers. The markers have shown wider allelic variations among the guava genotypes (Table 4). All the markers were found to be polymorphic, and the average number of alleles per locus ranged from 2 to 9 (mean = 3.89) in the group of 18 genotypes including wild species

Table 2
PCA analysis for fruits and quality traits in guava genotypes.

Variables	PC1	PC2	PC3	PC4	PC5
FWT	−0.334	0.3356	0.3425	−0.0362	0.0671
FL	−0.1749	−0.1676	0.1117	−0.5948	−0.0351
FD	−0.2782	0.3823	0.3474	−0.1461	0.0089
NOS	0.1901	−0.2705	0.1874	0.4015	−0.2171
TA	−0.0908	0.0401	0.2949	0.6243	0.1705
TSS	−0.0144	0.117	0.2464	0.0561	−0.7156
RS	−0.1582	−0.363	0.1817	−0.0857	−0.4862
AA	−0.2148	0.4996	−0.0896	0.1168	−0.1428
TPC	−0.3965	−0.0722	−0.3743	0.1262	−0.0897
FLC	−0.373	−0.386	0.2027	0.0442	0.1384
FLV	−0.391	−0.2981	0.2157	0.0527	0.2468
DPPH	0.3577	0.0124	0.3265	−0.1146	−0.0082
FRAP	−0.2959	−0.0074	−0.4375	0.1216	−0.2468
Standard deviation	1.9489	1.4973	1.4086	1.2747	1.1646
Proportion of variance	0.2922	0.1724	0.1526	0.125	0.1043
Cumulative proportion	0.2922	0.4646	0.6173	0.7422	0.8466
EigenValues	3.7984	2.2419	1.9842	1.6248	1.3563

FWT, fruit weight; FL, fruit length; FD, fruit diameter; NOS, number of seeds per fruits; TA, titratable acidity; TSS, total soluble solids; RS, reducing sugar; AA, ascorbic acid; TPC, total phenolic content; FLC, flavonoid content; FLV, flavonol content; DPPH, 1, 1-diphenyl-2- picrylhydrazyl; FRAP, ferric reducing antioxidant power.

Psidium guineense (Sw.). Similarly, the number of effective alleles also varied from 1.12 to 6.0 with mean value of 2.25. The allele frequency of the marker also varied form 0.12–0.69. The observed heterozygosity was lower than the expected heterozygosity and ranged from 0.00 to 0.94 and 0.10–0.83, respectively. Polymorphism information content ranged from 0.12 (mPgCIR14 and mPgCIR154) – 0.83 (mPgCIR19) with average polymorphism of 0.46. The values of Shannon information index were higher than 1.5 and their corresponding PIC value was also above 0.7. The highest PIC and Shannon information index was observed for the marker mPgCIR19 (0.83 and 1.98), mPgCIR22 (0.78 and 1.72) and mPgCIR09 (0.79 and 1.71), respectively. Out of 56 markers, 20 have shown the Shannon information index over 1.0.

3.5.2. Cluster analysis

The results of cluster analysis based on 56 microsatellite markers, the genotypes were grouped into 4 groups cluster –I was comprised of 15 accessions, while RCGH-1, Mizo Purple and Guinea guava were monogenotypic (Fig. 3). All the red fleshed genotypes RCGH-4, RCGS-1, Local Pink, Lalit and Allahabd Surkha were found closer to each other. The genetic distance among the genotypes ranges form 0.10 - 0.84 with mean value of 0.47. Among the guava genotypes, Guinea guava was found the most diverse from all the genotype with distance value of 0.84 with RCG-3 and found closer to cream colour genotype RCGH-1(0.66). The improved hybrids RCGH-1, RCGH-4, and RCGH-7 were found closer to Allahabad Safeda, RCGS-1 and L-49, respectively (Table 5). Further, Analysis of molecular variance (AMOVA) between two population *i.e.*, white (10 genotypes) and red fleshed (6 genotypes) guava also revealed the presence of the wider variability in among the population (7 %), among the individual (56 %) and within the individual (37 %) with F*st* value range of 0.069–0.562 and mean of 0.122.

3.5.3. Principal coordinate analysis

Under principal coordinate analysis (PCoA), the first three coordinates explained 39.76 % of the total variation, with 15.97% defined by the first coordinate and 13.02% by the second coordinate. Similar to

Table 3
Cluster mean value for fruits and quality traits of guava genotypes.

Groups	FWT	FL	FD	NOS	TA	TSS	RS	AA	TPC	FLC	FLV	DPPH	FRAP
1	79.94	60.03	54.17	353.25	0.35	10.63	3.76	108.35	160.84	182.62	22.64	60.2	3.89
2	126.55	62.80	64.36	190.06	0.40	10.71	4.24	141.45	240.65	430.32	28.66	42.4	4.90
3	135.85	59.57	67.52	290.29	0.46	10.69	4.21	133.54	168.05	341.75	24.23	55.3	3.44

FWT, fruit weight; FL, fruit length; FD, fruit diameter; NOS, number of seeds per fruits; TA, titratable acidity; TSS, total soluble solids; RS, reducing sugar; AA, ascorbic acid; TPC, total phenolic content; FLC, flavonoid content; FLV, flavonol content; DPPH, 1, 1-diphenyl-2- picrylhydrazyl; FRAP, ferric reducing antioxidant power.

