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Genetic diversity of Pakistani guava (*Psidium guajava* L.) germplasm and its implications for conservation and breeding

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ABSTRACT

Cultivated guava (*Psidium guajava* L.) is an important commercial fruit crop. One hundred and thirty two accessions were collected from 12 regions in Pakistan for study of genetic diversity. A total of 33 traits (18 qualitative and 15 quantitative) enabled an assessment of the genetic variability and structure of this guava germplasm. The measured traits of fruit acidity, fruit diameter, seed weight, non-reducing sugars, thickness of outer flesh, number of seeds, fruit sweetness, longitudinal grooves, leaf twisting, fruit skin color, fruit shape at the stalk, longitudinal ridges, and flesh color were found highly variable. Many of these traits are of significant economic importance and could be used as breeding targets to increase fruit yield and fruit quality. There were strong positive correlations detected among the 15 quantitative traits related to fruit yield and fruit quality. These included fruit length and diameter, fruit weight and diameter, length and width of the leaf blade, number of seeds and seed weight, fruit weight and diameter of the fruit cavity, and seed weight and fruit weight. On the other hand, there were some negative correlations among the 18 qualitative traits studied. The 2D PCA plot successfully grouped the samples according to their phenotypic resemblance and morphological characteristics. The morphological dendrogram generated from agglomeration hierarchical clustering (AHC) grouped the 132 accessions into 3 major clusters.

1. Introduction

Cultivated guava (*Psidium guajava* L. $2n = 2x = 22$) which belongs to the genus *Psidium* (Myrtaceae) is one of the most important fruit crops grown commercially across the tropics and sub-tropics (Hayes, 1970; Pathak and Ojha, 1993; Rodríguez et al., 2010). Having high vitamin A and B contents and being exceptionally rich in vitamin C, guava fruit is generally known as the 'Apple of Tropics and Sub-tropics' (Prakash et al., 2002; Rai et al., 2010). Guava originates from Mexico, the Caribbean, and Central and South America. Other less cultivated relatives include Brazilian guava (*Psidium guineense*), Mountain guava (*Psidium montanum*), Strawberry or Cherry guava (*Psidium cattleianum*), Pineapple guava (*Acca sellowiana*) and Chilean guava (*Ugni myricoides*). Approximately 130 genera and 3000 species are categorized within the Myrtaceae and genus *Psidium* has more than 150 species, many of which are edible. (Watson and Dallwitz, 2007; Jaiswal and Jaiswal, 2005).

Botanically, guava fruit is a berry which may be rounded, ovate, or pear shaped. The fruit varies from 25 to 102 mm in diameter and from 56 to about 450 g in weight. The skin color of the ripe fruit is usually yellow and the flesh color may be white, pink, yellow or cream. Guavas vary from thick fleshed fruits with only a few seeds in a small central cavity, to thin fleshed fruits with numerous seeds imbedded in a large mass of pulp. The fruits range in flavor from quite sweet in some varieties, to sour and highly acidic in others. The characteristic musky guava aroma and flavor are quite evident in most forms, however in some types they are milder and more pleasant. In others, the aroma and flavor may be too strong and penetrating for most tastes (Menzel, 1985).

The chemical and biochemical characterizations of guava fruit are important in providing information useful in defining its use. For instance, guava fruit is highly nutritious, excellent in taste and trees produce fruit all year round (Zamir et al., 2003; Rahman

Abbreviations: PCA, principal-components analysis; AHC, agglomeration hierarchical clustering.

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et al., 2003). Quality and shelf-life of guava enhanced by chemical treatment (Muhammad et al., 2013). Guava fruit is five times richer in ascorbic acid compared to citrus –100 g of guava fruit contains more than 260 mg of ascorbic acid. It is a very good source of vitamin A, vitamin C, citric, lactic, malic, oxalic and acetic acids as well as sugars and mineral salts (Rahman et al., 2003). Guava can be consumed as a fresh fruit or can be processed into sweets, jelly, jam and juice. High soluble solids and titratable acidity in fruits are desirable for industry because they reduce processing costs, whereas low acidity and high solids content are desirable for fresh consumption (Padilla-Ramirez et al., 2012). Development of nutrient-rich cultivars has been a focus of plant breeding programs.

India has the world's largest mass production of guava, followed by Pakistan, Mexico, Brazil, Egypt, Thailand, Columbia, and Indonesia. Production in these countries has increased 10-fold in the last five years (Pommer and Murkami, 2009). In Pakistan, guava is extensively grown in Punjab and Sindh provinces and occupies third position after citrus and mango in terms of area and production. The two main types of cultivated guava in Pakistan are Gola (round shape fruit) and Surahi (pear shape fruit). According to the Pakistan Statistical Yearbook (2010), guava is grown on 62,300 ha giving 512,300 t of annual production with a yield of 8223 kg per hectare. Punjab province grew 49,700 ha with a total production of 422,300 t and yield of 8497 kg per hectare. Although guava is cultivated across a large area, its production remains low, probably due to a lack of superior varieties along with other environmental and disease stresses (Imran et al., 2013).

