RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (5) 2219-2231, 2025
http://doi.org/10.54910/sabrao2025.57.5.43
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

PHYSICO-CHEMICAL DIVERSITY IN WHITE-FLESHED ROUND GUAVA GERMPLASM AND IMPLICATIONS FOR BREEDING

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SUMMARY

Guava (*Psidium guajava* L.) is a nutraceutical commercial fruit crop of the subtropics. Twenty-one accessions of round or Gola cultivars were collected from Punjab and Khyber Pakhtunkhwa (KPK) provinces having diverse climatic conditions. This indigenous germplasm bore investigation for 17 physicochemical fruit traits. The assessment sought to estimate variability and population structure. Accessions collected from Punjab had greater genotypic diversity and a wider genetic base than accessions from KPK. Several accessions proved superior for economically important consumer-related fruit traits, including fruit weight (FW), fruit size (FS), total soluble solids (TSS), ratio of TSS to titratable acidity (TA), and total sugars (TS). Most physical traits displayed positive correlations, whereas chemical traits had a negative correlation. Accessions with larger fruit size and fewer seeds indicated an association with lower TSS. Prevailing low temperatures in both areas enhanced fruit size and decreased total sugars. The principal component analysis (PCA) depicted higher loadings of FW, seed cavity weight (SCW), number of seeds (NS), and TS and grouping of most accessions of a locality in one cluster. The selected accessions, as vegetatively propagated, could serve as candidate varieties used for genetic association studies and as parental lines for the development of hybrids with better horticultural traits.

Keywords: Guava germplasm, biodiversity, fruit quality, principal component analysis, breeding

Communicating Editor: Dr. Akshaya Kumar Biswal

Manuscript received: July 15, 2024; Accepted: March 29, 2025.

Citation: Riaz N, Usman M, Fatima B, Bokhari SAM, Rana MA, Shahid M (2025 Physico-chemical diversity in white-fleshed round guava germplasm and implications for breeding. *SABRAO J. Breed. Genet.* 57(5): 2219-2231. http://doi.org/10.54910/sabrao2025.57.5.43.

Key findings: The Round accessions, G_1, G_5, G_{13}, and G_{18}, are suitable candidates for better fruit weight and size. Meanwhile, the apple guava G_9 could be a strong parental candidate for reduced number of seeds, higher TSS, TSS:TA, and dark red skin—a consumer-preferred trait.

INTRODUCTION

Genetic resources provide raw materials for selection, breeding, and other crop improvement programs to enhance genetic variability (Gangappa *et al.,* 2022). Available diversity will undergo essential collection, characterization, conservation, and cultivation under varied agroclimatic conditions to evaluate varietal potential. Introduction and adoption of economically important varieties is useful for a short-term strategy to widen the germplasm base; however, it may also lead to genetic erosion of the indigenous material needing conservation (Ahuja and Jain, 2017). Exploring the existing diversity and utilization of the selected material for breeding programs could develop hybrids with better fruit quality and yield attributes. Selection and breeding objectives for commercial varieties of guava include better fruit size (≥ 7 cm in diameter), fruit weight (200–300 g), uniform fruit shape, and attractive skin and pulp color (white for table varieties and pink to red flesh for the processing industry). Other targets are high yield (40–60 t/ha), more soluble solids (9%–12%), lower acidity for table varieties (0.2%–0.6%), more ascorbic acid content (250–300 mg/100 g fruit), few and soft seeds, dwarf varieties, resistance to wilt and fruit fly, frost tolerance, and long shelf life (Pereira and Nachtigal, 2002). Great genetic variability is available in guava due to its reproductive behavior and seminal propagation (Pereira *et al*., 2017; Usman *et al*., 2021), which is necessary for exploration, selection, and conservation.

Pakistan stands among the top five producers of fresh mangoes and guavas after India, China, and Indonesia, with a 4.72% share in global production (FAOSTAT, 2024; www.tridge.com/intelligences/guava/production). Pakistan has produced 0.79 million tons of fresh guavas (MNFSR, 2024), despite having a low yield in Asia and yield gaps ranging from 5 to 10 tons per hectare (FAO, 2024; Usman *et al*., 2020). Since 2017–2018, a massive decrease in area under cultivation has emerged (~11000 ha). However, annual production has increased many folds (210,836 tons) and per hectare yield has also increased up to 14 t/ha^{-1} (MNFSR, 2024). Along with other factors, this sharp increase in production could refer to the adaptation of high-density plantation technology (Awan *et al*., 2024). In Pakistan, guava production has concentrated in Punjab (>80%), including Lahore, Sheikhupura, Faisalabad, Pakpattan, Sahiwal, and Bahawalpur districts. Few pockets of guava production in Khyber Pakhtunkhwa (KPK) exist, including Peshawar and Kohat.