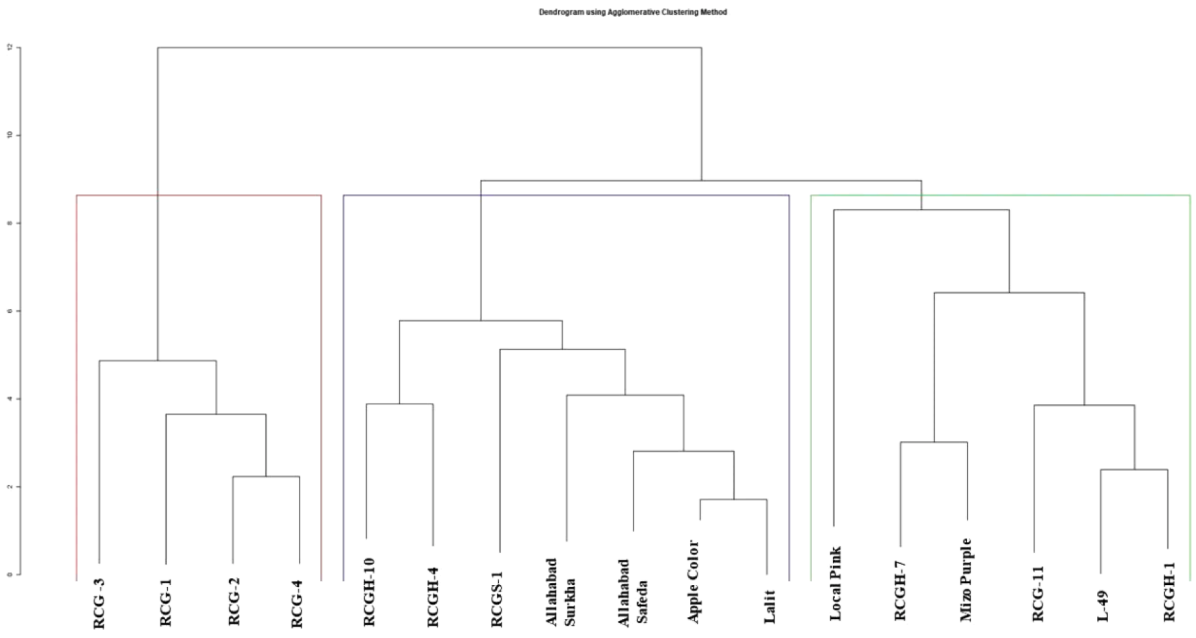

Fig. 2. Genetic relationship among the guava accessions based on fruits and quality attributes.

Table 4
Details of SSR makers used for genetic diversity analysis of guava genotypes .

Primer	Size	Na	Ne	Ho	He	uHe	PIC	Allele frequency	I
mPgCIR01	210–250	3	1.57	–	0.36	0.37	0.33	0.33	0.65
mPgCIR02	205–225	3	2.79	0.44	0.64	0.66	0.55	0.48	1.06
mPgCIR03	110–150	3	2.63	0.13	0.62	0.64	0.54	0.38	1.02
mPgCIR04	110–150	4	2.39	0.50	0.58	0.60	0.46	0.38	1.05
mPgCIR05	240–260	3	1.59	–	0.37	0.38	0.34	0.33	0.68
mPgCIR07	155–160	2	1.25	–	0.20	0.20	0.18	0.50	0.35
mPgCIR08	210–260	4	1.89	0.12	0.47	0.48	0.46	0.26	0.92
mPgCIR09	140–210	7	4.63	0.33	0.78	0.81	0.79	0.19	1.71
mPgCIR10	215–160	5	2.13	0.22	0.53	0.55	0.53	0.24	0.96
mPgCIR11	260–290	3	2.95	–	0.66	0.68	0.59	0.33	1.09
mPgCIR13	220–230	2	1.25	–	0.20	0.20	0.18	0.50	0.35
mPgCIR14	180–200	2	1.12	–	0.10	0.11	0.10	0.50	0.21
mPgCIR15	110–160	3	1.12	0.06	0.11	0.11	0.19	0.35	0.25
mPgCIR16	260–290	4	3.07	0.39	0.67	0.69	0.66	0.35	1.23
mPgCIR17	160–240	6	4.18	0.06	0.76	0.78	0.75	0.18	1.53
mPgCIR18	170–240	6	4.13	0.61	0.76	0.78	0.72	0.27	1.53
mPgCIR19	220–300	9	6.00	0.11	0.83	0.86	0.83	0.12	1.98
mPgCIR20	260–310	4	1.58	0.17	0.37	0.38	0.41	0.29	0.69
mPgCIR21	110–160	6	3.70	0.28	0.73	0.75	0.71	0.21	1.50
mPgCIR22	230–280	6	5.36	0.06	0.81	0.84	0.78	0.18	1.72
mPgCIR23	160–180	2	1.53	–	0.35	0.36	0.29	0.50	0.53
mPgCIR25	100–120	2	1.86	0.39	0.46	0.47	0.36	0.69	0.65
mPgCIR26	160–210	3	1.63	0.50	0.39	0.40	0.39	0.50	0.65
mPgCIR31	110–150	4	1.82	0.06	0.45	0.46	0.46	0.26	0.88
mPgCIR46	80–160	4	2.03	0.33	0.51	0.52	0.50	0.33	0.94
mPgCIR48	90–150	5	2.45	0.11	0.59	0.61	0.59	0.22	1.11
mPgCIR93	100–140	4	2.12	0.33	0.53	0.54	0.48	0.33	0.88
mPgCIR101	110–130	3	1.74	–	0.43	0.44	0.37	0.33	0.73
mPgCIR108	110–180	4	2.31	0.11	0.57	0.58	0.54	0.28	0.96
mPgCIR109	105–120	4	2.75	–	0.64	0.65	0.57	0.25	1.13
mPgCIR111	105–150	4	2.71	0.11	0.63	0.65	0.58	0.28	1.13
mPgCIR133	140–210	5	1.60	0.11	0.37	0.38	0.45	0.22	0.77
mPgCIR139	210–250	3	1.78	0.22	0.44	0.45	0.50	0.41	0.78
mPgCIR150	105–125	3	1.26	–	0.20	0.21	0.19	0.33	0.43
mPgCIR154	110–150	2	1.12	–	0.10	0.11	0.10	0.50	0.21
mPgCIR161	220–250	3	1.74	0.00	0.43	0.44	0.37	0.33	0.73
mPgCIR175	60–80	3	2.07	0.33	0.52	0.53	0.43	0.44	0.79
mPgCIR179	110–170	7	5.10	0.06	0.80	0.83	0.80	0.15	1.73
mPgCIR180	90–140	4	1.49	0.28	0.33	0.34	0.55	0.56	0.61
mPgCIR182	150–170	3	1.33	0.06	0.25	0.26	0.26	0.35	0.50
mPgCIR184	180–230	5	2.41	0.06	0.58	0.60	0.58	0.21	1.19
mPgCIR192	140–160	2	1.18	0.06	0.15	0.16	0.17	0.53	0.29
mPgCIR194	150–200	4	2.32	0.44	0.57	0.59	0.53	0.36	1.01
mPgCIR195	130–160	5	2.38	–	0.58	0.60	0.54	0.20	1.16
mPgCIR200	160–180	2	1.60	0.39	0.38	0.39	0.34	0.69	0.56
mPgCIR201	120–160	3	2.23	0.94	0.55	0.57	0.45	0.65	0.87
mPgCIR205	90–125	4	2.17	0.28	0.54	0.55	0.49	0.32	0.89
mPgCIR206	170–200	3	2.66	0.44	0.62	0.64	0.53	0.48	1.04
mPgCIR218	140–200	5	1.51	0.11	0.34	0.35	0.40	0.22	0.74
mPgCIR220	200–250	3	1.40	0.11	0.29	0.29	0.30	0.37	0.53
mPgCIR240	160–220	4	1.91	0.17	0.48	0.49	0.46	0.29	0.90
mPgCIR243	150–190	4	1.78	0.06	0.44	0.45	0.43	0.26	0.81
mPgCIR245	180–210	4	2.32	0.28	0.57	0.59	0.55	0.32	1.01
mPgCIR321	110–150	3	1.55	0.06	0.36	0.37	0.36	0.35	0.61
mPgCIR325	150–170	2	1.46	0.17	0.31	0.32	0.30	0.58	0.49
mPgCIR326	100–140	3	1.26	0.17	0.20	0.21	0.30	0.39	0.43
Average	–	3.8	2.25	0.18	0.47	0.49	0.46	0.35	0.88
Range	–	2–9	1.12–6.0	0.0–0.94	0.10–0.83	0.11–0.86	0.10–0.83	0.12–0.69	0.21–1.98

