



RESEARCH ARTICLE

# Genetic diversity and trait correlation analysis among guava (*Psidium guajava* L.) parents and their F<sub>1</sub>s differing in pulp color

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## Abstract

Guava (*Psidium guajava* L.) is popularly known as 'Super Fruit' and 'Apple of the Tropics' due to its nutritive richness. The pulp color of the guava fruit is associated with its pigment composition. In the present study, twenty-two guava hybrids and their 5 parental genotypes were evaluated for various fruit quality-related traits. The fruit weight (FW), fruit length (FL), fruit width (WF), pulp thickness (PT), seed core diameter (SCD) and FL/width ratio in guava hybrids varied from 78.93-207.10 g, 49.66-83.95 mm, 50.07-73.08 mm, 9.58-18.97 mm, 17.46-44.95 mm and 0.83-1.30, respectively. Total soluble solids (TSS), ascorbic acid (ASC) content, lycopene (LYC) content, total anthocyanins (TAN) and total carotenoids (TCR) in the hybrid fruits ranged from 10.17-18.80 °B, 143.79-275.99 mg/100 g, 0.17-9.51 mg 100 g<sup>-1</sup>, 0.01-4.61 mg 100 g<sup>-1</sup> and 0.16-2.49 mg 100 g<sup>-1</sup>, respectively. In general, white-pulped genotypes had higher ASC content than the pink/red pulped ones. Pulp-color-related traits, viz., LYC content, TCR and TAN, showed high heritability (H) and mean genetic advance (GA). Correlation analysis revealed that LYC content was positively correlated with TCR. The observed phenotypic coefficient of variation (PCV) for all traits exceeded the respective genotypic coefficient of variation (GCV), indicating genetic diversity among the studied guava genotypes. Cluster analysis differentiated guava genotypes into distinct clusters based on pulp color as well as other fruit-related traits. The hybrids, red/pink pulp: 'HSU/SH-16-8-2', 'HSU/SH-16-8-3', 'PPT/HSU-16-9-16' and white pulp: 'HSU/SH-16-8-18', 'SH/BG-14-1-2', excelled for fruit-related traits, having potential to be utilized in future breeding programs.

**Keywords:** correlation; genetic variation; heritability; lycopene; *Psidium guajava* L.

## Introduction

Globally, there is an increased demand for phytochemical-rich sources for the human diet, driven by growing awareness of the health benefits associated with these compounds, i.e., antioxidant, anti-inflammatory, anticancer and cardioprotective functions. Guava (*Psidium guajava* L.) offers a variety of health benefits due to its nutrient-rich profile. The fruit is exceptionally high in vitamin C (3-4 times higher than oranges) (1), which is important for immune function, skin health and antioxidant protection (2). Besides, the fruit is also rich in dietary fiber (5.4 mg 100 g<sup>-1</sup>) and nutrients including calcium (18 mg 100 g<sup>-1</sup>), magnesium (22 mg 100 g<sup>-1</sup>), phosphorus (40 mg 100 g<sup>-1</sup>) and potassium (417 mg 100 g<sup>-1</sup>) (3).

In recent times, the global guava industry has witnessed a shift from white-pulped cultivars to red/pink-pulped cultivars, rich in health-promoting bioactive compounds, i.e., LYC/anthocyanins, developed through systematic breeding efforts (4). Varietal

improvement in guava is limited by inherent constraints, such as heterozygosity, cross-incompatibility, juvenility and epigynous flower structure (5). Most of the pink/red pulped guavas in the world are a result of selection, such as Hong Kong pink in Thailand (6); Selection 25-5 and Selection 25-9 in Mexico (7); Ruby in the USA (8). Besides, several red- or pink-pulped guava hybrids have been developed worldwide, including 68/4 and 61/5 in Israel (9). Despite the challenges associated with conventional hybridization breeding, it has been a crucial approach for improving both qualitative and quantitative traits in guava and for generating genetic variability for selection (10).

Genetic diversity plays a critical role in selecting superior genotypes for future breeding programs. In guava, genetic diversity studies have primarily focused on variations in a limited number of morphological and fruit quality traits that are imperative for consumer preference and marketability, such as fruit size, shape, pulp color, ASC content and total soluble solids

(TSS). Considering the traits and requirements of the guava industry, a systematic guava improvement program was initiated at the Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi, India to breed coloured guava cultivars/hybrids enriched with bioactive compounds through inter-varietal hybridization. In the present study, we systematically assessed the qualitative and quantitative fruit traits of 22 guava hybrids developed through inter-varietal hybridization, alongside their parental genotypes, to identify elite hybrids with high commercial potential. Genetics-based information, viz., correlation, genotypic variance, H, GA, etc., will provide new insights into the composition and inheritance of pulp colour in guava.

## Materials and Methods

### Plant material

The present study was conducted at the guava germplasm and hybrid block, Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi, during 2021-2022. The experimental site is located at an altitude of 228 m above the mean sea level with a latitude of 28° 40' N and a longitude of 77° 13' E. The experimental site is typically subtropical, characterized by alluvial soil with a clay loam texture that is slightly alkaline. The hybrid seedlings were planted at 2 m x 2 m. All guava hybrids and their parental genotypes were irrigated and fertilized uniformly, following the recommended practices for guava cultivation, including pest and disease management, to ensure reliable assessment of fruit quality traits in the same agro-climatic zone.

The analysis was carried out on 22 guava hybrids (11 red/pink pulped and 11 white-pulped) and their 5 parents ('Black guava'; 'Hisar Sorkha'; 'Punjab Pink'; 'Pant Prabhat'; and 'Shweta'), which were evaluated for several fruit traits. The specific

information pertaining to guava hybrids and their parents can be found in Table 1. All the hybrids in the present study were aged between 3-6 years at the time of the experiment. Also, the transverse sections of different hybrids and their parents are shown in Fig. 1. Observations were recorded in triplicate, with 5 fruits per replication (n = 15). The fruits from the selected genotypes were harvested at the physiologically mature stage during the winter season. In North India, the winter season is preferred for guava cultivation because cool, dry weather produces fruit of superior quality. Harvested fruits were carried to the division laboratory and each fruit was washed thoroughly and wiped with tissue paper to remove surface impurities, if any. The samples were stored at -20 °C until analysis.

### Morphological parameters

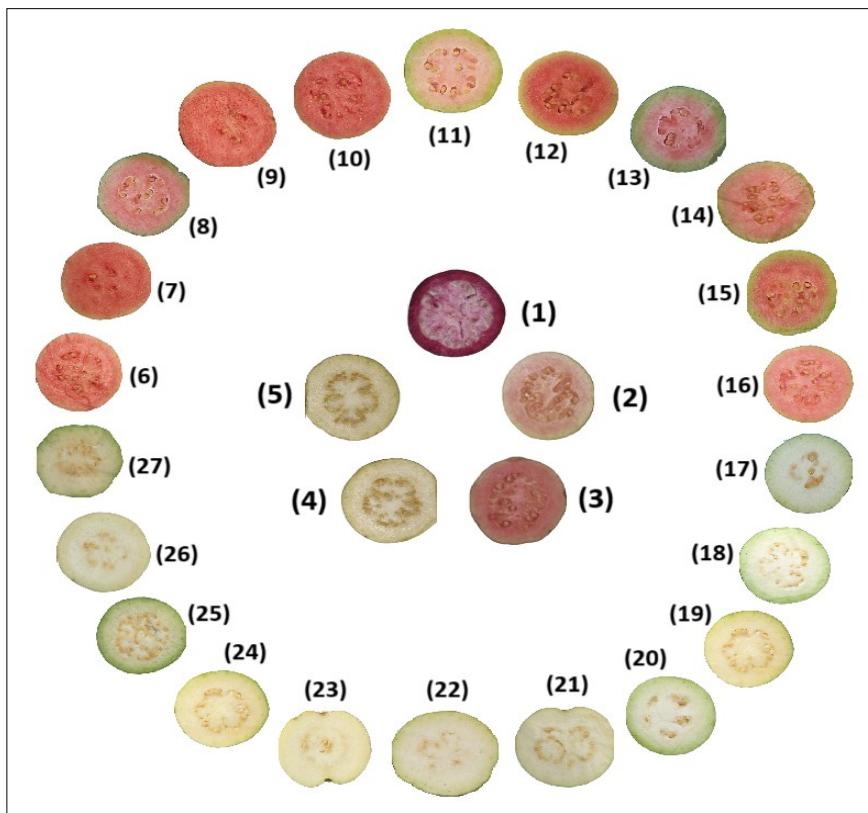
The International Union for the Protection of New Varieties of Plants (UPOV) published guidelines for testing distinctness, uniformity and stability in guava to characterize crop germplasm worldwide. Guava hybrids and their parents were characterized for 11 qualitative traits and classified according to the UPOV descriptors for guava (11). The Royal Horticultural Society (RHS) colour chart was also used to classify guava genotypes based on peel and pulp colour.