Despite the recent increase in production, key reasons for low productivity include limited certified nurseries, seedling populations, a narrow gene pool, insufficient introduction and varietal selection programs, high postharvest losses, dieback, other diseases and fruit flies, and abrupt climatic changes (Shah *et al*., 2019; Ur Rahman *et al*., 2023). Guava is a highly nutraceutical fruit crop rich in ascorbic acid, calcium, essential oils, carotenoids, and flavonoids. Guava has become an attractive fruit crop for the farmers, and its production is highly economical compared with other fruit crops (Usman *et al*., 2013). Commercial table varieties in Pakistan comprised the White Flesh Round (Gola) and Pyriform (Surahi) cultivars, which further have different accessions, viz., Sadabahar Gola, Sadabahar Surahi, Chinese Gola, White Gola, Large Gola, Large Surahi, and others (Shah *et al*., 2019). These accessions have been named by the amateur growers based on phenotypic diversity in their shape, size, flesh color, skin color, and fruit quality.

Fruit characterization is vital for several reasons, including selection for new varieties, parental material for breeding programs, and accessions having novel traits. Additionally, it helps to classify promising accessions as a table or processing variety. Though guava is mainly a self-pollinated (75%–80%) crop, reports stated cross-pollination by insects occurs enhancing diversity in seedlings (Omar *et al*., 2021). Seedlings live longer and bear fruit with diverse sizes and qualities (Pommer and Murkami, 2009) due to inherent variability and the metaxenia effects of the pollen parent (Usman *et al*., 2013). This further emphasizes the need for selection of better accessions and their clonal multiplication (Awan *et al*., 2024). For improvement of the target traits, genetically diverse parents are the key requirement (Pommer, 2012) to understand their level of divergence and variability for better utilization of the genetic resources in crop improvement programs. Quantitative and qualitative traits are beneficial to evaluate the phenotypic diversity and fruit quality for stringent selection and utilization in guava germplasm improvement (Usman *et al*., 2013).

The first choice utilized by professional guava growers as a tool is the selection, and they have selected better accessions like Sadabahar Gola and Sadabahar Surahi, which continue to bear fruit several months a year (Usman *et al*., 2013). The indigenous germplasm has presumably evolved from a limited genetic resource due to lack of extensive research work. Generally, crop improvement in guava could be successful by a) selection among the available germplasm or the seedling populations for fruit attributes and chemical characters and b) breeding programs for developing better-performing varieties. The screened material could proceed further in propagating using clonal propagation methods, including tissue culture (Usman *et al*., 2012) and softwood cuttings (Kareem *et al*., 2013; Awan *et al*., 2024), and their conservation for future use. Efficient utilization of plant genetic resources is the key to crop improvement programs; hence, the well-adapted indigenous guava germplasm incurred exploring for physicochemical diversity to widen the germplasm base for future breeding applications and conservation of genetic resources.

MATERIALS AND METHODS

Plant material and locations

Fruits of 21 accessions of white-fleshed round (Gola) guava reached collection at market maturity from different localities of Punjab and KPK (Figure 1 and Table 1) and attained characterization for physicochemical variation.

Physical traits

The determination of physical parameters of fruits followed the standard descriptors (UPOV, 1987). These include the fruit weight ‘FW’ (g), flesh weight ‘FlW’ (g), seed core weight ‘SCW’ (g), fruit length ‘FL’ (mm), fruit diameter ‘FD’ (mm), ratio of ‘FL:FD,’ flesh thickness ‘FT’ (mm), seed weight ‘SW’ (g), and number of seeds ‘NS,’ as described in Usman *et al*. (2020).

Chemical traits

Fruit juice, as extracted from five different fresh fruit samples per treatment, underwent processing to measure parameters, such as total soluble solids ‘TSS’ (°Brix), titratable acidity ‘TA’ (%), ratio of ‘TSS:TA,’ ascorbic acid ‘AA’ (mg/100 ml), total sugars ‘TS’ (%), reducing sugars ‘RS’ (%), and non-reducing sugars ‘NRS’ (%) following standard procedures described briefly.

Total soluble solids ‘TSS’ and titratable acidity ‘TA’

The placement of several drops of guava juice extracted from fresh fruit samples proceeded on the prism of a digital refractometer (RX 5000, ATAGO, Japan), before closing the lid and then taking a reading in °Brix. Titratable acidity determination on the guava juice extracted from fresh fruit samples employed the titration method against alkali (1N NaOH) and 1% phenolphthalein (indicator), as