Na, No. of different alleles; Ne, No. of effective alleles; PIC, Polymorphism information content; I, Shannon's Information Index; Ho, observed heterozygosity; He, Expected heterozygosity; uHe, Unbiased expected heterozygosity.

cluster analysis, the PCoA also differentiated the accessions with each other (Fig. 4). PCoA-I differentiated the Guinea guava, RCGH-1 and Mizo Purple form rest of the genotype, and coloured genotypes were found close to each other and differentiated by PCoA-II.

3.5.4. *DNA fingerprinting of the improved cultivars*

The improved cultivars of the guava RCGH-1(Megha Supreme), RCGH-4 (Megha Magenta), RCGH-7 (Megha Wonder) and RCG-11 (Megha Seedless) were identified by a set of the markers (Supplementary Figure 2). RCGH-4 by marker mPGCIR-184 and mPgCIR-194; RCGH −7 by marker mPgCIR-19 and mPgCIR-16; RCG −11 by marker mPgCIR-206 and mPgCIR-325; and RCGH-1 by two set of the markers mPgCIR-108 and mPgCIR-243, mPgCIR-182 and mPgCIR-220. Out of 18 genotypes, 12 were identified by private alleles (Supplementary Table 2). The maximum number of private allels were recorded in Guinea guava (29) and minimum in RCG-1, RCG-3, RCGH-7, RCGH-10 and Local Pink (1 each). Genotypes L-49, Allahabd Safeda, RCGH-4, RCG-11, Lalit and Allahabad Surkha were without any private alleles.