### Physical parameters

Fruit physical characters, i.e., FW, WF, FL, SCD, PT and FL-to-width ratio (L/W) were recorded for individual fruit, as per standard procedures. Fruit weight was measured using a digital weighing balance (Adiar Dutt-1620C, USA) and the average weight was computed. LF, WF and SCD were measured with the help of a digital Vernier Caliper (Mitutoyo Model 500-147) and the average values of the replicates were computed. Data on total soluble contents, as degree Brix, was determined using a Digital Refractometer (MA871; Milwaukee Instruments, Inc., Rocky Mount,

**Table 1.** Details pertaining to the characteristics and parentage of guava genotypes utilized in the study

S. No.	Guava genotypes	Details
1.	Black guava	Germplasm collection, maintained at Guava germplasm block, Todapur orchard, Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi-110 012.
2.	Hisar surkha	It is a cross between apple colour x Banarasi Sorkha made at CCSHAU, Hisar, Haryana, India. Its fruits are pink-pulped and roundish.
3.	Punjab pink	It is a hybrid between Portugal x L 49 = F1 x Apple colour, released in 2009. The fruit is medium to large in size, with an attractive golden-yellow colour.
4.	Pant prabhat	Selection from GBPUAT, Pantnagar, Uttarakhand, India. The cultivar has white pulp with a distinct aroma and soft seeds. It is released for commercial cultivation in 2004.
5.	Shweta	Selection from half-sib population of apple colour, CISH, Lucknow. Fruits are medium in size, globose-shaped, with a creamy-white mesocarp, high TSS and vitamin C and good keeping quality.
6.	PP/BG-18-7-8	Punjab pink x Black guava
7.	PP/BG-19-14-6	Punjab pink x Black guava
8.	PP/BG-19-15-1	Punjab pink x Black guava
9.	PP/BG-19-16-8	Punjab pink x Black guava
10.	HSU/SH-16-8-2	Hisar Sorkha x Shweta
11.	HSU/SH-16-8-3	Hisar Sorkha x Shweta
12.	SH/PP-16-7-15	Shweta x Punjab Pink
13.	PP/HU-16-8-14	Punjab pink x Hisar Sorkha
14.	PP/HU-19-17-1	Punjab pink x Hisar Sorkha
15.	PPT/PP-16-7-5	Pant Prabhat x Punjab Pink
16.	PPT/HU-16-9-16	Pant Prabhat x Hisar Sorkha
17.	PP/BG-19-20-2	Punjab pink x Black guava
18.	PP/BG-19-20-11	Punjab pink x Black guava
19.	PP/BG-19-23-13	Punjab pink x Black guava
20.	HSU/SH-16-8-18	Hisar Sorkha x Shweta
21.	SH/BG-14-1-2	Shweta x Black guava
22.	SH/BG-14-1-5	Shweta x Black guava
23.	PPT/BG-19-21-4	Pant Prabhat x Black guava
24.	PPT/SH-16-7-6	Pant Prabhat x Shweta
25.	PP/SH-18-9-12	Punjab pink x Shweta
26.	PP/SH-19-11-2	Punjab pink x Shweta
27.	PP/SH-19-16-4	Punjab pink x Shweta



**Fig. 1.** Transverse section of fruits of the studied guava hybrids and their parents.

(1) = Black guava; (2) = Hisar Surkha; (3) = Punjab Pink; (4) = Pant Prabhat; (5) = Shweta; (6) = PP/BG-18-7-8; (7) = PP/BG-19-14-6; (8) = PP/BG-19-15-1; (9) = PP/BG-19-16-8; (10) = HSU/SH-16-8-2; (11) = HSU/SH-16-8-3; (12) = SH/PP-16-7-15; (13) = PP/HSU-16-8-14; (14) = PP/HSU-19-17-1; (15) = PPT/PP-16-7-5; (16) = PPT/HSU-16-9-16; (17) = PP/BG-19-20-2; (18) = PP/BG-19-20-11; (19) = PP/BG-19-23-13; (20) = HSU/SH-16-8-18; (21) = SH/BG-14-1-2; (22) = SH/BG-14-1-5; (23) = PPT/BG-19-21-4; (24) = PPT/SH-16-7-6; (25) = PP/SH-18-9-12; (26) = PP/SH-19-11-2; (27) = PP/SH-19-16-4.

NC, USA).

#### Ascorbic acid

The procedure determined by AOAC method no. 967.21 was followed for the estimation of ASC concentration (12). A 3 % metaphosphoric acid solution was freshly prepared and 100 mL was used per replication, corresponding to 5 g of the guava fruit sample. Further, a 10 mL aliquot of the metaphosphoric extract was titrated with a standard solution of freshly prepared 2,6-dichlorophenol-indophenol dye. The titration endpoint is characterized by the development of a pink color, which should persist for at least 10-15 sec. Ascorbic acid (mg 100 g<sup>-1</sup>) was calculated using the following equation (13):

$$\text{Ascorbic acid (mg 100 g}^{-1}\text{)} = \frac{\text{Titre value} \times \text{Dye factor} \times V_{(\text{total})}}{V_{(\text{extract})} \times m_{(\text{extract})}} \times 100 \quad (\text{Eqn. 1})$$

#### Pigment content: Lycopene, total anthocyanins and total carotenoids

For LYC estimation, extraction was carried out with a hexane:ethanol: acetone (2:1:1) (v/v) mixture following the standardized protocol (14), with some slight modifications. Fresh fruit samples (5 g) were dissolved in 10 mL of distilled water and vortexed in a water bath at 30 °C for 1 hr, then 8.0 mL of hexane:ethanol: acetone (2:1:1) was added. After that, the samples were capped and vortexed immediately, then incubated in the dark. After at least 10 min, water was added to each sample and vortexed again. Samples were allowed to stand for 10 min to allow phase separation and for air bubbles to

dissipate and the samples were then absorbance at 503 nm. The total LYC content (mg 100 g<sup>-1</sup>) was calculated as follows:

$$\text{Lycopene (mg 100 g}^{-1}\text{)} = \frac{\text{Abs 503nm} \times 537 \times 8 \times 0.55}{0.10 \times 172} \quad (\text{Eqn. 2})$$

Total anthocyanin content was analyzed using a standardized spectrophotometric method (15). Briefly, anthocyanins were extracted from samples (5 g) using a solvent mixture of 95 % ethanol and 1.5 N HCl (85:15, v/v). The samples were macerated in the extraction solvent overnight at 4 °C, then filtered. The absorbance was measured at 535 nm and anthocyanin content was calculated using the molar extinction coefficient (e=98.2).

$$\text{Total anthocyanins (mg 100 g}^{-1}\text{)} = \frac{\text{Abs}535\text{nm} \times \text{Dilution} \times \text{Final volume} \times 100}{\text{Weight of the sample} \times e} \quad (\text{Eqn. 3})$$

Total carotenoids were quantified following a standardized method with slight modifications (16). 2 g of crushed fruit were combined with 20 mL of acetone and allowed to stand overnight. The next day, a hexane (15 mL): water (10 mL) mixture was added to the solution and the hexane layer was isolated. The pigmented hexane layer was collected through filtration. A portion was transferred to a cuvette and the carotenoid content was determined at 450 nm.

$$\text{Total carotenoids (mg 100 g}^{-1}\text{)} = \frac{\text{Abs}450\text{nm} \times \text{Volume of separated solution} \times 3.8 \times 100}{\text{Weight of the sample} \times 1000} \quad (\text{Eqn. 4})$$

## Statistical analysis

The data recorded from the present experimental design followed a randomized block design (RBD). For statistical analysis, one-way analysis of variance (ANOVA) was used to determine significant differences among genotypes for multiple fruit attributes, using the PROC GLM in SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). Tukey's HSD test was also used to identify significant pairwise differences among genotypes at  $p \leq 0.05$ . The assumptions of normality and homogeneity of variance were checked using the Shapiro-Wilk test and Levene's test, respectively. Principal component analysis (PCA) and correlation analysis were performed using the mean values of physical and biochemical fruit traits in RStudio v. 2022.07.1-554. The genetic estimation of traits was carried out by calculating genetic parameters, including PCV, GCV, H and GA (17). The cluster analysis based on different parameters was conducted using the unweighted pair-group method with arithmetic mean (UPGMA). The statistical analyses and graphical representations were performed using RStudio (RStudio, PBC, Version 2022.07.1-554) software.

## Results

### Morphological characterization

UPOV descriptors of guava have been considered for distinctiveness, uniformity and stability and are presented in Fig. 2. Significant variation was observed across the various morphological parameters. Round fruit shape and smooth relief of the fruit surface were predominantly present. Longitudinal ridges were absent in all guava genotypes except some hybrids ('PP/BG-19-16-8', 'HSU/SH-16-8-2', 'PP/HSU-19-17-1', 'PP/BG-19-

20-11' and 'PP/SH-19-16-4'), which had a weak prominence. Longitudinal grooves of fruit and discoloration of flesh after cutting were absent in all the guava genotypes. The size of the sepals of the fruits ranged from small to medium. There were variations regarding the diameter of the calyx cavity in relation to that of the fruit. Except for 'Pant Prabhat', all the guava cultivars had an inconspicuous ridged collar around the calyx cavity, whereas the hybrids had variations. Fruit puffiness was absent in all guava parents and hybrids except 'PPT/BG-19-21-4'. Also, all the guava parents produced juicy fruits, except 'Black guava', whereas the hybrids belonged to both the juicy and the medium categories. All the studied guava hybrids and parents were even for pulp color except 'Black guava'.