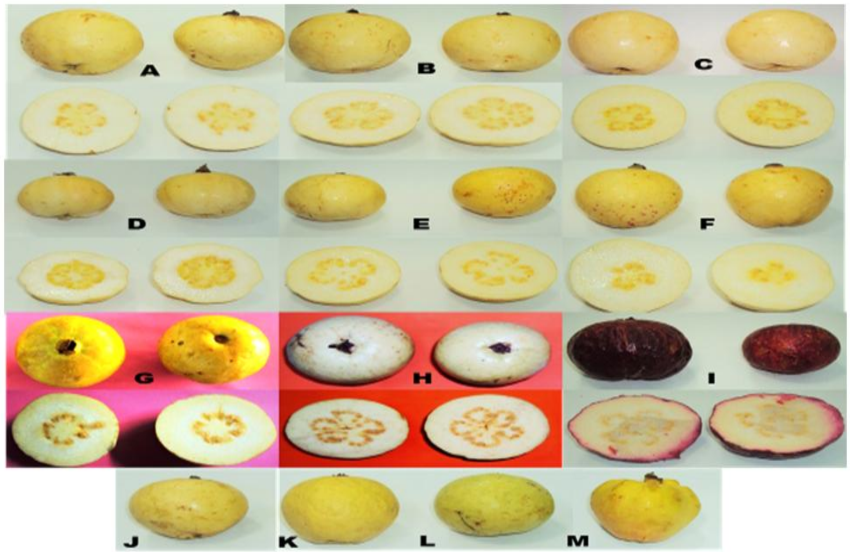

Figure 1. Morphological variation in different selected accessions of cv. Round (Gola types) collected from Faisalabad, Sahiwal, and Sharaqpur areas (Punjab) and Kohat and Peshawar areas (KPK). The accessions include A) Chinese (G_{13}), B) Allahabadi (G_{14}), C) White (G_{10}), D) Red Skinned (G_{16}), E) Gola (G_{12}), F) Sadabahar (G_{11}), G) Large Gola (G_{6}), H) White Gola (G_{19}), I) Red Skinned/Apple Gola (G_{9}), J) Large Gola (G_{1}), K) White Gola (G_{2}), L) Sadabahar Gola (G_{3}), and M) Gola Surahi (G_{8}). These accessions are named and classified by growers based on their phenotypic variability.

Table 1. Elite round guava germplasm collections from different localities of KPK and Punjab provinces.

Regions	Strains	Accessions	Key characters	Locality	Latitude/Longitude
KPK	LGKT	G_{1}	Large size, white flesh	Kohat	33.58°N/71.44°E
	WGKT	G_{2}	White skin, white flesh		
	SBGKT	G_{3}	Sadabahar, medium size, white flesh		
	GKT	G_{4}	Small size, white flesh		
	SGKT	G_{5}	Small size, white flesh		
	LGNP	G_{6}	Large size, white flesh	Peshawar	34.01°N/
	SGNP	G_{7}	Small size, white flesh		71.52°E
	GSNP	G_{8}	Medium size, white flesh		
Punjab	RSAGCW	G_{9}	Dark red skin, medium to large size, white flesh	Chichawatni	30.53°N/ 72.69°E
	WGCW	G_{10}	White skin, white flesh		
	SBGKM	G_{11}	Sadabahar, medium size, white flesh	Kamalia	30.72°N/ 72.64°E
	GDJ	G_{12}	Small to medium size, white flesh	Samundari	31.06°N/
	CNGSMJ	G_{13}	Large size, white skin, white flesh		72.95°E
	ABGSMJ	G_{14}	Medium to large size, white flesh		
	DGSMJ	G_{15}	Small size, white flesh		
	RSGSMJ	G_{16}	Red blush skin, medium size, white flesh		
	RSGSP	G_{17}	Red blush skin, white flesh	Sharaqpur	31.46°N/
	PGSP	G_{18}	Green to creamy skin, pink flesh		74.10°E
	WGSP	G_{19}	White skin, medium size, white flesh		
		G_{20}	Red blush skin, medium size, white flesh		
	RSFGSP				
	DGSP	G_{21}	Small size, white skin		

described by Hortwitz (1960). The ratio of TSS:TA, as calculated, occurred by dividing TSS values by the corresponding TA values.

Ascorbic acid 'AA' and total sugars 'TS'

The AA content measurement as mg 100 mL^{-1} juice used a solution of oxaloacetic acid, titrated with dye (2,6-dichlorophenolindophenol) solution, as described in AOAC (1990). The taking of 100 µL of fresh juice sample continued from an Eppendorf tube with 900 µL of distilled water. The TS and RS, as determined in fresh juice samples, followed the method of Sadasivam and Manickam (1992).

Experimental design and data analysis

The experiment layout had a randomized complete block design (RCBD). Five uniformly mature fruits attained selection for evaluation from different plants growing under the RCBD system. The least significant difference (LSD) test as employed compared the mean values (Steel *et al*., 1997). The values are presented as means ± standard error (SE). In identifying critical factors, the morphological and chemical data set was submitted to undergo the principal component analysis (PCA), as described by Franco and Hidalgo (2002), with data analysis done using Statistica (vr. 8.0). The study regarded factor loading >0.35 (physicochemical attributes) and >1.00 (genotypes) as significant (Mehmood *et al*., 2013). Pearson's correlation analysis helped analyze the quantitative physicochemical parameters in correlation. The data of the quantitative traits served for the construction of the dendrogram.

RESULTS

Physical traits

Significant genotypic differences were evident in the accessions (Table 1) collected from geographically distant localities in Punjab and KPK provinces for most physical traits. Fruit length (FL), fruit diameter (FD), and FT were significantly higher in G_{18} (77.49, 73.18, and 15.11 mm, respectively), followed by G_1 (Table 2). FW and FlW were maximum in G_1 (235.92 and 184.05 g), followed by G_{18}. Meanwhile, the minimum FW (89.11 g) and FlW (37.29 g) resulted in G_7 and G_{15}, respectively (Figure 1). Maximum SCW appeared in G_1 and G_5 (64.34 g), while it was the minimum in G_{19} (14.53 g). Regarding SW, minimum values occurred in G_{11} (0.95 g), and the maximum was in G_6 (4.54 g). Similarly, the lowest NS was notable in G_9 (92.67), whereas seed number per fruit was maximum in G_{13} (355.33) (Table 2).