3.6. *Stability analysis for fruit traits*

The analysis of variance has revealed the significant effects ($p <$

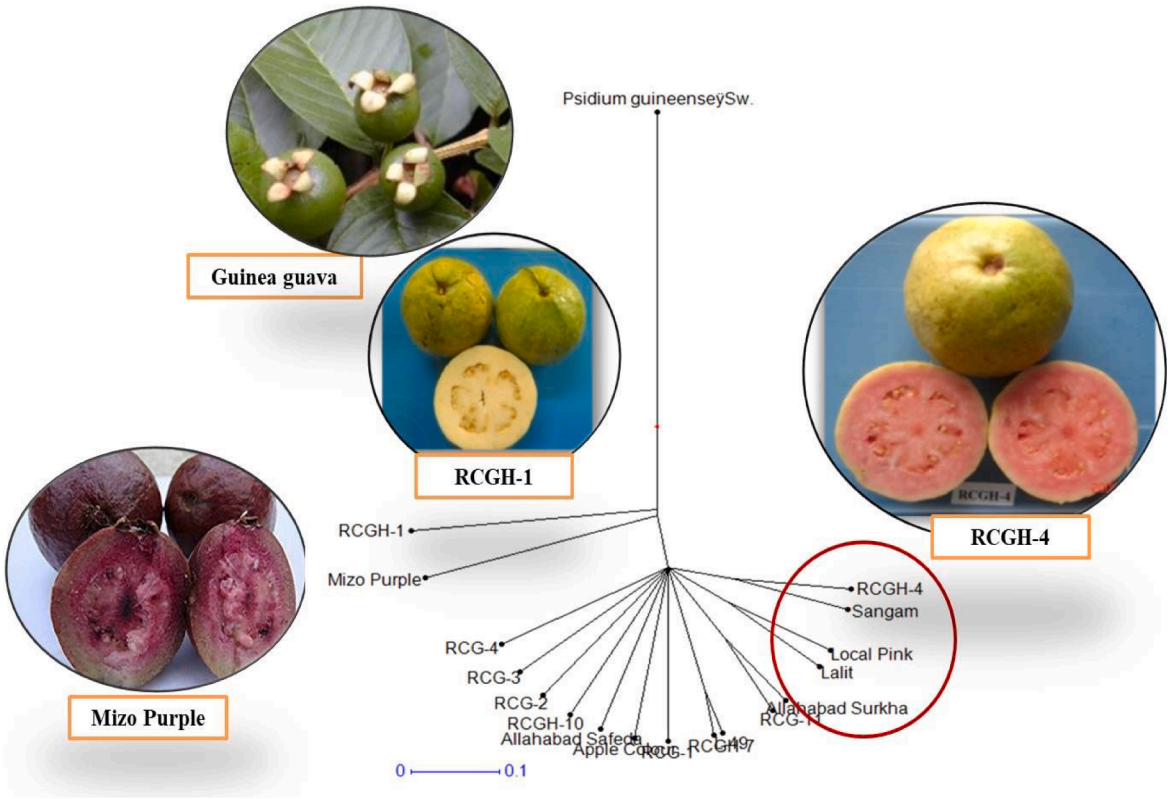

Fig. 3. Dendogram depicting genetic relationship among the guava accessions based on 56 SSR markers.

0.01) for genotypes (fixed), years/environments (random) and genotypes by environments interaction (GEI) on the fruit traits (Supplementary Table 3). Among the factors, genotype has explained significantly, *i.e.*, 69.74 for fruit weight, fruit length (48.70 %), fruit diameter (77.44 %) and number of seeds (94.68 %) of the total variation. The $G \times E$ interaction component was partitioned into first two interaction principal components (IPCA) that explain 100 % of the $G \times E$ variation. IPCA1 explained 65.0, 79.1, 58.7 and 83.3 % and IPCA2 explained 35.0, 20.9, 41.3 and 16.7% of the $G \times E$ interaction for fruit weight, fruit length, fruit diameter and number of seeds, respectively. The guava accessions have also shown wide range of variations for AMMI stability value (ASV, Supplementary Table 4). Genotype with lowest ASV is considered as a stable genotype for the traits, as identified in Mizo Purple, Local Pink, -49, RCGH-1, Lalit and RCGH-4 for fruit weight; RCGS-1, RCGH-4, Mizo Purple, and Allahabad Surkha for fruit length; RCG-4, Apple Color, RCGH-10 and RCGS-1 and RCG-3, RCGH-1, Lalit, RCG-4 and RCGH-7 for number of seeds/100 g pulp (Supplementary Table 3). In AMMI1 biplot, the main effects (genotype mean and environment mean) are plotted against IPCA1 scores for both genotypes and environments. For fruit weight (FW), Lalit, RCGH-1, L-49, RCGH-7, RCGH-4 were found close to the centre point (Fig. 5a) and stable across the years. Likewise, AMMI 2 biplot (Fig. 5b) based on IPCA1 vs. IPCA2 showed that genotypes Mizo Purple, RCGH-4, L-49, RCGH-1, and Lalit were closer to the centre and stable for FW over the years, while genotypes RCG-1, RCG-2, RCG-3 and RCG-4 shown differences in mean FW over the years. Similarly, for fruit length (FL) AMMI 1 biplot (Fig. 5c), genotypes RCGH-4, RCGH-10, and RCGS-1 were found stable while genotypes Local Pink, RCG-11 and RCGH-10 were higher for FL. AMMI2 biplot (Fig. 5d) differentiated the most stable genotypes RCGS-1 with other genotypes for average FL across the years. The biplot AMMI 1 (Fig. 5e) elucidated that genotypes Apple Color, Lalit, RCGH-1 and RCGS-1 were found stable. AMMI2 biplots (Fig. 5f) identified the stable genotypes least affected by GEI as RCGH-4, RCGH-1 and Lalit.

Likewise, AMMI1 for number of seeds (Fig. 5g) has also shown that Apple Color, RCG-2 and RCG-3 were the least affected by the environment. AMMI2 identified RCG-3 and RCGH-1 as stable genotype for number of seeds across the year. Genotypes Local Pink and RCGS-1 were identified as unstable with highly responsive to the environment with difference in number of seeds in pulp over the years (Fig. 5h).