### Physical parameters

Significant variation was observed for the physical fruit parameters among guava hybrids and parents (Table 2). Among the parents, the highest FW (127.40 g) and WF (64.21 mm) were recorded for 'Pant Prabhat', whereas the highest FL was recorded for 'Shweta' (54.71 mm). Among hybrids, highest FW and WF were recorded for the hybrid 'PPT/HSU-16-9-16', followed by 'HSU/SH-16-8-2', -while, lowest was observed for hybrid 'SH/PP-16-7-15' guava hybrids 'PP/BG-19-15-1', 'PP/BG-19-20-11' and 'PP/BG-19-16-8' had the greatest FL; the lowest was recorded for the hybrid 'PP/SH-19-11-2' (49.66 mm). Among the parents, 'Punjab Pink' (1.07) has a fruit L/W ratio greater than 1, indicating a slightly elongated fruit shape. In hybrids, the fruit L/W ratio ranged from 0.83 to 1.36. Except for hybrid 'PP/SH-19-11-2' (0.90), this ratio was greater than one in all hybrids where 'PP' was a parent (male/female).

In the present study, SCD among parents ranged from

Characters	FSS	RS	LR	LG	SS	DCC	RCC	EVN	DISC	PUFF	JUCE
Genotype/Status	Round	Smooth	Absent	Absent	Small	Small	Inconspicuous	Even	Present	Present	Medium
	Truncate	Rough			Medium	Medium					
	Broadly rounded	Bumpy	Present	Present	Medium	Large	Conspicuous	Uneven	Absent	Absent	Juicy
Black guava											
Hisar Surkha											
Punjab Pink											
Pant Prabhat											
Shweta											
PP/BG-18-7-8											
PP/BG-19-14-6											
PP/BG-19-15-1											
PP/BG-19-16-8											
HSU/SH-16-8-2											
HSU/SH-16-8-3											
SH/PP-16-7-15											
PP/HSU-16-8-14											
PP/HSU-19-17-1											
PPT/PP-16-7-5											
PPT/HSU-16-9-16											
PP/BG-19-20-2											
PP/BG-19-20-11											
PP/BG-19-23-13											
HSU/SH-16-8-18											
SH/BG-14-1-2											
SH/BG-14-1-5											
PPT/BG-19-21-4											
PPT/SH-16-7-6											
PP/SH-18-9-12											
PP/SH-19-11-2											
PP/SH-19-16-4											

**Fig. 2.** UPOV (1978) fingerprints of guava hybrids and their parents based on fruit morphological traits. FSS = Fruit shape at stalk end; RS = Relief of surface; LR = Longitudinal ridges; LG = Longitudinal grooves; SS = Size of sepal; DCC = Diameter of calyx cavity in relation to that of fruit; RCC = Ridged collar around calyx cavity; EVN = Evenness of pulp color; DISC = Discoloration of flesh after cutting; PUFF = Fruit puffiness; JUCE = Fruit juiciness.

**Table 2.** Physical fruit parameters of the studied guava hybrids and their parents

Genotype	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Fruit length / width ratio	Seed core diameter (mm)	Pulp thickness (mm)
<b>Parents</b>						
Black guava	85.71 ± 10.62jkl	55.45 ± 4.39ghijk	49.62 ± 1.58kl	0.90 ± 0.05hij	39.66 ± 4.67bcd	7.89 ± 0.97l
Hisar Surkha	89.69 ± 5.68jkl	57.36 ± 2.51ghi	47.94 ± 2.10l	0.84 ± 0.01j	35.24 ± 0.70defg	11.06 ± 0.95jk
Punjab Pink	65.21 ± 5.25l	45.88 ± 1.17l	48.86 ± 6.18kl	1.07 ± 0.16def	38.25 ± 1.22cde	5.81 ± 1.05m
Pant Prabhat	127.40 ± 8.44efghi	64.21 ± 5.69cde	47.34 ± 2.64l	0.74 ± 0.03k	38.53 ± 3.02cde	12.84 ± 1.37ghij
Shweta	110.07 ± 10.00ghij	63.36 ± 3.20def	54.71 ± 1.07ijk	0.86 ± 0.03ij	37.94 ± 0.27cdef	12.71 ± 1.55ghij
<b>Hybrids</b>						
PP/BG-18-7-8	103.20 ± 6.35hijk	56.90 ± 2.25ghi	59.06 ± 0.98fghij	1.03 ± 0.02efg	29.03 ± 1.70ijk	13.91 ± 0.29efgh
PP/BG-19-14-6	125.66 ± 13.80efghi	58.55 ± 2.63fgh	62.74 ± 2.29defg	1.07 ± 0.01def	22.13 ± 0.15lm	18.20 ± 1.38ab
PP/BG-19-15-1	196.50 ± 5.77a	66.56 ± 1.20bcd	83.95 ± 0.92a	1.30 ± 0.06a	35.60 ± 1.45defg	15.48 ± 0.38cde
PP/BG-19-16-8	192.70 ± 5.03ab	63.44 ± 1.25def	76.91 ± 0.31b	1.21 ± 0.02b	25.60 ± 0.53kl	18.91 ± 0.85a
HSU/SH-16-8-2	200.50 ± 15.22a	71.82 ± 2.52ab	73.97 ± 1.93bc	1.02 ± 0.03fg	43.66 ± 2.02ab	14.06 ± 0.60defgh
HSU/SH-16-8-3	165.66 ± 11.24bc	69.33 ± 2.88abc	62.15 ± 2.28efgh	0.89 ± 0.07hij	44.95 ± 2.02a	12.16 ± 1.59hij
SH/PP-16-7-15	78.93 ± 7.27kl	50.07 ± 0.07kl	56.28 ± 1.39hij	1.11 ± 0.04cdef	30.90 ± 0.66ghij	9.58 ± 0.30kl
PP/HSU-16-8-14	101.66 ± 15.00ijk	57.31 ± 4.43ghi	59.23 ± 0.57fghij	1.0 ± 0.093fg	29.76 ± 2.06hijk	13.74 ± 1.18efgh
PP/HSU-19-17-1	127.66 ± 3.79efghi	60.18 ± 0.30efg	66.65 ± 0.43de	1.10 ± 0.01cdef	29.66 ± 0.42hijk	15.25 ± 0.36cdef
PPT/PP-16-7-5	86.23 ± 26.80jkl	54.22 ± 5.01hijk	49.79 ± 6.01kl	0.91 ± 0.03hij	29.83 ± 5.97hijk	12.18 ± 0.57hij
PPT/HSU-16-9-16	207.10 ± 15.03a	73.08 ± 1.83a	68.38 ± 1.40cd	0.93 ± 0.01hi	41.56 ± 2.21abc	15.75 ± 0.23cde
PP/BG-19-20-2	81.43 ± 17.05jkl	51.06 ± 5.40jkl	57.29 ± 5.91ghij	1.12 ± 0.02cde	17.46 ± 3.87m	16.76 ± 0.94bc
PP/BG-19-20-11	142.50 ± 15.45cdef	58.37 ± 2.53fgh	79.52 ± 3.56ab	1.36 ± 0.01a	20.43 ± 1.51m	18.97 ± 0.54a
PP/BG-19-23-13	81.46 ± 3.00jkl	52.16 ± 0.35ijk	50.04 ± 0.75kl	0.95 ± 0.01gh	29.10 ± 1.39ijk	11.53 ± 0.53ijk
HSU/SH-16-8-18	148.06 ± 14.97cdef	66.69 ± 6.81bcd	61.93 ± 9.85efgh	0.92 ± 0.07hi	31.20 ± 3.56ghij	14.75 ± 4.31cdefg
SH/BG-14-1-2	159.83 ± 11.39cd	68.28 ± 3.42abcd	61.4 ± 4.02efgh	0.89 ± 0.02hij	38.79 ± 2.48bcde	14.74 ± 1.99cdefg
SH/BG-14-1-5	140.86 ± 14.86cdef	66.56 ± 4.89bcd	57.42 ± 7.71ghij	0.85 ± 0.07ij	34.41 ± 8.32efgh	16.07 ± 1.75cd
PPT/BG-19-21-4	153.53 ± 19.04cde	66.86 ± 5.83bcd	60.42 ± 4.33fghi	0.90 ± 0.02hij	37.43 ± 6.12cdef	14.70 ± 0.14cdefg
PPT/SH-16-7-6	138.26 ± 7.43cdefg	65.06 ± 1.95cde	54.18 ± 6.31jk	0.83 ± 0.12j	38.30 ± 1.66cde	13.36 ± 1.07fghi
PP/SH-18-9-12	122.00 ± 12.13fghi	56.48 ± 2.16ghij	64.53 ± 1.66def	1.13 ± 0.02bcd	33.03 ± 2.98fghi	12.00 ± 0.32hij
PP/SH-19-11-2	89.06 ± 9.74jkl	55.07 ± 0.78ghijk	49.66 ± 0.78kl	0.90 ± 0.01hij	27.23 ± 0.68jk	13.91 ± 0.35efgh
PP/SH-19-16-4	131.56 ± 9.64defgh	56.86 ± 1.22ghi	66.98 ± 3.08de	1.17 ± 0.03bc	27.70 ± 0.89jk	14.59 ± 0.17defg
Mean	127.87	60.42	60.60	1.00	32.88	13.66
C.D. (p ≤ 0.05)	19.57	5.52	6.17	0.08	4.94	2.10

34.25-39.66 mm. In the hybrids, maximum SCD was recorded in 'HSU/SH-16-8-3', followed by 'HSU/SH-16-8-2' and 'PPT/HSU-16-9-16'. Lowest SCD was recorded for hybrid 'PP/BG-19-20-2'. Among parents, the maximum PT was recorded in 'Pant Prabhat' and lowest in 'Punjab Pink'. However, among hybrids, PT ranged from 9.58 to 18.97 mm.