Fruit quality traits

The maximum TSS (12.66 °Brix) was prominent in G_9, followed by G_{19} (12.22 °Brix); however, TSS was minimum (8.32 °Brix) in G_{11}. The lowest values for TA (0.61%) manifested in G_{18}, followed by G_{19}, whereas the maximum TA values (0.91% to 0.93%) emerged in G_5 and G_{10}. The key quality trait TSS:TA was higher (19.75) in G_{19}, followed by G_{20} (17.73), whereas minimum values surfaced in G_5 and G_{10} (10.08). Maximum AA (303.27 to 306.02 mg/100 g) was dominant in G_{17}-G_{19} and G_{20}. Meanwhile, minimum AA (184.24–186.25 mg/100 g) was noticeable in G_1 and G_5 (Table 3). Regarding total sugars (TS), maximum sugars (7.29%–7.46%) were remarkable in G_7, followed by G_4 and G_2, whereas it was minimum (5.11%) in G_{10}. Reducing sugars (RS) were maximum (3.90%) in G_6, followed by G_8. However, the RS was minimum (2.49%) in G_{10}. Regarding non-reducing sugars (NRS), maximum values (4.58%) were notable in G_4, and minimum values (2.17%) appeared in G_{14} (Table 3).

Overall, average FW was higher in cultivar Round accessions collected from KPK than from Punjab. Regarding the key fruit quality trait TSS:TA, average values were higher in accessions collected from Punjab than with KPK.

Correlations of the quantitative traits

Strong positive correlations resulted in most physical traits, whereas most chemical traits displayed negative correlations (Table 4). The

Table 2. Variability in physical traits of round accessions collected from different localities of Punjab and KPK provinces.

Locations	Accessions	Fruit length (mm)	Fruit diameter (mm)	Flesh thickness (mm)	Fruit weight (g)	Flesh wt. (g)	Seed Core wt. (g)	Seed wt. (g)	No. of Seeds
KPK	G1	71.47±2.45	80.01±3.11	13.18±0.68	235.92±12.90	184.05±17.79	64.34±4.51	2.41±0.10	195.75±7.29
	G2	54.53±1.45	59.01±2.57	8.08±0.84	103.70±9.54	64.19±7.22	38.49±3.40	2.11±0.29	169.67±6.30
	G3	66.86±3.11	66.57±2.46	11.67±0.38	162.13±21.50	116.20±9.07	45.03±12.07	2.43±0.07	162.00±13.05
	G4	53.18±2.21	64.62±1.98	11.09±0.38	138.52±9.37	92.22±5.42	45.05±4.71	1.81±0.18	194.33±12.19
	G5	72.75±4.53	72.01±1.85	10.26±0.41	198.60±19.12	132.25±12.83	64.58±6.31	3.81±0.15	296.00±32.35
	G6	63.86±2.42	69.77±2.43	12.27±0.69	177.67±16.40	120.04±15.26	55.92±6.42	4.54±0.38	213.22±16.65
	G7	51.11±2.60	55.00±1.86	7.37±0.09	89.11±9.90	44.43±5.52	51.97±5.88	2.20±0.49	151.00±51.33
	G8	59.25±0.51	53.36±0.78	9.63±1.07	95.56±4.30	69.60±2.72	25.13±2.88	2.25±0.05	190.33±7.54
Punjab	G9	50.58±3.49	61.20±3.93	8.35±1.36	114.47±23.89	80.35±17.47	31.65±6.30	1.33±0.72	92.67±44.33
	G10	66.72±1.73	65.68±0.85	8.17±0.41	153.92±7.40	108.59±7.10	44.97±4.04	1.66±0.12	226.67±19.97
	G11	63.78±2.12	56.98±0.52	10.59±1.11	111.79±2.99	88.29±4.76	22.06±1.75	0.95±0.23	138.33±27.87
	G12	59.46±1.22	63.52±1.56	11.03±1.12	123.29±8.73	85.96±6.26	33.25±6.74	2.20±0.37	151.33±15.32
	G13	69.60±2.31	70.46±1.52	12.17±0.42	187.85±14.93	143.61±11.89	41.83±3.22	3.76±0.24	355.33±58.38
	G14	61.30±1.94	69.03±0.51	11.09±0.37	125.99±6.36	83.20±2.83	41.29±3.33	2.71±0.23	212.33±26.96
	G15	70.71±1.81	69.31±0.96	8.64±1.47	136.83±6.40	37.29±2.29	16.74±2.65	3.32±0.17	324.67±14.44
	G16	54.02±0.95	56.91±0.77	9.30±0.79	92.76±4.26	64.23±1.85	26.99±3.21	1.97±0.04	156.00±27.15
	G17	65.54±1.96	58.34±0.95	11.25±0.51	118.02±5.96	88.19±5.41	29.35±1.34	1.71±0.13	139.94±9.57
	G18	77.49±4.67	73.18±2.18	15.11±0.41	224.33±25.13	171.33±20.41	46.47±7.40	3.08±0.16	293.15±15.88
	G19	55.79±3.90	59.08±2.02	12.33±1.70	106.46±10.43	84.00±8.53	14.53±6.47	1.86±0.21	226.67±19.97
	G20	62.86±6.26	57.06±1.50	10.78±0.31	101.66±12.55	80.33±2.82	27.23±3.91	2.43±0.44	216.33±11.05
	G21	57.72±3.08	61.07±2.07	10.60±1.27	114.72±14.65	88.00±12.61	26.04±3.53	2.06±0.16	217.00±22.50
$LSD_{0.05}$		13.3993	9.8570	2.7911	53.4175	48.8713	17.8.95	0.9169	65.6356

Table 3. Variability in fruit quality traits of round accessions collected from different localities.