4. Discussion

Guava, being a highly nutritious fruit, has been found to be one of the most popular fruit crops for ensuring nutritional security, particularly in September–October in the mid-hills of the region, where few fruits are available. In order to ensure nutritional security, evaluation and characterization were carried out for 12 guava genotypes using SSR markers. In the present study, the F_1 hybrid RCGH-4 (Megha Magenta) developed from the cross between Red Fleshed (Red) × Allahabad Safeda (White) were found red fleshed and which indicated that red colour is governed by the dominant gene. This might be due to the fact that red colour is dominant over white pulp, and this character is governed monogenically (Subramanyam and Iyer, 1992). However, RCGH-1 (Megha Supreme) developed from Sour Type (White) × Red Fleshed (Red), having a pale-yellow pulp colour, which indicated that the pulp colour is probably from maternal inheritance or polygenic. A similar observation was reported by Thakre et al. (2023) that pulp colour in guava is governed by a polygenic trait and identified 12 distinct SNPs between pink and white-pulped guava genotypes in the Phytoene synthase 1 (PSY1) gene. Hybrid RCGH-7 (Megha Wonder) was a cross of L-49 and Pear Shaped also showed white colour pulp, where both the parents are white colour pulp and based on molecular analysis it was found closer to parental line L-49.

GCV and PCV are the most important parameters to estimate the level of variability present in the population. The present study had moderate (10–20 %) for fruit length and TSS, while all other fruits and quality traits had higher (> 20 %) GCV and PCV, which indicated the presence of wider variability for these traits as previously reported in the

Table 5
Genetic distance among the guava genotypes based on SSR markers.

	RCG-4	RCG-3	RCG-2	RCG-10	RCGH-7	L-49	Allahabad Safeda	RCGS-1	RCGH-1	Apple Colour	RCGH-4	RCG-11	Mizo Purple	RCG-1	Lalit	Allahabad Surkha	Local Pink
RCG-4	1																
RCG-3	0.33																
RCG-2	0.36	0.32															
RCGH-10	0.37	0.25	0.38														
RCGH-7	0.44	0.35	0.39	0.22													
L-49	0.49	0.40	0.44	0.27	0.10												
Allahabad Safeda	0.50	0.29	0.45	0.37	0.43	0.36											
RCGS-1	0.51	0.44	0.48	0.36	0.34	0.28	0.34										
RCGH-1	0.62	0.61	0.64	0.63	0.54	0.51	0.50	0.53									
Apple Colour	0.58	0.48	0.53	0.43	0.48	0.41	0.39	0.34	0.53								
RCGH-4	0.54	0.41	0.50	0.33	0.41	0.36	0.28	0.27	0.54	0.29							
RCG-11	0.49	0.45	0.51	0.39	0.34	0.29	0.41	0.25	0.50	0.31	0.26						
Mizo Purple	0.58	0.56	0.66	0.59	0.51	0.51	0.52	0.58	0.58	0.52	0.53	0.47					
RCG-1	0.55	0.54	0.42	0.54	0.50	0.42	0.41	0.44	0.51	0.43	0.36	0.48	0.58				
Lalit	0.45	0.41	0.55	0.42	0.46	0.42	0.40	0.44	0.56	0.51	0.39	0.38	0.52	0.43			
Allahabad Surkha	0.49	0.44	0.47	0.39	0.25	0.23	0.43	0.33	0.54	0.40	0.39	0.20	0.48	0.41	0.33		
Local Pink	0.50	0.42	0.55	0.43	0.51	0.49	0.43	0.42	0.62	0.39	0.41	0.37	0.54	0.46	0.25	0.34	
Psidium guineense Sw.	0.82	0.84	0.82	0.75	0.76	0.73	0.79	0.71	0.66	0.73	0.73	0.75	0.79	0.72	0.76	0.76	0.75

guavas of India (Rajan et al., 2005; Patel et al., 2011; Dinesh and Vasugi, 2010; Thakre et al., 2020). Further, the values of PCV were higher than their corresponding values of GCV for all the characters under consideration, indicating that the apparent variation is not only due to genotypes but also to the influence of environment in the expression of traits. Among the traits, the fruit length (FL) and fruit diameter (FD) contribution of genotype was comparatively lower than the other traits, indicating that these traits are under the higher influence of environmental factors. Hence, selection for such traits may not be reliable, and in this case, estimates of heritability and GA may only help in the selection of desirable genotypes. Similarly, findings were also observed by Rajan et al. (2005) in guava crops grown in Lucknow under subtropical conditions. The heritability of a trait is a key component in determining GA through selection (Nyquist and Baker, 1991). The additive gene action is mostly attributed to high heritability combined with high GA (Panse, 1957). According to our research, all traits exhibited high heritability (> 60 %) and high GA (> 20 %), indicating that additive gene action governs these characteristics. This suggests that selection on the basis of the phenotypic performance of these characters would be more effective for further breeding programs (Patel et al., 2015). Therefore, the genotypes with higher fruit sizes are superior in quality parameters such as TSS, TA, RS, TPC, TFLC, and TFC content, as well as antioxidant activity, and can be used for selection.