### Total soluble solids and ascorbic acid

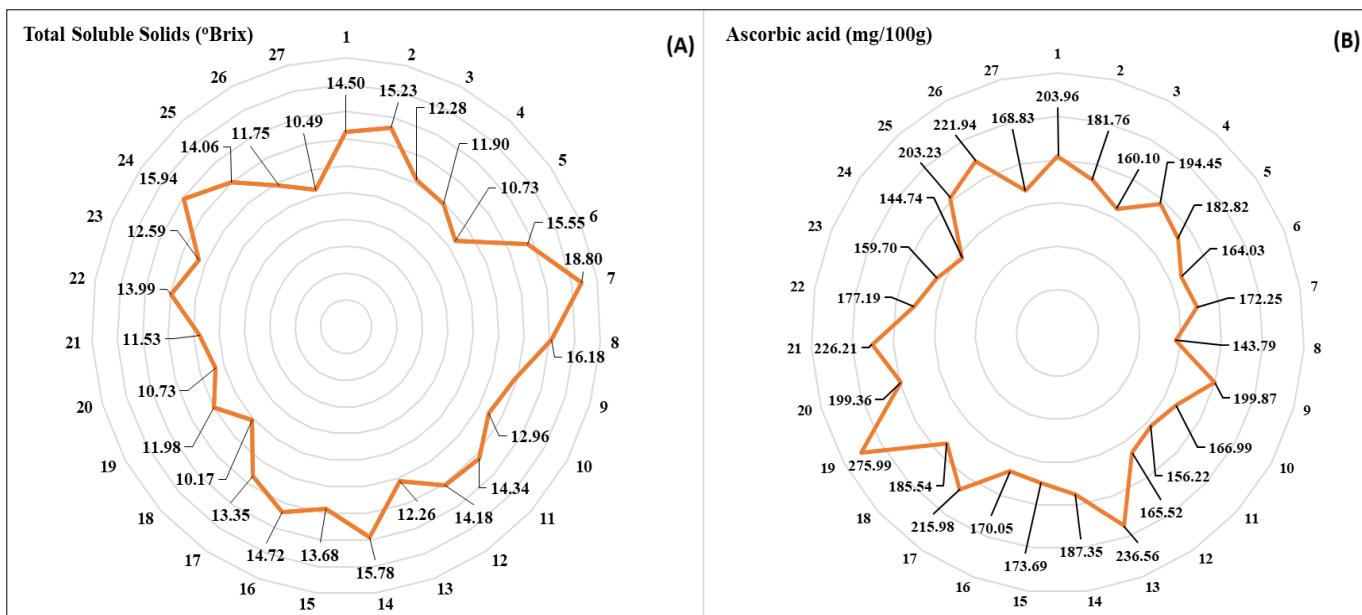
The data presented in Fig. 3A indicate significant variation in TSS among guava hybrids and their parents. Significantly, the highest TSS was recorded for guava hybrid 'PP/BG-19-14-6' (red/pink pulp; 18.80 °B), while the lowest was in the hybrid 'PP/BG-19-20-11' (white pulp; 10.17 °B). Among the parents, the highest TSS was found in 'Hisar Surkha' (red/pink pulp; 15.23 °B). Fig. 3B clearly shows differences in ASC content in the fruits of guava hybrids and their parents. The highest ASC content was recorded in hybrid 'PP/BG-19-23-13' (275.99 mg 100 g<sup>-1</sup>), followed by 'PP/HSU-16-8-14' (236.56 mg 100 g<sup>-1</sup>) and 'SH/BG-14-1-2' (226.21 mg 100 g<sup>-1</sup>), while hybrid 'PP/BG-19-14-6' (143.79 mg 100 g<sup>-1</sup>) had the lowest ASC content. Moreover, among the parents, significantly higher ASC content was found in 'Black guava' (203.96 mg 100 g<sup>-1</sup>).

### Pigment composition

In the parental genotypes, LYC content varied considerably, with 'Hisar Surkha' exhibiting the highest concentration and 'Pant Prabhat' the lowest. Among the hybrids, 'PP/BG-18-7-8' recorded the maximum LYC content (9.510 mg 100 g<sup>-1</sup>; red/pink pulp), followed by 'PPT/HSU-16-9-16' (7.796 mg 100 g<sup>-1</sup>; red/pink pulp)

and 'PP/BG-19-16-8' (7.788 mg 100 g<sup>-1</sup>; red/pink pulp) (Table 3). Overall, pink-pulp F1 hybrids exhibited substantially higher LYC concentrations F1 (4.295-9.510 mg 100 g<sup>-1</sup>) compared to white-pulp hybrids (0.169-1.359 mg 100 g<sup>-1</sup>), in which LYC levels were negligible. The purple-pulped parent 'Black guava' displayed a distinct pigment profile, with the highest total anthocyanin content (9.663 mg 100 g<sup>-1</sup>) (Fig. 4). Among the hybrids, 'HSU/SH-16-8-2' recorded the maximum total anthocyanin concentration (4.611 mg 100 g<sup>-1</sup>; pink/red pulp), which was significantly higher than that of 'PP/BG-19-15-1' (4.125 mg 100 g<sup>-1</sup>; pink/red pulp) and 'HSU/SH-16-8-3' (4.122 mg 100 g<sup>-1</sup>; pink/red pulp). Similar to LYC, pink-pulp hybrids consistently exhibited higher total anthocyanin content (2.883-4.611 mg 100 g<sup>-1</sup>) compared to white-pulp hybrids (0-1.154 mg 100 g<sup>-1</sup>).

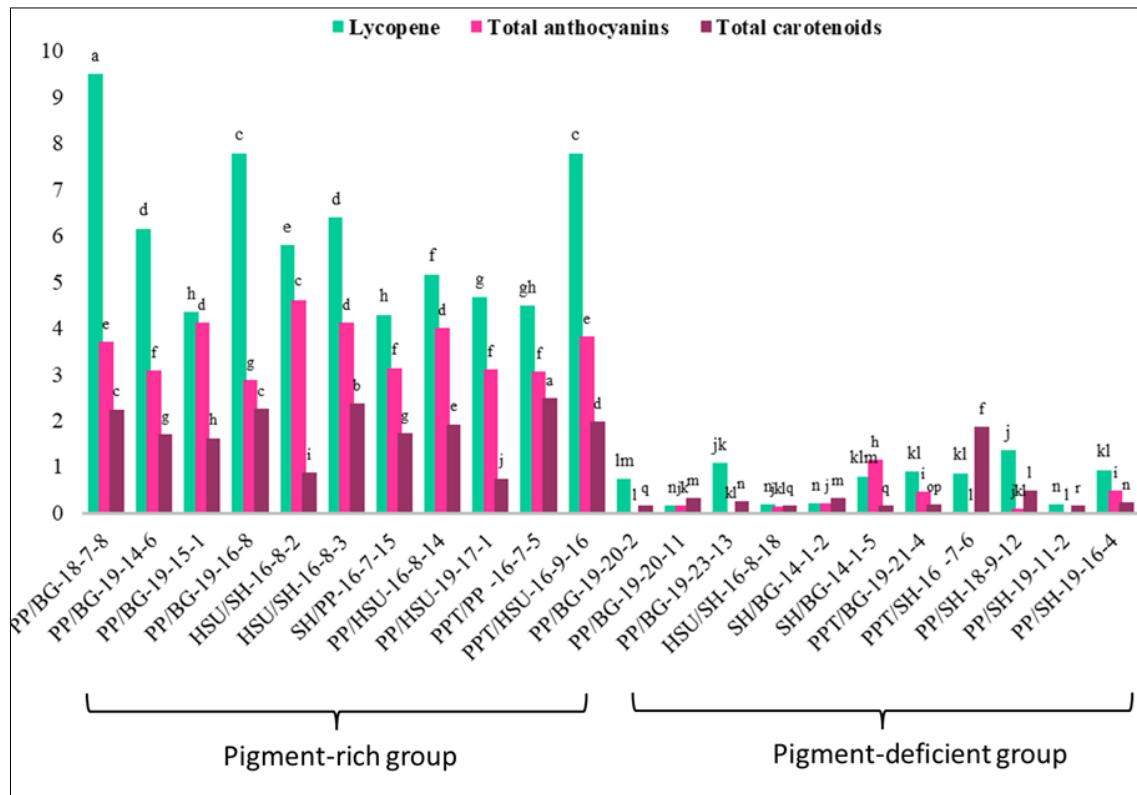
Total carotenoid content, however, exhibited a slightly different trend. Most red/pink-pulp hybrids had higher total carotenoid levels than white-pulp hybrids, except 'PPT/SH-16-7-6' (1.875 mg 100 g<sup>-1</sup>). The highest total carotenoid concentration was observed in 'PPT/PP-16-7-5' (2.493 mg 100 g<sup>-1</sup>; pink/red pulp), followed by 'HSU/SH-16-8-3' (2.371 mg 100 g<sup>-1</sup>; pink/red pulp), with significant differences between them. Among parental genotypes, total carotenoid content ranged from 0.21 to 0.73 mg 100 g<sup>-1</sup>. These results collectively highlight the strong association between pulp color and accumulation of LYC and anthocyanins, while carotenoid distribution appears to be influenced by additional genetic factors beyond pulp pigmentation.