Locations	Accessions	TSS (°Brix)	TA (%)	TSS:TA	Ascorbic acid (mg/100 g)	TS (%)	RS (%)	NRS (%)
KPK	G_1	9.83±0.01	0.850±0.020	11.63±0.30	186.25±4.38	6.84±0.21	2.82±0.22	4.03±0.35
	G_2	10.59±0.14	0.820±0.040	13.06±0.78	200.66±5.30	7.29±0.28	3.06±0.26	4.23±0.44
	G_3	10.51±0.01	0.700±0.060	15.30±1.36	198.89±1.10	6.80±0.42	2.77±0.35	4.04±0.08
	G_4	9.60±0.08	0.840±0.020	11.47±0.20	190.33±2.48	7.34±0.16	2.76±0.12	4.58±0.20
	G_5	9.25±0.11	0.910±0.010	10.11±0.04	184.24±4.65	6.92±0.33	2.86±0.24	4.05±0.50
	G_6	9.66±0.03	0.690±0.020	13.96±0.33	258.41±8.26	6.97±0.22	3.90±0.20	3.07±0.15
	G_7	8.59±0.03	0.680±0.010	12.71±0.22	239.33±1.85	7.46±0.25	3.58±0.08	3.88±0.22
	G_8	9.79±0.02	0.690±0.010	14.20±0.22	270.63±2.95	6.58±0.71	3.85±0.38	2.73±0.33

Table 3. (cont'd.)

Locations	Accessions	TSS (°Brix)	TA (%)	TSS:TA	Ascorbic acid (mg/100 g)	TS (%)	RS (%)	NRS (%)
Punjab	G9	12.66±0.04	0.830±0.010	15.34±0.11	250.02±3.43	5.48±0.22	2.79±0.14	2.68±0.32
	G10	9.40±0.09	0.930±0.010	10.08±0.13	280.93±3.23	5.11±0.39	2.49±0.22	2.62±0.28
	G11	8.32±0.02	0.630±0.060	13.65±1.43	261.44±3.86	5.90±0.24	2.64±0.23	3.27±0.40
	G12	11.22±0.06	0.850±0.010	13.16±0.10	271.18±2.00	5.98±0.44	3.08±0.19	2.90±0.57
	G13	10.34±0.02	0.890±0.010	11.65±0.18	250.03±2.48	5.54±0.27	2.74±0.31	2.80±0.09
	G14	10.68±0.04	0.800±0.010	13.41±0.13	240.91±2.28	5.30±0.36	3.12±0.06	2.17±0.40
	G15	11.55±0.02	0.840±0.020	13.73±0.25	246.93±5.01	5.91±0.20	3.10±0.50	2.81±0.31
	G16	10.76±0.03	0.810±0.010	13.30±0.19	250.02±3.43	5.51±0.22	2.79±0.14	2.72±0.33
	G17	9.20±0.00	0.630±0.010	14.60±0.24	303.27±1.55	6.58±0.10	3.11±0.07	3.46±0.12
	G18	9.55±0.05	0.610±0.010	15.62±0.39	305.09±5.65	6.45±0.24	2.68±0.34	3.77±0.13
	G19	12.22±0.07	0.620±0.030	19.75±0.88	306.02±1.57	6.32±0.17	3.10±0.06	3.22±0.19
	G20	11.58±0.02	0.660±0.010	17.73±0.44	298.10±5.59	6.32±0.17	2.88±0.11	3.44±0.26
	G21	9.91±0.03	0.660±0.010	15.07±0.26	305.71±2.87	6.67±0.18	2.78±0.45	3.88±0.60
LSD0.05		0.2053	0.09188	1.9170	17.766	0.8856	0.7626	1.1286

Table 4. Correlation coefficients among 15 quantitative traits in 21 accessions of the Round Guava.

Traits	FW	FL	FD	FlW	SCW	SW	NS	FlT	TSS	TA	TSS:TA	AA	TS	RS	NRS
FW	1														
FL	0.795	1													
FD	0.926	0.709	1												
FlW	0.912	0.677	0.762	1											
SCW	0.695	0.334	0.633	0.632	1										
SW	0.568	0.530	0.591	0.362	0.467	1									
NS	0.537	0.657	0.553	0.352	0.142	0.704	1								
FlT	0.646	0.557	0.545	0.763	0.211	0.340	0.330	1							
TSS	-0.235	-0.266	-0.046	-0.255	-0.456	-0.039	-0.011	-0.064	1						
TA	0.238	0.044	0.410	0.051	0.360	0.144	0.172	-0.371	0.164	1					
TSS:TA	-0.356	-0.200	-0.377	-0.212	-0.608	-0.183	-0.163	0.271	0.538	-0.724	1				
AA	-0.308	-0.002	-0.385	-0.156	-0.598	-0.175	0.030	0.161	0.140	-0.589	0.589	1			
TS	0.087	-0.102	-0.033	0.058	0.383	0.160	-0.110	0.097	-0.366	-0.266	0.009	-0.371	1		
RS	-0.232	-0.251	-0.197	-0.328	0.079	0.404	-0.127	-0.110	-0.047	-0.262	0.158	0.025	0.417	1	
NRS	0.259	-0.053	0.168	0.229	0.651	0.059	-0.186	-0.046	-0.406	0.228	-0.460	-0.709	0.757	0.132	1