The success of any crop improvement program depends on the identification of traits and the selection of diverse genotypes. Multivariate analyses such as PCA and cluster analysis have been deployed in many crop species to classify and order the genetic variability and phylogenetic relationships in the populations. Given the perennial nature of guavas, their long gestation period and breeding cycle, as well as the high heterozygosity in the population, it is crucial to identify the desirable traits, their inheritance, and diverse parents before the initiation of the breeding program. The PCA in our study revealed the presence of wider variability for the different traits. PCs with an eigenvalue > 1.0 are considered to be inherently more informative than any single original variable alone (Iezzoni and Pritts, 1991). The first 5 PCs (eigenvalue > 1.0) indicate the distribution of the variability over the PCs. The PC-I differentiated the genotypes for DPPH and seed number; the PC-II for fruit parameters (such as weight and size) and quality traits (TPC, TFC, TFLC, RS, and antioxidant activity FRAP) in the population. The PCA biplot indicated the selection of genotypes for various traits, which included RCG-11 (Megha Seedless) for minimum seed number and RCGS-1 and L-49 for quality traits (TPC, AA, and TSS). The cluster analysis for fruits and quality attributes differentiated the genotypes into three major clusters. The local genotypes were grouped together in cluster-I and were characterized as smaller in fruit size with the maximum seed number, and poor quality. This could be due to the maximum translocation of photosynthates towards the multiplication of seeds. This was further indicated by a negative correlation of seed number with FW (−0.284), FL (−0.240), and FD (−0.361). Meena et al. (2020) also observed a negative correlation between the number of seeds and pulp. The genotypes of this group, due to their better adoptability, could be utilized as rootstocks for the improved cultivars. The genotype of cluster-II was found superior for quality traits (TSS, TA, AA, TPC, TFC, TFLC, and antioxidant activities) with medium fruit weight (>125 g) and size. Out of 7 genotypes, 4 (RCGH-4, RCGS-1, Allahabad Surkha, and Lalit) were of the most valued traits, i.e., red flesh suitable for processing purposes. Lycopene is a major carotenoid in guava, responsible for the pink coloration of pink-fleshed guava (Rani and Vijayanchali, 2017), which has high antioxidant properties and has demonstrated many beneficial health effects (Rymbai et al., 2013). These colored genotypes can be utilized for processing industries as well as in further improvement programs. Further, the genotypes of cluster III, comprised of unique genotypes, *viz.*, RCGH-1 (light yellow pulp), Mizoram Purple (purple type, anthocyanin rich), and RCG-11 (less seeded cultivar, < 75 seeds/100 g pulp), could be utilized for crop production and future improvement programs. Our correlation study

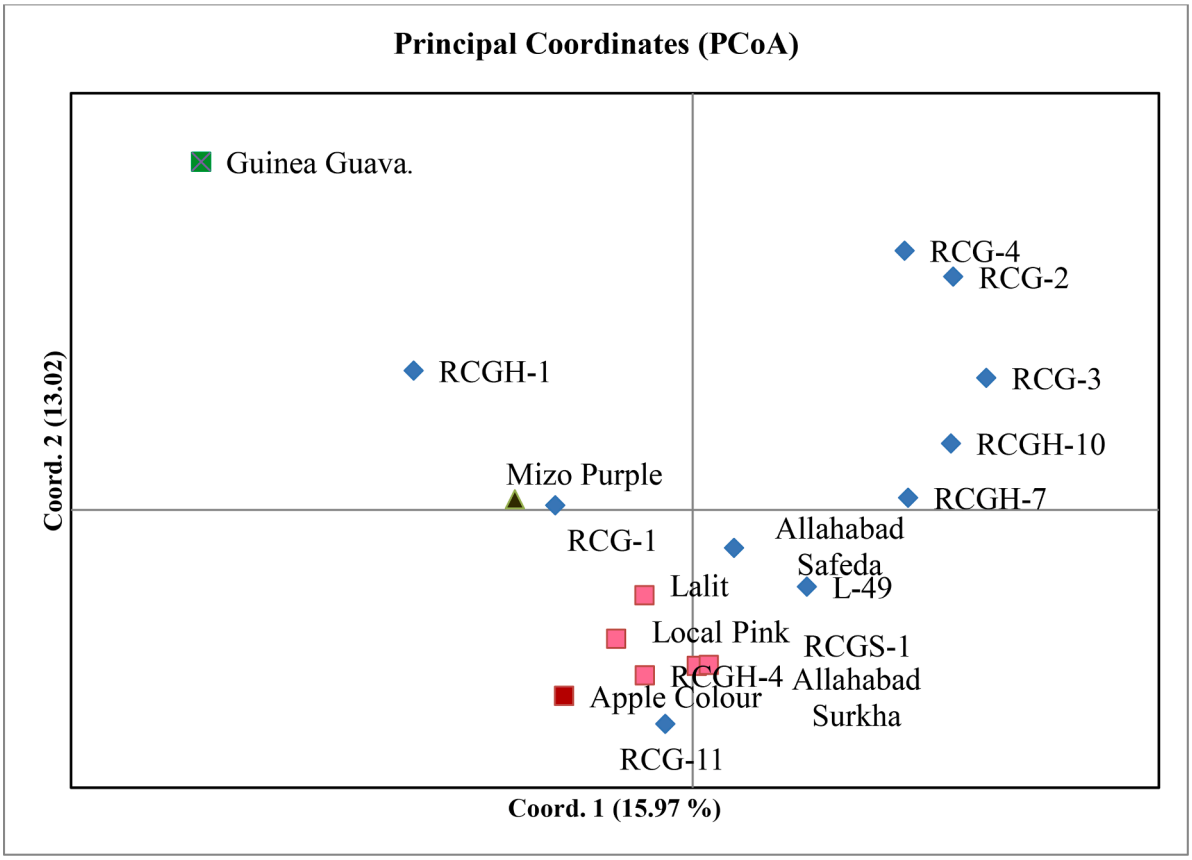

Fig. 4. Principal coordinate analysis of guava accessions based on SSR markers.

indicated that FRAP was positively correlated with TPC, AA, TFLC, and TFC, while DPPH was negatively correlated with quality traits (AA, TPC, TFC, TFLC, and antioxidant activity). It is well established that the IC50 value of DPPH is inversely proportional to the free radical scavenging activity (Rymbai et al., 2023). Corrêa et al. (2011) also observed a similar trend of correlation among the quality and antioxidant properties of guava fruits. Our study also suggested that the colored genotypes (cluster-II) were found to be rich in quality and antioxidant parameters, hence being identified as superior in quality traits over the white pulp genotypes.