**Fig. 3.** Total soluble solids (A) and ascorbic acid content (B) of studied guava hybrids and their parents. (1) = Black Guava; (2) = Hisar Surkha; (3) = Punjab Pink; (4) = Pant Prabhat; (5) = Shweta; (6) = PP/BG-18-7-8; (7) = PP/BG-19-14-6; (8) = PP/BG-19-15-1; (9) = PP/BG-19-16-8; (10) = HSU/SH-16-8-2; (11) = HSU/SH-16-8-3; (12) = SH/PP-16-7-15; (13) = PP/HSU-16-8-14; (14) = PP/HSU-19-17-1; (15) = PPT/PP-16-7-5; (16) = PPT/HSU-16-9-16; (17) = PP/BG-19-20-2; (18) = PP/BG-19-20-11; (19) = PP/BG-19-23-13; (20) = HSU/SH-16-8-18; (21) = SH/BG-14-1-2; (22) = SH/BG-14-1-5; (23) = PPT/BG-19-21-4; (24) = PPT/SH-16-7-6; (25) = PP/SH-18-9-12; (26) = PP/SH-19-11-2; (27) = PP/SH-19-16-4.

**Table 3.** Pigment composition of the studied guava hybrids and their parents.

	Fruit: color of peel	Fruit: color of pulp	Lycopene content (mg 100 g <sup>-1</sup> )	Total anthocyanins (mg 100 g <sup>-1</sup> )	Total carotenoids (mg 100 g <sup>-1</sup> )
<b>Parents</b>					
Black guava	Greyed orange group (174A)	Greyed orange group (186B)	0.52 ± 0.23m	9.66 ± 0.41a	0.73 ± 0.02j
Hisar Surkha	Yellow-orange group (18B)	Red Group (38C)	8.80 ± 0.92b	7.86 ± 0.03b	0.55 ± 0.03k
Punjab Pink	Green Yellow group (1B)	Red Group (37A)	3.33 ± 0.04i	0.11 ± 0.03jkl	0.47 ± 0.02l
Pant Prabhat	Yellow-green group (154B)	Yellow white group (158A)	0.05 ± 0.02n	3.09 ± 0.02f	0.57 ± 0.02k
Shweta	Yellow-green group N144	Yellow white group (158C)	0.08 ± 0.03n	0.21 ± 0.03j	0.21 ± 0.02o
<b>Hybrids</b>					
PP/BG-18-7-8	Yellow-green group (144C)	Red group (39C)	9.51 ± 0.02a	3.72 ± 0.01e	2.25 ± 0.01c
PP/BG-19-14-6	Yellow-green group (153D)	Red group (39A)	6.16 ± 0.07d	3.10 ± 0.01 f	1.70 ± 0.01g
PP/BG-19-15-1	Yellow-green group (144A)	Red group (37B)	4.35 ± 0.07h	4.13 ± 0.01d	1.63 ± 0.01h
PP/BG-19-16-8	Yellow-green group (153B)	Red group (40A)	7.79 ± 0.07c	2.88 ± 0.01g	2.27 ± 0.03c
HSU/SH-16-8-2	Yellow-green group (151D)	Red group (40A)	5.81 ± 0.06e	4.61 ± 0.02c	0.88 ± 0.01i
HSU/SH-16-8-3	Yellow-green group (153C)	Red group (39A)	6.41 ± 0.04d	4.12 ± 0.02d	2.37 ± 0.02b
SH/PP-16-7-15	Yellow-green group (151B)	Red group (39B)	4.30 ± 0.06h	3.15 ± 0.01f	1.73 ± 0.03g
PP/HSU-16-8-14	Yellow-green group (145A)	Red group (38B)	5.17 ± 0.04f	4.02 ± 0.01d	1.93 ± 0.01e
PP/HSU-19-17-1	Yellow-green group (144A)	Red group (39B)	4.68 ± 0.03g	3.12 ± 0.02f	0.75 ± 0.02j
PPT/PP-16-7-5	Yellow-green group (153C)	Red group (39A)	4.49 ± 0.06gh	3.06 ± 0.02f	2.49 ± 0.02a
PPT/HSU-16-9-16	Yellow-green group (151A)	Red group (37A)	7.80 ± 0.05c	3.83 ± 0.02e	1.99 ± 0.02d
PP/BG-19-20-2	Yellow-green group (145B)	White group (155B)	0.74 ± 0.03lm	0.01 ± 0.01l	0.17 ± 0.01pq
PP/BG-19-20-11	Yellow-green group (144C)	White group (155C)	0.17 ± 0.02n	0.18 ± 0.02jk	0.34 ± 0.03m
PP/BG-19-23-13	Yellow-green group (154C)	White group (155B)	1.09 ± 0.03jk	0.04 ± 0.02kl	0.25 ± 0.01n
HSU/SH-16-8-18	Yellow-green group (144C)	White group (NN155B)	0.18 ± 0.02n	0.14 ± 0.01jkl	0.17 ± 0.01pq
SH/BG-14-1-2	Yellow-green group (153C)	White group (155C)	0.21 ± 0.02n	0.22 ± 0.01j	0.32 ± 0.02m
SH/BG-14-1-5	Yellow-green group (145B)	White group (155B)	0.79 ± 0.02klm	1.15 ± 0.02h	0.17 ± 0.01pq
PPT/BG-19-21-4	Yellow-green group (154C)	White group (155B)	0.90 ± 0.05kl	0.47 ± 0.02i	0.19 ± 0.02op
PPT/SH-16-7-6	Yellow-green group (144A)	White group (NN155B)	0.86 ± 0.01kl	0.01 ± 0.01l	1.88 ± 0.03f
PP/SH-18-9-12	Yellow-green group (145A)	White group (155A)	1.36 ± 0.03j	0.11 ± 0.02jkl	0.49 ± 0.02l
PP/SH-19-11-2	Yellow-green group (153D)	White group (155B)	0.19 ± 0.03n	0.01 ± 0.01l	0.16 ± 0.01q
PP/SH-19-16-4	Yellow-green group (145A)	White group (155C)	0.93 ± 0.02kl	0.48 ± 0.01i	0.25 ± 0.01n
Mean			3.21	2.35	0.99
C.D. (p≤0.05)			0.30	0.13	0.03

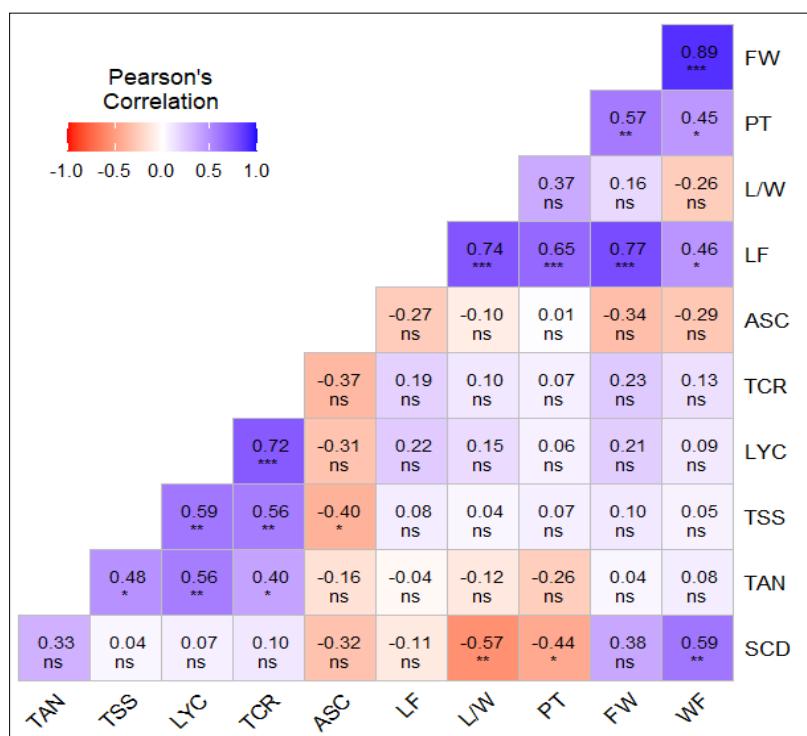


**Fig. 4.** Pigment-rich and pigment-deficient groups of guava hybrids based on pigment composition.

#### Correlation studies

Pearson's correlation was used to examine the relationships among various physical and biochemical parameters in guava hybrids and their parents (Fig. 5). Of the different physical parameters, FW had a significant positive correlation with WF ( $r = 0.89, p < 0.001$ ), LF ( $r = 0.77, p < 0.001$ ), SCD ( $r = 0.38, p < 0.05$ ) and PT ( $r = 0.57, p < 0.01$ ). WF had a significant positive correlation with LF ( $r = 0.46, p < 0.05$ ), SCD ( $r = 0.59, p < 0.01$ ) and PT ( $r = 0.45, p < 0.05$ ). LF had a significant positive correlation with L/W ( $r = 0.74, p < 0.001$ ) and PT ( $r = 0.65, p < 0.001$ ). L/W had a

significant negative correlation with SCD ( $r = -0.55, p < 0.01$ ). SCD is negatively correlated with PT ( $r = -0.44, p < 0.05$ ). For biochemical parameters, the correlation analysis showed significant positive correlations for LYC with TAN ( $r = 0.56, p < 0.01$ ), TCR ( $r = 0.72, p < 0.001$ ) and TSS ( $r = 0.566, p < 0.01$ ). The positive correlation between TSS and the LYC ( $r = 0.56, p < 0.01$ ), TAN ( $r = 0.48, p < 0.05$ ) and TCR ( $r = 0.56, p < 0.01$ ) showed improved fruit quality with higher pigment concentration. TSS is negatively correlated with ASC ( $r = -0.40, p < 0.05$ ).