Values in red color are significant ($P < 0.05$). Abbreviations: FW-fruit weight; FL-fruit length; FD-fruit diameter; FlW-flesh weight; SCW-seed core weight; SW-seed weight; NS-number of seeds; FlT-flesh thickness; TSS-total soluble solids; TA-titratable acidity; AA-ascorbic acid; TS-total sugars; RS-reducing sugars; and NRS-non-reducing sugars.

highest positive correlation occurred between FW with FD (0.926), FlW (0.912), and FL (0.795). Other positive correlations included SCW and NRS (0.651), TSS:TA and AA (0.589), and TS and NRS (0.757). Among negative correlations, the highest values were prominent for TA and TSS:TA (-0.724), AA and NRS (-0.709), SCW and TSS:TA (-0.608), and SCW and AA (-0.598). Likewise, strong positive correlations were visible in most physical traits, while most chemical fruit quality traits indicated negative correlatedness (Table 4). The highest positive correlations emerged between FW and FlW (0.935), FlW and FlT (0.913), FW and FD (0.842), TS and NRS (0.820), and TS and RS (0.642). Among negative correlations, the topmost values were distinct for TA and AA (-0.778), TA and TSS:TA (-0.652), SW and NS (-0.604), and SCW and TSS (-0.571).

Principal component analysis (PCA) for fruit physicochemical attributes

PCA put 15 variables of physicochemical attributes in five components (F1–F5) that explained 90.03% of the total variation in 21 Round accessions (Table 5). The first component accounted for 37.52% of the total variation, including FW, FD, FlW, SCW, FL, NS, TSS:TA, FT, AA, NRS, TA, and TSS. The second component accounted for 20.91% of the total variation, comprising NRS, AA, FT, TSS:TA, TS, FL, SCW, and TA. The third component explained 14.58% of the total variation and consisted of TA, TS, RS, TSS:TA, and FT. The fourth component made up for 9.87% of the total variation, which included SW, RS, NS, and FlW. The fifth component explained 7.14% of the total variation, including TSS and TSS:TA (Table 5).

The PCA plot based on the 1st and 2nd component factors of the fruit biochemical properties' data analysis showed greater variation, exhibiting accessions divided into different groups mainly based on localities, including Kohat, Peshawar, Faisalabad, Sharaqpur, and Chichawatni areas. Several accessions, such as PGSP (G_{18}), SGNP (G_7), WGSP (G_{19}), and SGKT (G_5) appeared as more divergent from the rest of the accessions and were outliers in the population under study (Figure 2a). The dendrograms as developed based on variance data, used the agglomerative hierarchical clustering (AHC) to estimate the genotypic dissimilarity using the phenotypic and fruit quality data. The dendrogram revealed two main groups (C1-C2) and seven subgroups (A-G), highlighting significant variation among different accessions. The cluster C2 contained more accessions (12) than the cluster C1. The subgroups C and F had numerous accessions (Figure 2b).

Table 5. First five components from the PCA analysis of 15 quantitative traits in 21 accessions of the Round Guava.

Traits	F1	F2	F3	F4	F5
Fruit weight	-0.955	0.211	0.018	0.099	0.092
Fruit length	-0.745	0.468	-0.007	0.036	-0.243
Fruit diameter	-0.902	0.197	-0.162	-0.071	0.202
Flesh weight	-0.833	0.277	0.123	0.361	0.139
Seed core weight	-0.807	-0.424	0.117	0.021	0.029
Seed weight	-0.64	0.18	0.176	-0.691	-0.069
No of seeds	-0.56	0.456	-0.139	-0.422	-0.239
Flesh thickness	-0.521	0.579	0.428	0.238	0.228
Total soluble solid	0.354	0.347	-0.327	-0.337	0.718
Fruit acidity	-0.358	-0.391	-0.785	-0.203	0.133
TSS:TA	0.542	0.562	0.445	-0.023	0.393
Vitamin C	0.479	0.698	0.183	0.066	-0.303
Total sugars	-0.191	-0.519	0.731	-0.044	0.127
Reducing sugars	0.153	-0.236	0.572	-0.663	-0.058
Non-reducing sugars	-0.416	-0.776	0.291	0.142	0.176
Variability (%)	37.523	20.914	14.587	9.871	7.144