The molecular analysis has shown the amplification of all 56 SSR markers in both the common guava genotypes (*Psidium guajava* L.) and Guinea guava (*Psidium guineense* Sw.). These makers showed wider allelic variations (2.0–9.0), indicating the presence of a wider diversity in the population. Allele frequencies in a population are a reflection of genetic diversity, and the allele frequency of the markers varied from 0.12 to 0.69, indicating a low to moderate gene variant in the population. The PIC value depends on the genetic diversity among the genotypes. The PIC value in our study ranged from 0.12 (mPgCIR14 and mPgCIR154) to 0.83 (mPgCIR19), with an average polymorphism of 0.46. Therefore, according to the categories of PIC values (Xie et al., 2010), the average PIC value (0.46), indicating the presence of moderate (0.25–0.50) genetic diversity in the population as per the categories of PIC values (Xie et al., 2010). Similar findings (0.46) were also observed by Kumar et al. (2020) in guava accessions based on SSR markers. The results of the PIC value, coupled with a higher Shannon information index, also prove the locus diversity in the population. The extent of genetic variations measured the amount of actual or potential heterozygosity existing in the population. The SSR marker, being a co-dominant marker, is most suitable to study the extent of heterozygosity in the population. Although guava is a cross-pollinated crop, the observed heterozygosity (0.18) was lower than the expected heterozygosity (0.47). Similar findings were also observed by Risterucci et al. (2005) in guavas. The lower observed heterozygosity over the expected value shows a departure from Hardy-Weinberg equilibrium (HWE) and the possibility of inbreeding (production of the single cultivar, open pollination among themselves and maintained through vegetative propagation), geographical isolation (cultivation of the dominant cultivars in a particular area), artificial selection, population structure and size, and the Wahlund effect, i.e., mixing of individuals from different genetic sources (Johnson and Black, 1984). The Nei genetic distances from 56 SSR markers have also shown wider variations among the genotypes, which range from 0.10 between RCGH-7 and L-49 (as it is one of the parents of RCGH-7) to 0.84 between RCG-3 and related species Guinea guava (*P. guineense* Sw.). The cluster analysis based on Nei genetic distance grouped the guava genotypes into 4 groups. Cluster I comprised 15 accessions, while RCGH-1, Mizo Purple, and Guinea Guava were monogenotypic. All the red-colored genotypes were found close to each other in cluster I. Similarly, all colored genotypes except Local Pink, RCGS-1, RCGH-4, Allahabad Surkha, and Lalit were also grouped together based on fruit and quality traits. Likewise, the PCoA also differentiated the accessions from each other. Further, fruit color-based analysis of molecular variance (AMOVA) between two populations, *i.e.*, white (10 genotypes) and red fleshed (6 genotypes), revealed the presence of wider variability among the population (7 %), among the individual (56 %), and within the individual (37 %). Moreover, the F*st* value range of 0.069–0.562 and mean of 0.122 also indicated a low to moderate level of genetic differentiation among the genotypes of the guava.

Additionally, these 56 SSR markers also differentiated the improved cultivars of guava, like RCGH-1 (Megha Supreme), RCGH-4 (Megha Magenta), RCGH-7 (Megha Wonder), and RCG-11 (Megha Seedless), by a set of markers. The improved cultivars were differentiated by one set of markers, exception of cv. RCGH-1 by two sets of markers mPgCIR-108