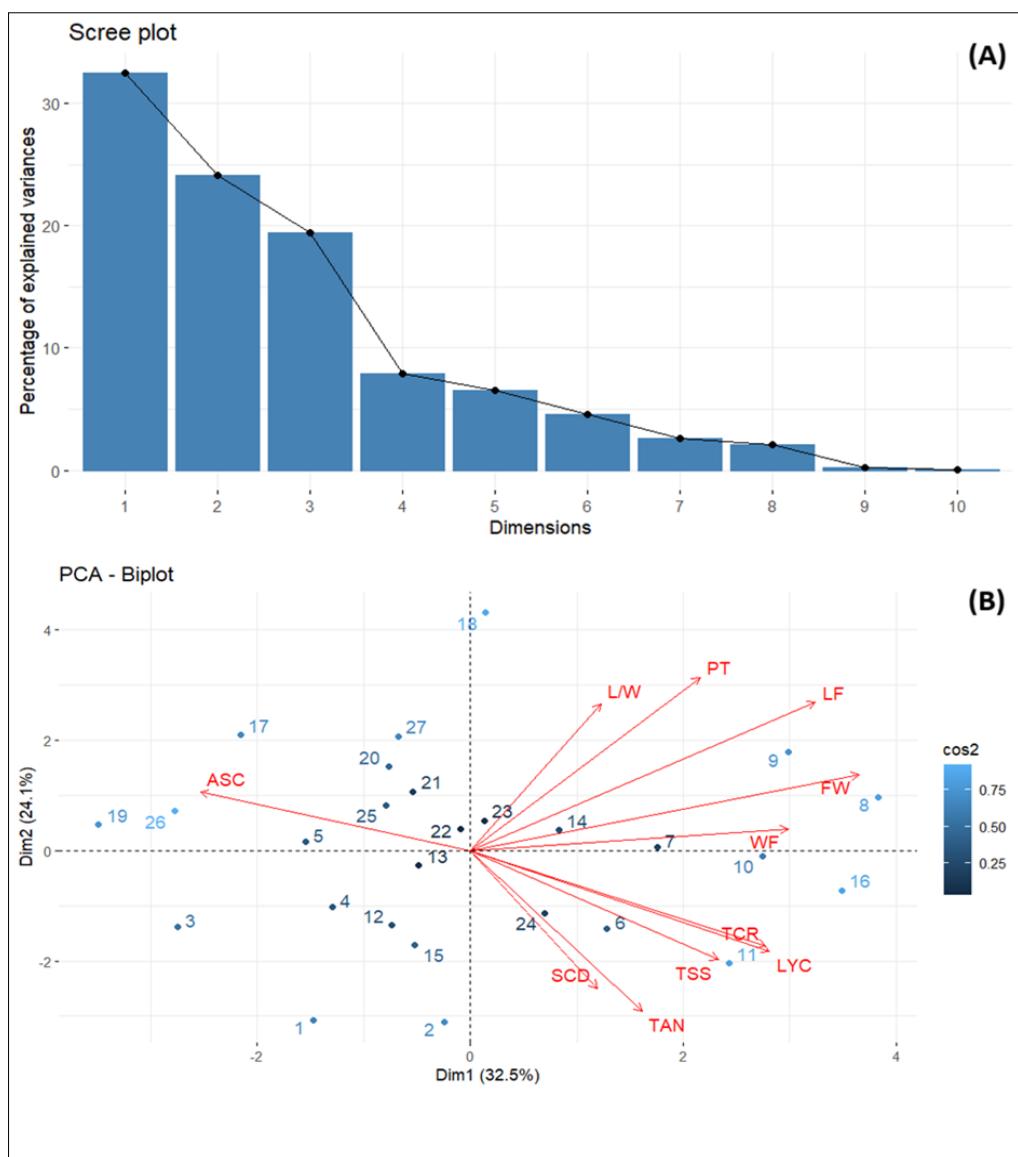


**Fig. 5.** Pearson's correlation coefficients for guava hybrids and parents based on physical and biochemical traits. ns = Not significant ( $p \geq 0.05$ ); \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$

## Principal component analysis

PCA was performed using the mean values of 22 guava hybrids and 5 parental genotypes for various physical and biochemical properties. A scatter plot was used to graph the percentage of explained variance of the principal components (PCs) (Fig. 6A). With a minimum eigenvalue threshold of 1, the 3 PCs (PC1, PC2 and PC3) were selected, which explain 76.04 % of the total variation (Fig. 6B). PC1 contributed 32.5 % of the total variation, followed by PC2 (24.1 %). Component loadings were evaluated to reveal potential relationships within the data by identifying representative PCs through sample grouping and differentiation and by explaining the variance. Each PC is associated with an eigenvalue and an eigenvector, representing a portion of the dataset's variation and the variation within the primary components, respectively. PCA indicated the primary fruit traits driving variation in the present study. PC1 was strongly defined by traits associated with intermediaries involved in pigment composition (LYC, TAN, TCR), along with soluble solids and ASC,

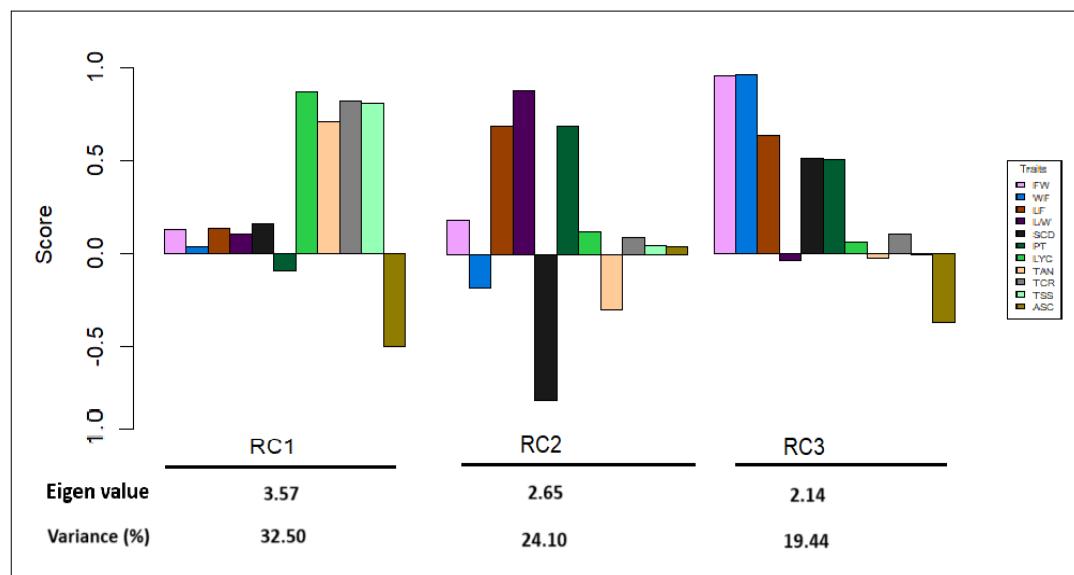
with the highest contribution to the variability (32.50 %) among the studied genotypes (Fig. 6A). The inverse association of ASC with PC1 may be attributed to the typical ripening pattern in guava, wherein organic acids decline while pigment accumulation and soluble solid content increase progressively with fruit maturation. In PC2 (24.10 %), SCD contributed significantly to high negative loadings; however, LF, L/W and PT contributed to significant variability with positive loadings, jointly explaining that fruits with larger seed cores generally possess lower pulp or vice versa. The distinction along PC3 (19.44 %) mainly depends on the high positive loadings of FW, LF and WF, suggesting that this component differentiated genotypes based on their overall fruit size and mass. The significant proportions of total variation (> 50 %) accounted for by PC1 and PC2 highlighted key traits influencing genotypic differentiation. Furthermore, the rotated component matrix showing loadings for the first 3 components of fruit physical and biochemical traits is depicted in Fig. 7.



**Fig. 6.** (A) Scree plot explaining principal component variances in terms of components. (B) PCA Biplot indicates the distribution of various traits in various guava hybrids and parents based on their calculated component loading values.

Parents: (1) = Black guava; (2) = Hisar Surkha; (3) = Punjab Pink; (4) = Pant Prabhat; (5) = Shweta

Hybrids: (6) = PP/BG-18-7-8; (7) = PP/BG-19-14-6; (8) = PP/BG-19-15-1; (9) = PP/BG-19-16-8; (10) = HSU/SH-16-8-2; (11) = HSU/SH-16-8-3; (12) = SH/PP-16-7-15; (13) = PP/HSU-16-8-14; (14) = PP/HSU-19-17-1; (15) = PPT/PP-16-7-5; (16) = PPT/HSU-16-9-16; (17) = PP/BG-19-20-2; (18) = PP/BG-19-20-11; (19) = PP/BG-19-23-13; (20) = HSU/SH-16-8-18; (21) = SH/BG-14-1-2; (22) = SH/BG-14-1-5; (23) = PPT/BG-19-21-4; (24) = PPT/SH-16-7-6; (25) = PP/SH-18-9-12; (26) = PP/SH-19-11-2; (27) = PP/SH-19-16-4.



**Fig. 7.** Rotated component matrix showing loading values of fruit physical and biochemical traits for the first 3 components (eigenvalue  $\geq 1$ ) obtained for guava genotypes and the percentage of variance explained by components.