With a low occurrence of self-pollination, guava is generally cross-pollinated and is usually be propagated from seed. Seedlings are generally long-lived and the trees bear fruit of variable size and quality (Singh and Sehgal, 1968; Pommer and Murkami, 2009). Rooting of cuttings is an effective vegetative propagation method (Kareem et al., 2013) that leads to get true-to-type progeny. Sexual propagation is still being commonly used by local growers in Pakistan for economic reasons. As growers name their cultivars according to a few morphological characters, the misnaming of synonymous and homonymous trees is a major problem in guava orchards. Accurate characterization of guava cultivars and rootstocks is essential for commercial orchards and nurseries and can guarantee uniformity in the establishment of new orchards. The high percentage of cross pollination in seed propagated guava orchards is one of the main reasons for low productivity in developing countries like Pakistan (FAO, 2002). Therefore, it is necessary to evaluate all the potential domestic guava germplasm resources for breeding superior varieties. Genetic diversity and discrimination among individual accessions or groups of individuals or populations can be analyzed by a specific method or combination of methods (Mohammadi and Prasanna, 2003; Coser et al., 2012).

The key to increase guava yield and quality is through widening the available genetic base. Breeders emphasize the importance of genetic divergence in the selection of parents for hybridization because the more diverse the parents are within a reasonable range, the more chance there is of improving target traits (Pommer, 2012). Knowledge of genetic variation and relationships between populations is important in understanding the available genetic variability and its potential use in breeding programs (Hayward and Breese, 1993). Quantitative traits provide an estimate of genetic diversity and various numerical taxonomic techniques have been used to classify and measure the pattern of phenotypic diversity among guava germplasm collections (Rodríguez et al., 2004; Hernandez-Delgado et al., 2007; Fernandes-Santos et al., 2010). Therefore, the aim of this study was to analyze both quantitative and qualitative traits in 132 elite Pakistani guava accessions in order to assess possibilities for improving guava fruit yield and quality.

2. Materials and methods

2.1. Plant materials

Fifty guava orchards were surveyed and identified, 132 accessions were collected from these orchards distributed across different districts of Punjab and Khuber Pakhtum Khwa (KPK) provinces (Table 1, Fig. 1). Districts included Faisalabad, Sahiwal, Sheikhupura, Toba Tek Singh, Chechawatni, Vehari, Chishtian, Multan, Bahawalpur, Rahim Yar khan, Kasur and Peshawer. The investigated area covered 68,315 km^2 (Table 1).

2.2. Quantitative traits evaluation

The 15 quantitative traits evaluated, included length of leaf blade [LLB], width of leaf blade [WLB], fruit length [FL], fruit diameter [FD], fruit weight [FW], diameter of cavity on fruit [DCF], thickness of outer flesh [TOF, number of seeds [NS] in each fruit, seed weight from a single fruit [SW], percentage fruit acidity [FA], total soluble solids [TSS] measured in Brix, vitamin C [VC] calculated in mg/100 ml of juice, percentage total sugars [TS], percentage reducing sugars [RS] and percentage non-reducing sugar [NRS].

The total soluble solids [TSS] of the guava juice were estimated in Brix at harvest using a digital refractometer (RX 5000, ATAGO, Japan). The total titratable acidity component was measured by the Hortwitz (1960) method, and the ascorbic acid component in pulp tissue was estimated using the Ruck (1969) method. The total fruit sugar content was calculated as the sum of reducing and non-reducing sugars using methods described by Hortwitz (1960) and Ronald and Sawyer (1981).

2.3. Evaluation of qualitative traits

The 18 qualitative traits investigated included fruit shape at the stalk [FSS], width of neck of fruit [WNF], fruit skin color[FCS], longitudinal ridges [LR], prominence of longitudinal ridges [PLR], longitudinal grooves [LG], color of flesh [CF], evenness of color of flesh [ECF], juiciness [J], attitude of branches [AB], young shoot color [YSC], young leaf anthocyanin [YLA], leaf shape [LS], leaf twisting [LT], leaf variegation [LV], color of midrib on leaf [CML], leaf shape on base [LSB] and leaf shape on tips [LST]. The method used to grade or rate phenotypes of each of these 18 traits was based on the plant descriptor from UPOV (1987).