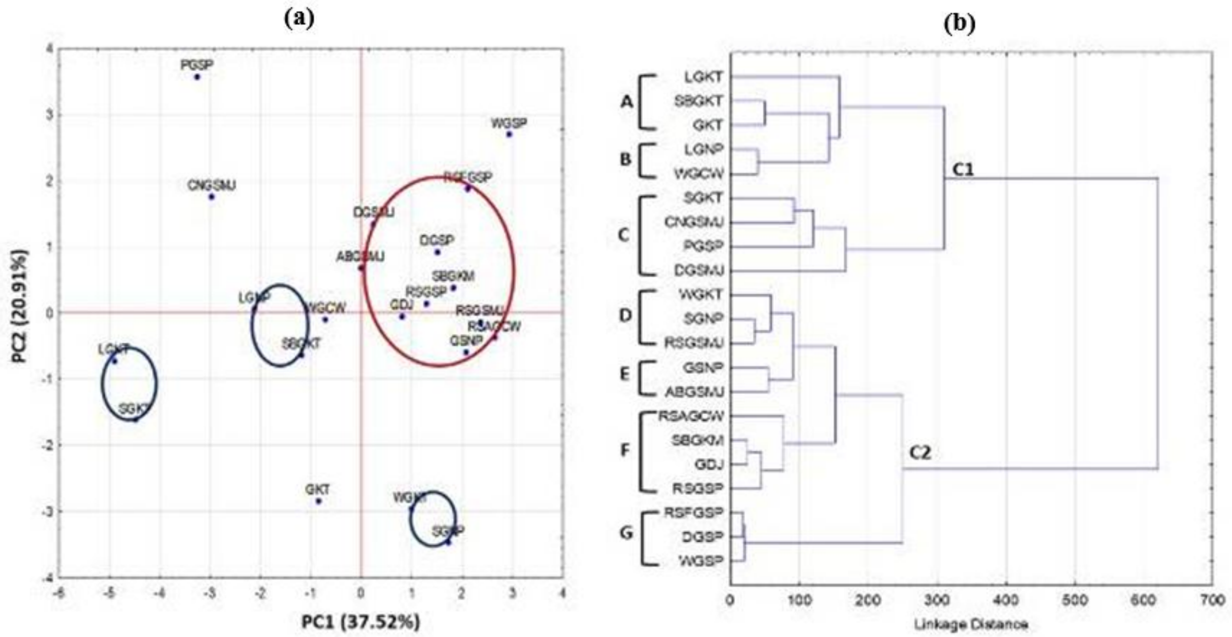

Figure 2. Two dimensional PCA plots based on first two components for quantitative traits of 21 accessions of the Round Guava a) collected from geographically different localities of Punjab and KPK provinces of Pakistan and b) developed from the similarity matrix based on Ward's (1963) method.

DISCUSSION

Germplasm comprising 21 accessions collected from two provinces succeeded in investigating for 10 morphological and seven biochemical traits for varietal identification and in estimating the extent of available variability in the fruit quality. Identification of economically important traits, including fruit weight, fruit size, yield, fruit quality, and pest resistance has been highly effective in guava, just like in other fruit crops (Pelea *et al*., 2016; Usman *et al*., 2020) for the growers and breeders to enhance crop productivity and quality. Findings of this study also support the viewpoint that the identification of key morpho-chemical traits could be efficiently applicable for the varietal characterization in large and heterozygous populations. Similar findings about the significance of quantitative and qualitative traits in genotypic characterization have also emerged in guava germplasm (Correa *et al.*, 2012; Aslam *et al*., 2024).

The presented study revealed wide variability in fruit morphology and quality among the germplasms. Genotypic diversity was higher in accessions collected from Punjab than in the KPK regions. Similarly, reports have also stated morphochemical diversity from different parts of the world, including Cuba (Pelea *et al*., 2016), Mexico (Hernández-Delgado *et al*., 2018), Venezuela (Aranguren *et al*., 2010), Brazil (Santos *et al*., 2011), Kenya (Chiveu *et al*., 2019), and Bangladesh (Alam *et al*., 2018). The relevant study efficiently categorized individual accessions which highlights great potential in the available germplasm for making better selections for target traits. These selections could apply to asexual propagation for enhanced productivity and utilization in future breeding programs.

Knowledge of the range of the available phenotypic and fruit quality variation in the germplasm is highly valuable for the breeders to select parents and initiate breeding programs. Among key commercial fruit traits, FW has a direct relation to the yield and profitability. Maximum fresh FW, as reported in Mexican germplasm, was 120 g (Hernández-Delgado *et al*., 2007); in Indian germplasm, it was 150 g (Singh *et al*., 2018); and in Cuban germplasm, it was 250 g (Pelea *et al*., 2016).

The current study showed four accessions which had FW ranging from 190 to 235 g, a considerably better fruit weight. Similarly, considerable variation was also evident in Pakistani germplasm for other key traits like fruit size, NS, TSS, TSS:TA, and TS. Traits, i.e., skin color, FlT, and SW are consumers' preferences, while fruit size is important for packing and shipment plans (Padilla-Ramirez *et al*., 2012).

In Pakistan, guava growers have named varieties based on variability in their size (large, medium, and small), shape (round and pear-shaped), bearing habit (Sadabahar, i.e., those that bear year-round or for most of the months), and skin and flesh color (red-skinned Gola, pink-fleshed Gola, and Apple guava). Similar accessions were available under different names in various areas. It highlights the need for genetic characterization and categorization of different accessions according to the phenotypic similarities. The latest study has also revealed phenotypic similarities more precisely in accessions of Pakistani germplasm.