### Heritability studies and hierarchical cluster analysis

In the present study, a broad spectrum of variability was observed for both physical and biochemical traits (Table 4). The highest PCV was observed for TAN (106.91 %), followed by LYC (96.39 %) and TCR (84.47 %), while lower PCV values were observed for WF (12.45 %), L/W (15.69 %), TSS (16.28 %), ASC (16.55 %) and LF (17.23 %). Similarly, GCV was high ( $> 80$  %) for LYC, TAN and TCR and low ( $< 20$  %) for WF, LF, L/W, TSS and ASC. The highest mean value of GA was estimated for TAN (220.01 %), followed by LYC (197.85 %) and TCR (173.95 %), FW (57.76 %) and PT (44.05 %).

Based on the studied fruit quality characteristics, hierarchical cluster analysis was performed to estimate the interrelation among the studied guava genotypes. The evaluated citrus genotypes were grouped into 2 main clusters, viz. A (white-pulped genotypes; sub-clusters A1 and A2) and B (red-pulped genotypes; sub-clusters B1 and B2) using Euclidian distances (Fig. 8) and were compared using the cluster mean value for different traits (Table 5). All the clusters have 7 entries except A2 (6 entries). The genotype in cluster A1 exhibited the highest cluster mean values for SCD and the lowest for FL/width ratio and LYC content. Cluster A2 genotypes had the highest cluster

mean values for FL/width ratio and ASC and the lowest values for SCD, TSS, TAN and TCR. Genotypes in Cluster B1 had the highest cluster mean for TAN and the lowest for FW, FL, WF and PT. Cluster B2 genotypes had the highest cluster mean values for FW, WF, FL, PT, TSS, LYC content and TCR; the lowest for ASC. In general, Cluster A/white-pulped genotypes had higher ASC and lower TSS values. In contrast, Cluster B/pink/red-pulped genotypes had higher TSS and pigment concentrations (LYC, TAN and TCR) along with comparable ASC content. The FL/width ratio was also  $\approx 1$ , depicting a round fruit shape.

### Discussion

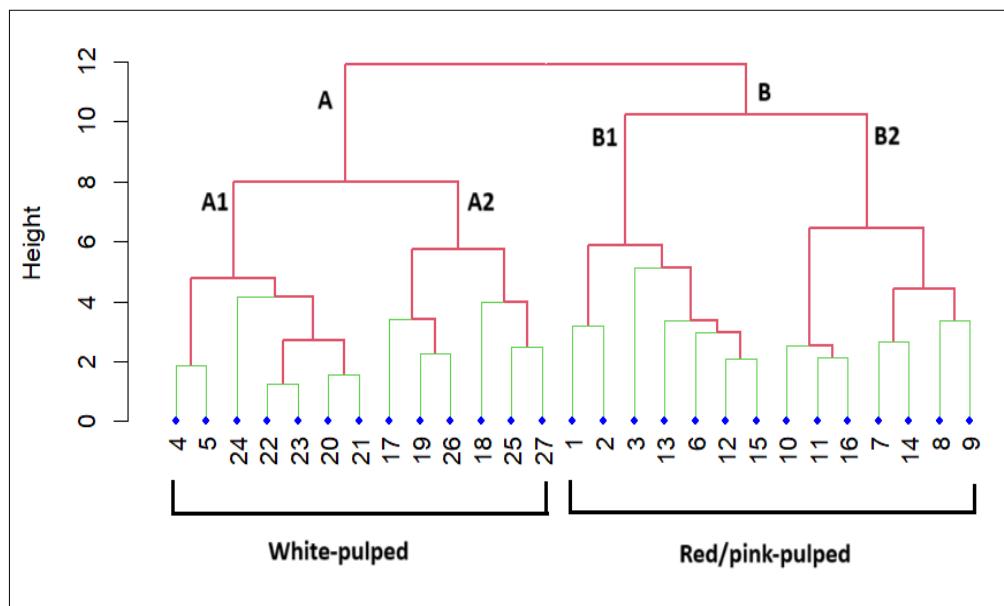
Morphological characterization is the most prevalent traditional approach for determining and analyzing genetic variation in crop improvement program. The approach is simple, easy and cost-effective, serving as the foremost step in germplasm characterization. The availability of published descriptor lists has simplified morphological characterization. In the present study, different color codes were assigned to traits that showed variation for each character and a similar pattern was observed across the studied genotypes. Previously, several researchers have used

**Table 4.** Estimation of descriptive statistics, heritability and genetic advance of guava hybrids for fruit traits

Parameter	Range	Mean	SEM	CV/ %	GCV/ %	PCV/ %	H <sup>2</sup> / %	GA	GAM/ %
FW (g)	60.04-224.20	127.87	10.42	14.11	30.84	33.91	0.83	73.85	57.76
WF (mm)	44.72-75.14	60.42	1.94	5.57	11.13	12.45	0.80	12.39	20.51
LF (mm)	43.93-84.63	60.40	2.17	6.23	16.07	17.23	0.87	18.64	3.09
L/W	0.70-1.37	1.00	0.03	5.17	14.81	15.69	0.89	0.29	28.80
SCD (mm)	13.00-47.25	32.88	1.74	9.17	20.49	22.45	0.83	12.67	38.53
PT (mm)	2.43-19.9	13.66	0.74	9.40	23.27	25.10	0.86	6.07	44.05
TSS (°B)	9.55-19.45	13.46	0.54	7.00	14.69	16.28	0.81	3.68	27.33
ASC (mg 100 g <sup>-1</sup> )	133.44-288.43	186.60	4.99	4.63	15.89	16.55	0.92	58.64	31.43
LYC (mg 100 g <sup>-1</sup> )	0.027-9.53	3.21	0.11	5.73	96.21	96.39	0.99	6.35	197.85
TAN (mg 100 g <sup>-1</sup> )	0.01-9.66	2.35	0.046	3.40	106.86	106.91	0.99	5.17	220.01
TCR (mg 100 g <sup>-1</sup> )	0.16-2.45	0.99	0.01	1.65	84.46	84.47	0.99	1.73	173.95

SEM = Standard error of the mean; CV = Coefficient of Variation; GCV = Genotypic coefficient of variation; PCV = Phenotypic Coefficient of Variation; H<sup>2</sup> = Broad-Sense Heritability; GA = Genetic Advance; GAM = Mean Value of Genetic Advance.

Physical properties: FW = Fruit weight; WF = Fruit width; LF = Fruit length; L/W = Fruit length/width ratio; SCD = Seed core diameter; PT = Pulp thickness; LYC = Lycopene; TAN = Total anthocyanins; TCR = Total carotenoids; TSS = Total soluble solids; ASC = Ascorbic acid



**Fig. 8.** Dendrogram showing relationships between studied guava hybrids and their parents. Parents: (1) = Black guava; (2) = Hisar Surkha; (3) = Punjab Pink; (4) = Panti Prabhat; (5) = Shweta.

Hybrids: (6) = PP/BG-18-7-8; (7) = PP/BG-19-14-6; (8) = PP/BG-19-15-1; (9) = PP/BG-19-16-8; (10) = HSU/SH-16-8-2; (11) = HSU/SH-16-8-3; (12) = SH/PP-16-7-15; (13) = PP/HSU-16-8-14; (14) = PP/HSU-19-17-1; (15) = PPT/PP-16-7-5; (16) = PPT/HSU-16-9-16; (17) = PP/BG-19-20-2; (18) = PP/BG-19-20-11; (19) = PP/BG-19-23-13; (20) = HSU/SH-16-8-18; (21) = SH/BG-14-1-2; (22) = SH/BG-14-1-5; (23) = PPT/BG-19-21-4; (24) = PPT/SH-16-7-6; (25) = PP/SH-18-9-12; (26) = PP/SH-19-11-2; (27) = PP/SH-19-16-4.

**Table 5.** Mean values of the fruit properties of the clusters of guava hybrids and parents obtained based on both physical and biochemical parameters

Physical parameters	N (group) <sub>mean</sub>			
	Cluster A	Cluster B	Cluster A	Cluster B
A1	A2	B1	B2	
Fruit weight (g)	139.72	108.00	87.23	173.68
Fruit width (mm)	65.86	55.00	53.88	66.14
Fruit length (mm)	56.77	61.34	52.97	70.68
Fruit length /width ratio	0.86	1.11	0.98	1.07
SCD (mm)	36.66	25.83	32.67	34.74
Pulp thickness (mm)	14.17	14.63	10.60	15.69
<b>Biochemical parameters</b>				
TSS (°B)	12.49	11.97	13.95	15.23
Ascorbic acid (mg 100 g <sup>-1</sup> )	183.50	211.92	183.66	170.93
Lycopene content (mg 100 g <sup>-1</sup> )	0.44	0.75	5.16	6.14
Total anthocyanins (mg 100 g <sup>-1</sup> )	0.75	0.14	4.51	3.68
Total carotenoids (mg 100 g <sup>-1</sup> )	0.50	0.28	1.45	1.66

morphological characterization to define important guava cultivars based on fruit traits (18-21). In general, a round fruit shape with a smooth surface, even pulp color and the absence of longitudinal ridges, puffiness and pulp discoloration are preferred in guava. Morphological characterization enables the detection of phenotypic diversity and provides a fundamental basis for the identification of superior genotypes for hybridization programs.