2.4. Data scoring and analysis

Data from the 132 guava accessions of involving 33 traits were analyzed by XLSTAT (2013) XLSTAT software (version 2013.1). In the principal components analysis (PCA), factor loadings >0.55 were regarded as significant. In the correlation analyses, parametric Pearson correlations were used to analyze the quantitative characters whereas non-parametric Spearman correlations were used to analyze the qualitative characters. Genetic similarity computing and the construction of respective 2D plots were also performed. The combined data from the quantitative and qualitative traits were then used for dendrogram construction. Euclidean distance was used to analyze the genetic dissimilarity component and Ward's method was used for the agglomerative hierarchical clustering (AHC).

Table 1
Details of 132 elite guava (*Psidium guajava* and *Psidium cattleianum*) germplasm collections.

Accession	Cultivar name	Origin	Species name	Characteristics
		Punjab		
SHS_1	Mota Gola	Sheikhupura	*P. guajava*	Round (big size)
SHS_2	Gola	Sheikhupura	*P. guajava*	Round (medium size, pink flesh)
SHS_3	Surahi	Sheikhupura	*P. guajava*	Pear shape(medium size, pink flesh)
SHS_4	Surahi	Sheikhupura	*P. guajava*	Pear shape (medium size, pink flesh)
SHS_5	Gola	Sheikhupura	*P. guajava*	Round (medium in size, pink flesh)
SHS_6	Gola	Sheikhupura	*P. guajava*	Round (medium size)
SHS_7	Surahi	Sheikhupura	*P. guajava*	Pear shape (medium size)
SHS_8	Surahi	Sheikhupura	*P. guajava*	Pear shaped (low seeded)
SHS_9	Sad Bahar Surahi	Sheikhupura	*P. guajava*	Pear shaped (medium size)
SHS_{10}	Sadabahar Gola	Sheikhupura	*P. guajava*	Round (medium size)
SHS_{11}	Surahi	Sheikhupura	*P. guajava*	Pear shape (medium size)
SHS_{12}	Chota Gola	Sheikhupura	*P. guajava*	Round (small size)
SHS_{13}	Mota Gola	Sheikhupura	*P. guajava*	Round (big size, golden skin)
SHS_{14}	Chota Gola	Sheikhupura	*P. cattleianum*	Round (small size, red blush)
SHS_{15}	Gola	Sheikhupura	*P. cattleianum*	Round (medium size, red blush)
SHS_{16}	Moti Surahi	Sheikhupura	*P. guajava*	Pear shaped (big size)
SHS_{17}	Surahi	Sheikhupura	*P. guajava*	Pear shape (low seeded)
SHS_{18}	Moti Surahi	Sheikhupura	*P. guajava*	Pear shape (big size)
SHS_{19}	Gola	Sheikhupura	*P. guajava*	Round (red flesh)
SHS_{20}	Gola	Sheikhupura	*P. guajava*	Round (golden skin)
SHS_{21}	Sadabahar Surahi	Sheikhupura	*P. guajava*	Pear shape (medium size)
CWS_{22}	Gola	Chichawatni	*P. guajava*	Round (medium size, shining skin)
CWS_{23}	Gola	Chichawatni	*P. guajava*	Round (medium size, golden skin)
CWS_{24}	Mota Gola	Chichawatni	*P. guajava*	Round (big size)
CWS_{25}	Gola	Chichawatni	*P. cattleianum*	Round (red blush)
CWS_{26}	Gola	Chichawatni	*P. guajava*	Round
CWS_{27}	Choti Surahi	Chichawatni	*P. guajava*	Pear shape (small size)
CWS_{28}	Surahi	Chichawatni	*P. guajava*	Pear (medium size, rough skin)