Interesting relationships were notable in correlations of the physical data. More positive correlations existed among physical traits and more negative correlations were among the chemical traits. Large-sized fruits typically have poor accumulation of the chemical contents, including TSS and TS, resulting in poor fruit taste and quality. FL showed a more negative correlation to TSS than with FW, FD, and FlW. Most accessions with greater fruit size and FW had lower TSS values. Similar trends were reports given by Mehmood *et al*. (2013) in guava germplasm. SCW has a negative association to TSS, indicating that by lowering the SN and SW, the fruit TSS may have to be compromised. Non-reducing sugars (NRS) play a major role in TS of fruit crops. NRS provided a positive correlation to SCW, indicating the role of FW and sugar accumulation. The PCA showed following traits in the first two components, such as FW, FD, FlW, SCW, FL, SW, NS, TS, and NRS, had the highest loadings. These findings signify such traits could be more useful for assessment of the physicochemical diversity and characterization of the germplasm in guava.

In this study, a clear correlation was notable among most accessions collected from different localities. Most accessions from one locality reached grouping together, except for a few outliers. The locations of Punjab and KPK have environmental variations, including temperature, day length, rainfall patterns, and relative humidity. In KPK, guava-culture areas have lower temperatures, shorter days, and prolonged winters compared with those of Punjab. Like other fruit crops, higher atmospheric temperature and moisture developed higher TSS in guava (Dinesh and Reddy, 2012). In another study, TSS exhibited a positive correlation to the annual mean temperature (Chiveu *et al*., 2019); however, no such trends were evident in an extensive study on guava, and no correlation of TSS to temperature, rainfall, and relative humidity emerged (Usman *et al*., 2021). TSS has a negative linkage to FW and size (Mehmood *et al*., 2013; Chiveu *et al*., 2019). In the promising study, FW and SW were higher in accession from KPK, whereas the TSS:TA ratio was low. Similarly, a report of a negative correlation of temperature to FW resulted in strawberry (Aguero *et al*., 2015). Fruit size was greater at higher altitudes in grapes (Regina *et al*., 2010). Total sugars were also higher in accessions from KPK areas situated at elevated altitudes compared with Punjab areas. Low temperature enhanced fruit size and sugars in guava in Punjab during winter (Usman *et al*., 2021). These reports support the development of higher FW, TSS:TA, and TS in KPK areas than in Punjab in this study. Furthermore, the same variety may also behave differently under varying agroclimatic conditions.

The correlations of the fruit yield and quality-related attributes of this study could be very useful for breeders in their crop improvement programs. The promising accessions having more FW, large fruit size, a smaller NS, and higher TSS could be applicable for both inter-varietal and intra-varietal crossing for crop improvement. Other traits of interest for the consumers include fruit skin, flesh color, and flesh thickness (Correa et al., 2012). A large variation is available in the fruit skin color (white, creamy, yellow, pink, and red) in both varieties, which could also be favorable to develop more diversified skin colors in guava. Likewise, regarding flesh color, the pink-to-red flesh varieties are available, which could benefit from further stringent selections for more pigment and higher lycopene content, which are highly useful for the processing industry (Mondragón-Jacobo *et al*., 2010). The parental selections for breeding can proceed based on the genetic distance rather than phenotypic variability and geographical distance. Genetic divergence among parents is reliant on the allelic diversity (Dias *et al*., 2003). In cluster analysis based on genetic divergence, the individuals grouped in a single cluster have less divergence compared with the individuals of the other cluster. Hence, to obtain more heterosis in hybrids, better accessions of one cluster require crossing with the selected accession of the other cluster (Roy *et al*., 2013). In this regard, the introduction of elite exotic cultivars, their evaluation under local environmental conditions for adaptability, and the use of promising cultivars in breeding programs may also be effective for greater genetic divergence, higher heterosis, and the development of better hybrids.

It is crucial to select the parental genotypes for crossing that are more stable under diverse environmental conditions to minimize the impact of the environment. The guava accessions from both regions in this study are already well-adapted to the local environmental conditions, which were selected from their native environments. These accessions have shown great genetic variability, and selected accessions could be beneficial for breeding programs, including marker-assisted breeding (MAB). Minimizing environmental impact can succeed by studying the environment and genotype interaction in the selected accessions. Genetic analysis using suitable molecular markers will further indicate the extent of genetic divergence (Mehmood *et al*., 2013; Usman *et al*., 2020) in the guava germplasm.

CONCLUSIONS

Wide diversity was evident in the germplasm for physical and chemical traits. Accessions G_1, G_5, G_{13}, and G_{18} could be suitable parental candidates for better FW and size, and G_7, G_9, and G_{12} could be effective for improvement in the TSS, TSS:TA, and TS. The Apple Guava G_9 could be a strong candidate for a smaller number of seeds, higher TSS and TSS:TA, and induction of dark red skin, which is a consumer-preferred trait. The distinct accessions could be beneficial for clonal multiplication, genetic characterization and association studies, and incorporation in breeding programs for crop improvement and stress tolerance.

ACKNOWLEDGMENTS

The authors are thankful to the Agriculture Linkage Program (ALP) of the Pakistan Agriculture Research Council (PARC), Islamabad, for financial assistance for this study under the Guava Polyploidization project # CS-121.

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