Physical fruit parameters are important not only because they affect yield but also determine fruit quality. In general, it has been noticed that very large fruits are poor in quality attributes. In our study, significant variation was observed for the physical fruit parameters among the guava genotypes. Fruit weight is an important parameter used in crop improvement programs for the selection of superior genotypes, but it is subject to differential translocation of photosynthates from the source, leading to variation (22). Variation in the physical fruit parameters may be due to both phenotypic and genotypic influences. Similar variations in

guava germplasm were also reported in other studies (23-25). The FL/WF ratio is a quantitative measure of fruit shape that helps distinguish between elongated ( $>1$ ), round (1) and flat ( $<1$ ) fruits. The fruit shape index indicates the degree of elongation or roundness in fruits. In this study, 'Punjab Pink' showed a value greater than one, denoting a slightly elongated form. This trait was largely inherited by its hybrids, suggesting a strong genetic influence of 'Punjab Pink' on the expression of fruit shape. SCD generally corresponds to fruit size, as larger fruits tend to possess a greater seed core due to the proportional growth of internal tissues. In guava breeding, however, a smaller seed core is preferred over a reduced seed number, since the latter is often associated with irregular fruit shape. PT is another key quality attribute, as consumers favor fruits with a thicker pulp relative to the seed core. In the present study, hybrids generally exhibited higher PT than their parents. Overall, desirable guava types are characterized by larger fruits with a small or proportionate seed core and a higher pulp-to-seed cavity ratio (26).

Total soluble solids and ASC concentration are pivotal determinants of guava fruit quality, as they collectively influence its nutritional composition, sensory appeal and suitability for processing. The data presented in Fig. 3A and 3B reveal significant variation in both TSS and ASC content among the evaluated genotypes. Typically, guava fruits harvested in winter exhibit higher TSS levels, contributing to enhanced sweetness and consumer preference. Moreover, the fruit is recognized as one of the richest natural sources of ASC, a potent antioxidant that plays a crucial role in mitigating oxidative stress and protecting against free radical-induced cellular damage (27). A previous study has also characterized guava germplasm for variation in TSS and ASC concentration, underscoring the genetic diversity and environmental factors that govern these nutritional traits (28).

The sweet scent and fleshy texture characterize *Psidium* fruits and the pulp can vary significantly in color. According to the oldest classification, there are 4 basic classes of guava based on pulp color: white, yellow, red/pink and purple (29). It has been reported

that around 400 guava cultivars are grown worldwide with variations in fruit peel and pulp color (30). Guava's pulp color is an important economic trait that directly affects consumer preferences. Colored-pulp guava fruits are a rich source of natural antioxidants and are highly demanded by health-conscious people. The pigment composition of all guava genotypes, along with color codes for peel and pulp color according to the RHS color chart, is presented in Table 5. Two groups were formed based on pigment composition (Fig. 4). One is the pigment-rich group, which consists of colored guava parents (i.e., 'BG', 'HSU', 'PP') and pink/red pulped hybrids. The second group is the pigment-deficient group, consisting of parents 'PPT' and 'SH' along with white pulped hybrids. The parent 'Black guava' had the highest total anthocyanin content, which corresponds to its purplish pulp color.

Lycopene, the pigment responsible for the pink pulp color in guava (31), is a highly stable singlet oxygen-quenching compound known for its strong antioxidant activity. Beyond its antioxidant potential, LYC also exhibits anti-inflammatory, anticarcinogenic, antimutagenic and cardioprotective properties (32, 33). The higher LYC content observed in hybrids such as 'PP/BG-18-7-8', 'PPT/HSU-16-9-16' and 'PP/BG-19-16-8' indicates successful transgressive segregation for this trait. This enhancement in LYC accumulation not only reflects the genetic potential of these hybrids for improved pigment contents but also underscores their superior nutritional and functional value. The concentrations exceeding those reported for 'Arka Kiran' and Mexican guava genotypes further demonstrate the scope for developing high-LYC guava cultivars, catering to both consumer health preferences and industrial demand for value-added products (34).

In the present study, the pink/red pulped hybrids had higher TAN content. Previously, several researchers have reported that LYC, along with other carotenoids, is responsible for the pink/red pulp color of guavas (35, 36). However, another study found that both LYC and anthocyanins are responsible for the pink pulp colour of guava (37). The total carotenoid content followed a different trend compared to LYC and total anthocyanin content.

The observed correlation patterns highlight the interdependence among key morphological and quality attributes in guava. The strong positive association between fruit dimensional traits and FW indicates that fruit size is a major determinant of overall yield potential. The positive relationship between FL and the length-to-width ratio reflects the contribution of longitudinal growth to fruit shape elongation. Conversely, negative correlations between SCD and both the FL/width ratio and PT suggest that an increase in seed core size compromises pulp development and fruit shape desirability. These findings emphasize that selection for reduced SCD and enhanced PT can simultaneously improve both fruit quality and consumer acceptability, thereby aiding breeders in developing superior guava genotypes with optimal fruit architecture and higher market value.

For biochemical parameters, LYC exhibited a significant positive correlation with TCR. As LYC serves as a key intermediate in the carotenoid biosynthetic pathway, derived from geranylgeranyl pyrophosphate (GGPP), a higher total carotenoid content typically reflects enhanced pathway activity and, consequently, increased LYC accumulation (38). The positive association between LYC and total anthocyanin content suggests that both pigments contribute to the development of pink-to-red pulp coloration, consistent with previous studies (39, 40). Additionally, the positive correlation

between TSS and pigment content indicates that fruits with higher pigment concentration often exhibit superior quality. During ripening, soluble solids tend to increase due to sugar accumulation, whereas ASC levels generally decline owing to oxidative degradation (41). This might define the negative association observed between TSS and ASC. The findings of the present study are consistent with previous studies on guava for different quality traits (42). The correlation studies reveal significant associations among various fruit physical and biochemical traits, offering valuable insights into the factors that drive fruit quality. Understanding these associations is crucial for breeding programs, as it enables the identification of traits that can be selected simultaneously. This facilitates the development of fruit cultivars/hybrids with optimal combinations of desirable attributes. Principal component analysis revealed complex trait interrelationships and effectively separated guava genotypes based on fruit quality. Moreover, the relationships among the studied fruit traits and guava genotypes observed in the PCA biplot generally aligned with results from Pearson's correlation and hierarchical clustering analyses.

Heritability estimates play a fundamental role in the breeding of perennial crops by quantifying the proportion of phenotypic variance attributable to genetic factors within a given population. It is imperative in fruit crops, as a lack of knowledge about inheritance patterns remains a bottleneck in fruit breeding (43). In the present study, the PCV for all traits exceeded the respective GCV, suggesting that, in addition to genetic factors, environmental factors also contribute significantly to observed variation (44). Moreover, the small difference between these two estimates (GCV and PCV) indicates a lower impact of the environment on the observed variability. *Psidium guajava* exhibits high heterozygosity, resulting in extensive genotypic and phenotypic diversity in the hybrid population. The current study revealed higher broad-sense heritability ( $\geq 80\%$ ) for all fruit traits (both physical and biochemical) studied, strengthening the evidence for the limited impact of environmental factors on these traits. Also, high heritability values suggest a high potential for genetic improvement of these traits. The high mean values of GA for TAN, LYC, TCR, FW and PT indicate that these traits are predominantly governed by additive gene action with minimal environmental influence. This suggests a high potential for effective selection and genetic improvement through conventional breeding methods (16, 45). In contrast, the remaining fruit quality traits exhibited low to moderate percent GAM ( $< 30\%$ ) despite high heritability estimates ( $> 80\%$ ), suggesting the predominance of non-additive gene action in their inheritance. Such traits are less amenable to direct selection and may benefit more from heterosis breeding approaches. Overall, the findings of the present study confirm the significant influence of genetic factors on key commercially important fruit traits in guava, providing valuable insights for strategic breeding interventions. Previously, several researchers have estimated heritability, GCV and PCV for fruit-related traits in guava (46-48).

Based on cluster analysis, the genotypes grouped in Cluster B2 (red/pink-pulped) emerged as particularly significant, highlighting their potential utility as pre-breeding lines for developing colored guava hybrids or parental lines. This cluster is characterized by medium-sized fruits with higher PT. Moreover, the genotypes had greater soluble solids and the highest pigment contents (LYC, carotenoids and anthocyanins). These genotypes offer considerable promise for generating elite hybrids in segregating

populations, particularly those exhibiting a higher concentration of desirable fruit quality traits. Moreover, the clustering results align well with the findings from the physical and biochemical analyses of fruit quality traits presented in the preceding section, thereby reinforcing the classification's reliability.

## Conclusion

The present study revealed substantial variation in fruit quality traits among newly developed guava hybrids and their parental genotypes, highlighting the role of genetic constitution in trait expression. The identification of promising hybrids based on overall data on fruit physical parameters and pigment composition (HSU/SH-16-8-2; red/pink pulped, HSU/SH-16-8-3; red/pink pulped, PPT/HSU-16-9-16; red/pink pulped, HSU/SH-16-8-18; white-pulped and SH/BG-14-1-2; white-pulped), underscores their potential application in future breeding programs, aimed at improving fruit quality in guava. Multivariate analyses, including PC analysis and hierarchical clustering, effectively identified the primary traits contributing to genotypic divergence. Additionally, correlation analysis elucidated key inter-relationships among key physical and biochemical fruit quality parameters. High heritability estimates for several fruit traits further confirm a strong genetic influence, supporting the feasibility of targeted selection strategies to enhance guava fruit quality-related traits in future breeding programs.

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## Authors' contributions

A, MT, RS and AMS conceptualized the research work. MT, SSK, AN, MKV and SGK designed the experiments. A executed all experiments. EV performed the statistical analysis and interpreted the results. A, MT, RS and AMS prepared the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** The authors do not have any conflicts of interest to declare.

**Ethical issues:** None

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