CWS_{29}	Chota Gola	Chichawatni	*P. guajava*	Round (small size)
TTS_{30}	Moti Surahi	Toba Tek Singh	*P. guajava*	Pear shape (big size)
TTS_{31}	Surahi	Toba Tek Singh	*P. guajava*	Pear shape (medium size)
BWS_{32}	Chota Gola	Bahawalpur	*P. guajava*	Round (small size)
BWS_{33}	Larkana Gola	Bahawalpur	*P. guajava*	Round (medium size)
BWS_{34}	Larkana Surahi	Bahawalpur	*P. guajava*	Pear shape (medium skin)
BWS_{35}	Larkana Surahi	Bahawalpur	*P. guajava*	Pear shaped (rough skin)
BWS_{36}	Gola	Bahawalpur	*P. guajava*	Round (medium size, pink flesh)
BWS_{37}	Kareala	Bahawalpur	*P. guajava*	Bitter gourd shape
BWS_{38}	Choti Surahi	Bahawalpur	*P. guajava*	Pear shape (small size)
RYS_{39}	Larkana Surahi	Rahim Yar Khan	*P. guajava*	Pear shape (medium size)
RYS_{40}	Larkana Surahi	Rahim Yar Khan	*P. guajava*	Pear shaped (low seeded)
RYS_{41}	Desi Gola	Rahim Yar Khan	*P. guajava*	Round shape (small size)
FDS_{42}	Gola	Faisalabad	*P. guajava*	Round shape (medium size)
FDS_{43}	Surahi	Faisalabad	*P. guajava*	Pear shape (medium size)
FDS_{44}	Surahi	Faisalabad	*P. guajava*	Pear shape (small size, short neck)
FDS_{45}	Surahi	Faisalabad	*P. guajava*	Pear shape (pink flesh
FDS_{46}	Surahi	Faisalabad	*P. guajava*	Pear shape (long neck)
FDS_{47}	Surahi	Faisalabad	*P. guajava*	Pear shape (low seed)
FDS_{48}	Bangladeshi Variety	Faisalabad	*P. guajava*	Round shape (extra large size)
FDS_{49}	Desi Gola	Faisalabad	*P. guajava*	Round (small size)
FDS_{50}	Sour Gola	Faisalabad	*P. guajava*	Round (sour taste)
FDS_{51}	Sour Surahi	Faisalabad	*P. guajava*	Pear shape (sour taste, pink flesh)
FDS_{52}	Sandhuri Surahi	Faisalabad	*P. guajava*	Pear shape (light pink blush)
FDS_{53}	Kareala	Faisalabad	*P. guajava*	Bitter gourd shape
KSS_{54}	Surahi	Kasur	*P. guajava*	Pear shape (medium size)
KSS_{55}	Sour Gola	Kasur	*P. guajava*	Round (sour taste, pink flesh)
KSS_{56}	Gola	Kasur	*P. guajava*	Round (pink flesh)
KSS_{57}	Sour Surahi	Kasur	*P. guajava*	Pear shape (sour taste)
KSS_{58}	Sour Gola	Kasur	*P. guajava*	Round (sour taste, pink flesh)
KSS_{59}	Gola	Kasur	*P. guajava*	Round (red flesh)
SWS_{60}	Surahi	Sahiwal	*P. guajava*	Pear shape (medium size)
SWS_{61}	Safaida	Sahiwal	*P. guajava*	Round (golden flesh)
SWS_{62}	Surahi	Sahiwal	*P. guajava*	Pear shape (medium size)
SWS_{63}	Moti Surahi	Sahiwal	*P. guajava*	Pear shape (large size)
SWS_{64}	Gola	Sahiwal	*P. guajava*	Round (medium size)
SWS_{65}	Surahi	Sahiwal	*P. guajava*	Pear shape (medium size)
SWS_{66}	Gola	Sahiwal	*P. guajava*	Round (pink flesh)
SWS_{67}	Surahi	Sahiwal	*P. guajava*	Pear shape (medium size)
SWS_{68}	Gola	Sahiwal	*P. guajava*	Round (pink flesh)
SWS_{69}	Surahi	Sahiwal	*P. guajava*	Pear shape (pink flesh)
SWS_{70}	Gola	Sahiwal	*P. guajava*	Round (pink flesh)
SWS_{71}	Surahi	Sahiwal	*P. guajava*	Pear shape (medium size)
SWS_{72}	Gola	Sahiwal	*P. guajava*	Round (medium size)
SWS_{73}	Surahi	Sahiwal	*P. guajava*	Pear shape (medium size)