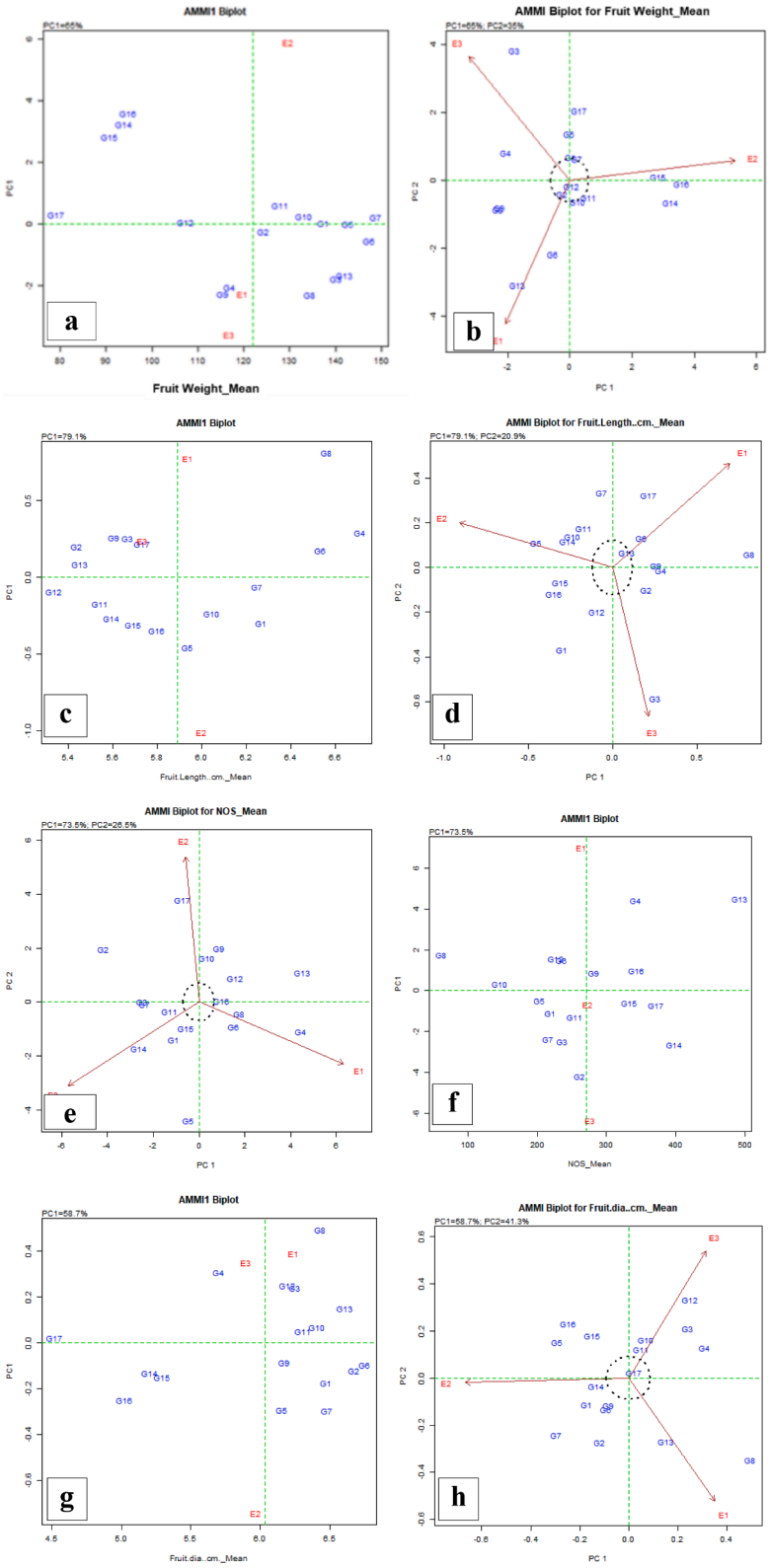

Fig. 5. AMMI1 biplot for fruit traits. (a) AMMI biplot for additive effect vs. IPCA1 for fruit weight, (b) AMMI2 biplot showing interaction between IPCA1 vs. IPCA2 for fruit weight, (c) AMMI biplot for additive effect vs. IPCA1 for fruit length, (d) AMMI2 biplot showing interaction between IPCA1 vs. IPCA2 for fruit length, (e) AMMI biplot for additive effect vs. IPCA1 for fruit diameter, (f) AMMI2 biplot showing interaction between IPCA1 vs. IPCA2 for fruit diamter, (g) AMMI biplot for additive effect vs. IPCA1 for number of seeds, (h) AMMI2 biplot showing interaction between IPCA1 vs. IPCA2 for number of seeds. .

and mPgCIR-243; mPgCIR-182 and mPgCIR-220. About 12 of the genotypes was identified by private alleles with the maximum number of private allels was recorded in Guinea guava (29) and the minimum in RCG-1, RCG-3, RCGH-7, RCGH-10, and Local Pink (1 each). Genotypes L-49, Allahabd Safeda, RCGH-4, RCG-11, Lalit, and Allahabad Surkha were without any private alleles. Further population studies and hybrid purity tests can be conducted using these genotype-specific alleles.

Guava is a sub-topical crop; the fruits and quality traits are affected by the soil and climate conditions of the growing pockets. Hence, identification of the stable genotype is very important, especially for commercial production. In our study, under humid subtropical climatic conditions, the AMMI model of stability for fruit traits was analyzed, and analysis of variance has shown the significant contribution of the genotypes and genotype-environment interaction on the expression of all the fruit traits over the years under the mega environment. Among the fruit traits, the number of seeds was least affected by environmental factors. However, none of the genotypes were found stable for all the traits, which was also observed by Miller et al. (2004) in apples. Based on AMMI stability values for fruit weight, the genotypes Mizoram Purple, Local Pink, L-49, RCGH-1, and RCGH-4 were found to be most stable, with the least AMMI stability value. Among the genotypes for multi-traits, the red-fleshed cultivar RCGH-4 (Megha Magenta) was found stable for fruit weight, fruit length, and medium in seed number; therefore, such genotypes can be promoted for commercial production suitable for processing. While RCGH-1 (Megha Supreme) has pale yellow pulp for fruit weight, fruit diameter, and medium in seed number suitable for table purposes.

5. Conclusion

The present investigation showed wider variability among the genotypes of guava on the basis of fruit morphological, biochemical, and molecular analyses. The majority of traits have high heritability and genetic advance, are controlled by additive gene action, and are highly responsive to selection. The pulp color is polygenic in inheritance. The red-fleshed genotypes are superior for quality attributes (TSS, TA, AA, TPC, TFC, and TFLC) as well as antioxidant activity. The markers specific to the genotype could be utilized for the testing of hybrid purity. Superior cultivars Megha Magenta (RCGH-4) and Megha Supreme (RCGH-1) were found stable for fruit weight and can be promoted for future breeding program, as well as for commercial cultivation in other similar agro-ecological conditions.

CRediT authorship contribution statement

Veerendra Kumar Verma: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Heiplanmi Rymbai:** Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Nishant Anandrao Deshmukh:** Visualization. **Bijoya Bhattacharjee:** Visualization. **Anjani Kumar Jha:** Visualization, Validation. **Ram Kishor Patel:** Resources. **Joiedevivreson Mawlein:** Data curation. **Biydut Chandan Deka:** Visualization, Resources. **Samarendra Hazarika:** Supervision. **Vinay Kumar Mishra:** Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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