Table 1 (*Continued*)

Accession	Cultivar name	Origin	Species name	Characteristics
SWS_{74}	Gola	Sahiwal	*P. guajava*	Round (pink flesh)
$CHSS_{75}$	Sadabahar Surahi	Chishtian	*P. guajava*	Pear shape (medium size)
$CHSS_{76}$	Desi Gola	Chishtian	*P. guajava*	Round (small size)
$CHSS_{77}$	Surahi	Chishtian	*P. guajava*	Pear shape (pink flesh)
$CHSS_{78}$	Gola	Chishtian	*P. guajava*	Round (medium size)
$CHSS_{79}$	Surahi	Chishtian	*P. guajava*	Pear shape (medium size)
$CHSS_{80}$	Gola	Chishtian	*P. guajava*	Round (medium size)
$CHSS_{81}$	Surahi	Chishtian	*P. guajava*	Pear shape (medium size)
$CHSS_{82}$	Surahi	Chishtian	*P. cattleianum*	Pear shape (pink blush)
$CHSS_{83}$	Surahi	Chishtian	*P. guajava*	Pear shape (pink flesh)
$CHSS_{84}$	Sadabahar Surahi	Chishtian	*P. guajava*	Pear shape (medium size)
$CHSS_{85}$	Sadabahar Gola	Chishtian	*P. guajava*	Round (medium size)
$CHSS_{86}$	Surahi	Chishtian	*P. guajava*	Pear shape (pink flesh)
$CHSS_{87}$	Sadabahar Surahi	Chishtian	*P. guajava*	Pear shape (medium size)
$CHSS_{88}$	Gola	Chishtian	*P. guajava*	Round (medium size)
$CHSS_{89}$	Gola	Chishtian	*P. guajava*	Round (pink flesh)
$CHSS_{90}$	Surahi	Chishtian	*P. guajava*	Pear shape(pink flesh)
$CHSS_{91}$	Choti Surahi	Chishtian	*P. guajava*	Pear shape (small size)
$CHSS_{92}$	Surahi	Chishtian	*P. guajava*	Pear shape (medium size)
MTS_{93}	Surahi	Multan	*P. guajava*	Pear shape (medium size)
MTS_{94}	Gola	Multan	*P. guajava*	Round (pink flesh)
MTS_{95}	Gola	Multan	*P. guajava*	Round (medium size)
MTS_{96}	Gola	Multan	*P. guajava*	Round (medium size)
MTS_{97}	Gola	Multan	*P. guajava*	Round (medium size)
MTS_{98}	Larkana Surahi	Multan	*P. guajava*	Pear shape (medium size)
MTS_{99}	Larkana Surahi	Multan	*P. guajava*	Pear shape (medium size)
MTS_{100}	Gola	Multan	*P. guajava*	Round (pink flesh)
MTS_{101}	Mota Gola	Multan	*P. guajava*	Round (big size)
VHS_{102}	Gola	Vehari	*P. guajava*	Round (golden skin)
VHS_{103}	Chota Gola	Vehari	*P. guajava*	Round (small size)
VHS_{104}	Gola	Vehari	*P. guajava*	Round (medium size)
VHS_{105}	Gola	Vehari	*P. guajava*	Round (pink flesh)
VHS_{106}	Surahi	Vehari	*P. guajava*	Pear shape (medium size)
VHS_{107}	Gola	Vehari	*P. guajava*	Round (pink flesh)
VHS_{108}	Gola	Vehari	*P. guajava*	Round (medium size)
VHS_{109}	Surahi	Vehari	*P. guajava*	Pear shape (medium size)
VHS_{110}	Surahi	Vehari	*P. guajava*	Pear shape (medium size)
VHS_{111}	Choti Surahi	Vehari	*P. guajava*	Pear shape (small size)
VHS_{112}	Gola	Vehari	*P. guajava*	Round (medium size)
VHS_{113}	Gola	Vehari	*P. guajava*	Round (pink flesh)
VHS_{114}	Chota Gola	Vehari	*P. guajava*	Round (small size)
VHS_{115}	Surahi	Vehari	*P. guajava*	Pear shape (medium size)
VHS_{116}	Desi Gola	Vehari	*P. guajava*	Round (small size)
VHS_{117}	Surahi	Vehari	*P. cattleianum*	Pear shape (pink blush)
VHS_{118}	Choti Surahi	Vehari	*P. cattleianum*	Pear shape (small size, pink blush)
VHS_{119}	Mota Gola	Vehari	*P. guajava*	Round (big size)
VHS_{120}	Gola	Vehari	*P. guajava*	Round (medium size)
VHS_{121}	Surahi	Vehari	*P. guajava*	Pear shape (medium size)
VHS_{122}	Moti Surahi	Vehari	*P. guajava*	Pear shape (large size)
VHS_{123}	Choti Surahi	Vehari	*P. guajava*	Pear shape (small size)
		KPK		
$PSHS_{124}$	Sindhi	Peshawar,	*P. guajava*	Round
$PSHS_{125}$	Gulabi	Peshawar	*P. guajava*	Pear shape (pink flesh)
$PSHS_{126}$	Ramzani	Peshawar	*P. guajava*	Round
$PSHS_{127}$	Riazi	Peshawar	*P. guajava*	Round
$PSHS_{128}$	Thandiani	Peshawar	*P. guajava*	Pear shape
$PSHS_{129}$	PG-001	Peshawar	*P. guajava*	Pear shape (medium size)
$PSHS_{130}$	PG-005	Peshawar	*P. guajava*	Pear shape (medium size)
$PSHS_{131}$	PG-013	Peshawar	*P. guajava*	Round (medium size)
$PSHS_{132}$	Gola	Peshawar	*P. guajava*	Round (low seeded)

3. Results

3.1. Descriptive statistics and correlations for the quantitative variables

The 15 quantitative traits were measured and the descriptive statistics of minima, maxima, means, standard deviations and coefficients of variation (CV) are shown in Table 2. The results revealed extensive morphological variability. Some traits had high CVs. These included fruit acidity (44.31%), fruit width (36.93%), seed weight (36.12%), non-reducing sugars (34.22%), thickness of outer flesh (32.17%), number of seeds (31.46%) and fruit sweetness (30.95%). The remaining traits showed comparatively low CV values (<30%). Total sugars and leaf blade width had the lowest CVs of 16.72% and 15.62%, respectively.

Accession FDS_{53} had the highest fruit acidity (1.9%), followed by RYS_{39} (1.6%) and $CHSS_{86}$ (1.43%). The lowest fruit acidities were recorded in SHS_{12} (0.228%), BWS_{38} (0.24%) and FDS_{47} (0.25%). FDS_{48} had the heaviest fruits (390 g), followed by VHS_{122} (187.43 g) MTS_{101} (182.45 g) and VHS_{119} (174.78 g). The lightest fruits were in BWS_{32} (34.6 g) which also had a distinct sweet taste, followed by KSS_{56} (50.83 g) and CWS_{29} (52.5 g). Accessions

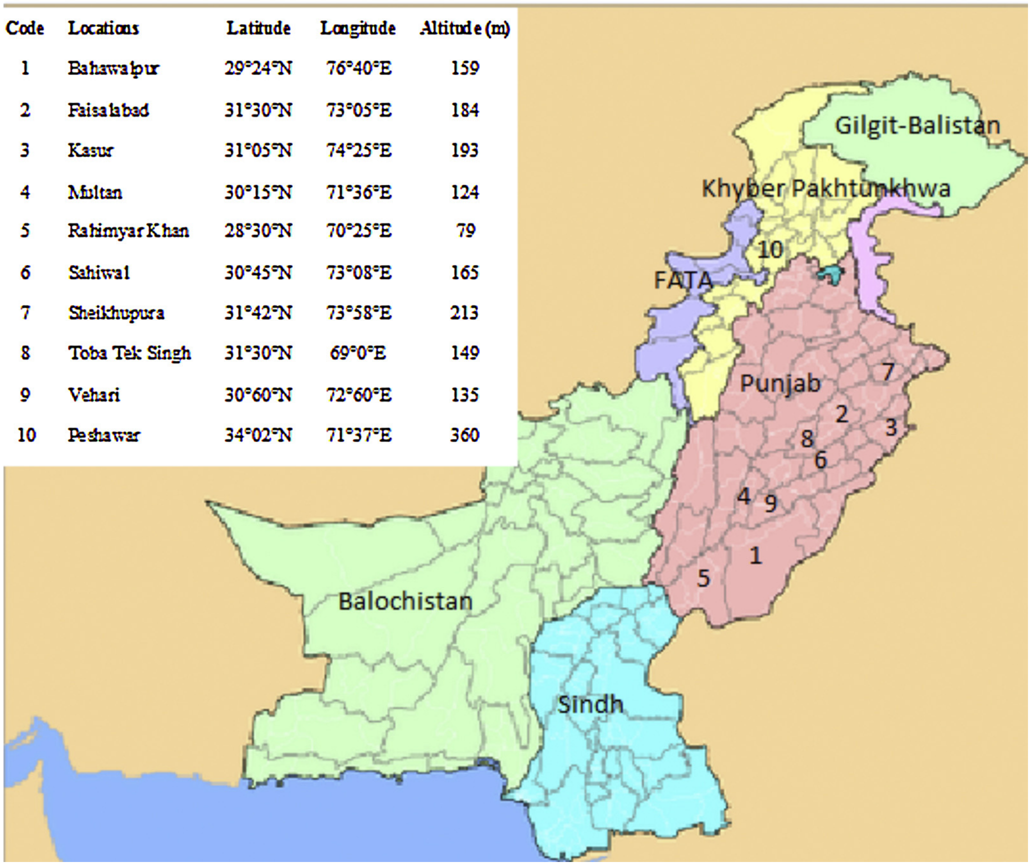

Fig. 1. Map of Pakistan with collection sites for 132 guava accessions from Punjab and KPK provinces. Numbers indicate districts from which samples were taken.

with low seed weight included BWS_{32} (8.33 g), BWS_{37} (9.23 g), CWS_{29} (9.8 g), FDS_{47} (9.8 g), RYS_{40} (10.0 g), TTS_{30} (10.3 g), FDS_{42} (10.8 g) and $PSHS_{132}$ (11.5 g). Accessions with abundant seed weights were FDS_{48} (65 g), SHS_{13} (50.3 g), MTS_{101} (47.3 g) and $CHSS_{78}$ (43. g). The maximum fruit diameters were for VHS_{119} (94.43 mm), followed by VHS_{122} (89.54 mm), MTS_{101} (88.65 mm) and MTS_{98} (81.77 mm). Maximum fruit length was observed in BWS_{37} (120.5 mm), followed by FDS_{48} (103.5 mm), VHS_{122} (101.32 mm) and VHS_{119} (97.43 mm). BWS_{38} (Sour Gola) had the minimum fruit length of 35.15 mm. The widest cavity on fruits was observed in FDS_{48} (52.5 mm), followed by $PSHS_{132}$ (39.54 mm), $CHSS_{91}$ (38.32 mm), MTS_{101} (37.54 mm) and MTS_{99} (37.32 mm).

Strong positive correlations were observed among all the 15 quantitative traits (Table 3). The highest positive correlation was between fruit length and fruit diameter (0.820). Positive correlations were also observed between fruit weight and fruit diameter (0.672), length of leaf blade and width of leaf blade (0.629), number of seeds and seed weight (0.587), fruit weight and fruit cavity diameter (0.579), seed weight and fruit weight (0.567), fruit weight and fruit length (0.566), total sugar and non-reducing sugar (0.549), total sugar and reducing sugar (0.477), thickness of outer flesh and fruit diameter (0.455), seed weight and diameter of cavity on fruit (0.443), and thickness of outer flesh and fruit length (0.385).

By contrast, there were also negative correlations between some quantitative traits (Table 3). These included reducing sugars and

Table 2
Descriptive statistics for 15 quantitative traits in 132 Pakistani guava accessions.

Trait	Minimum	Maximum	Mean	Std. deviation	CV
Fruit acidity (%)	0.22	1.90	0.73	0.32	44.3
Total soluble solid (°Brix)	6.50	12.50	9.79	3.03	31.00
Vitamin C (mg/100 ml Juice)	49.20	233.30	106.62	30.48	28.59
Total sugar (%)	4.00	10.00	5.76	0.96	16.72
Reducing sugar (%)	1.20	5.67	3.01	0.88	29.40
Non reducing sugar (%)	1.11	7.25	2.62	0.90	34.22
Number of seeds	47.00	532.00	255.42	80.36	31.46
Seed weight (g)	8.33	65.00	22.99	8.31	36.12
Thickness of outer flesh (mm)	0.42	22.21	11.64	3.74	32.17
Fruit weight (mm)	34.60	390.00	112.28	41.47	36.93
Diameter of cavity (mm)	13.32	52.50	28.09	6.59	23.48
Fruit length (mm)	35.15	120.50	60.98	16.11	26.42
Fruit diameter (mm)	29.21	94.43	52.02	12.76	24.52
Length of leaf blade (cm)	6.76	15.50	10.98	2.031	18.51
Width of leaf blade (cm)	3.50	7.80	5.32	0.83	15.62

Table 3
Correlation coefficients among 15 quantitative traits in 132 Pakistani guava accessions.

Trait	FA	TSS	VC	TS	RS	NRS	NS	SW	TOF	FW	DCF	F L	FD	LLB	WLB
FA	**1**														
TSS	−0.104	**1**													
VC	**0.209**	−0.032	**1**												
TS	0.062	−0.104	0.027	**1**											
RS	**−0.184**	−0.103	−0.019	**0.477**	**1**										
NRS	0.114	−0.022	−0.017	**0.549**	**−0.352**	**1**									
NS	0.065	−0.159	0.012	−0.100	−0.015	0.045	**1**								
SW	0.135	**−0.192**	**0.175**	0.077	0.025	0.075	**0.587**	**1**							
TOF	−0.148	−0.083	**−0.233**	0.034	**0.220**	−0.052	0.039	0.148	**1**						
FW	−0.037	**−0.207**	0.010	0.096	0.113	0.064	**0.194**	**0.567**	**0.552**	**1**					
DCF	−0.015	−0.098	0.097	0.093	−0.064	**0.265**	**0.292**	**0.443**	0.086	**0.579**	**1**				
F L	0.033	−0.008	0.029	**0.196**	0.013	**0.187**	0.042	**0.242**	**0.385**	**0.566**	**0.327**	**1**			
FD	−0.042	−0.047	−0.013	**0.189**	0.103	0.145	**0.176**	**0.302**	**0.455**	**0.672**	**0.422**	**0.820**	**1**		
LLB	**0.261**	0.113	**0.195**	**0.177**	−0.020	0.114	0.057	0.055	0.024	−0.034	−0.019	**0.235**	0.149	**1**	
WLB	−0.046	0.009	0.027	0.124	0.095	0.074	**0.182**	0.103	**0.199**	**0.174**	0.090	0.133	**0.243**	**0.629**	1

Values in bold are different from 0 with a significance level alpha = 0.05. Abbreviations: FA, fruit acidity; TSS, total soluble solid; VC, vitamin C, TS, total sugar; RS, reducing sugars; NRS, non-reducing sugars; NS, number of seeds; SW, seed weight, TOF, thickness of outer flesh; FW, fruit weight; DCF, diameter of cavity on fruit; FL, fruit length; FD, fruit diameter; LLB, length of leaf blade; WLB, width of leaf blade.

non-reducing sugars (−0.352), vitamin C and thickness of outer flesh (−0.233), fruit sweetness and fruit weight (−0.207), fruit sweetness and seed weight (−0.192), and fruit acidity and reducing sugars (−0.184).

3.2. Principal component analysis (PCA) of quantitative variables

PCA put the 15 quantitative traits into six components that explained 74.54% of the total variation (Table 4). The first component, which accounted for 24.21% of the total variation, included fruit weight, fruit diameter, fruit length, diameter of cavity on fruit, seed weight, thickness of outer flesh and number of seeds. The second component, which explained 12.38% of the total variation, included length of leaf blade, fruit acidity, non-reducing sugar, thickness of outer flesh, vitamin C and reducing sugar. The third component, explaining 11.71% of the total variation, included total sugar, number of seeds, seed weight, reducing sugar, length of leaf blade and width of leaf blade. The fourth component, accounting for 9.69% of the total variation, included non-reducing sugar, width of leaf blade, total sugar, length of leaf blade and number of seeds. The fifth component, accounting for 9.23% of the total variation, included reducing sugar, fruit sweetness, total sugar and fruit length. The sixth component, explaining 7.33% of the total variation, included vitamin C, number of seeds, width of leaf blade and non-reducing sugar.

A 2D PCA plot was constructed based on the first two components (see Fig. 2). The plot grouped the samples according to their phenotypic resemblance and morphological characteristics. For example, accessions FDS_{48}, VHS_{122}, VHS_{119}, MTS_{98}, MTS_{99} and MTS_{101} with the highest fruit weight, largest fruit diameter and longest fruit length were placed closely in the lower right plane while accessions BWS_{32}, CWS_{29}, FDS_{47}, KSS_{56}, KSS_{58} and KSS_{59} with the lowest fruit weight, smallest fruit diameter and shortest fruit length were placed close together in the upper left plane. These results demonstrate that fruit length, fruit weight and fruit diameter are highly positively correlated and as a result, these morphological traits led to the highest loading factors in this PCA analysis.

3.3. Descriptive statistics and correlations for qualitative traits

The 18 qualitative traits were also analyzed. These were polymorphic and generally showed more than two phenotypes. Their descriptive statistics shown in Table 5 indicate extensive morphological variability. Some traits had high CVs; for example, longitudinal grooves (117.1%), leaf twisting (86.24%), fruit color of skin (71.8%), fruit shape at stalk (58.96%), longitudinal ridges (43.02%) and color of flesh (41.54%). Some traits showed comparatively low CV values (<41%); for example, leaf shape on tips at 10.22% and juiciness at 8.45%.

Most of the accessions in this guava germplasm collection displayed a weak prominence of longitudinal ridges, but 11 accessions had a medium prominence of longitudinal ridges (SHS_{17}, CWS_{28}, BWS_{33}, BWS_{35}, BWS_{37}, BWS_{38}, RYS_{39}, RYS_{40}, FDS_{50}, MTS_{98} and MTS_{99}). The majority of accessions had no longitudinal ridges, whereas 26 accessions did have longitudinal ridges on their fruits (SHS_{17}, SHS_{19}, SHS_{20}, SHS_{21}, CWS_{22}, CWS_{24}, CWS_{27}, CWS_{28}, TTS_{30}, BWS_{32}, BWS_{33}, BWS_{34}, BWS_{35}, BWS_{36}, BWS_{37}, BWS_{38}, RYS_{39}, RYS_{40}, FDS_{46}, FDS_{48}, FDS_{50}, FDS_{51}, FDS_{53}, FDS_{57}, MTS_{98} and MTS_{99}). Most of the accessions had no longitudinal grooves except for 31 accessions which did (SHS_{3}, SHS_{8}, SHS_{18}, CWS_{28}, BWS_{33}, BWS_{35}, BWS_{37}, BWS_{38}, RYS_{39}, RYS_{40}, FDS_{42}, FDS_{45}, KSS_{57}, KSS_{58}, KSS_{59}, SWS_{61}, SWS_{64}, SWS_{66}, SWS_{67}, $CHSS_{76}$, $CHSS_{82}$, $CHSS_{83}$, $CHSS_{85}$, $CHSS_{86}$, MTS_{93}, MTS_{95}, MTS_{96}, MTS_{98}, MTS_{100}, VHS_{120} and VHS_{121}). There were only 16 accessions with acute leaf shape on tips (BWS_{38}, RYS_{39}, RYS_{40}, FDS_{44}, KSS_{54}, KSS_{55}, SWS_{63}, $CHSS_{77}$, $CHSS_{90}$, MTS_{98}, MTS_{99}, MTS_{100}, MTS_{101}, VHS_{106}, VHS_{119} and VHS_{123}), as most of the accessions had obtuse leaf shapes at the tips.

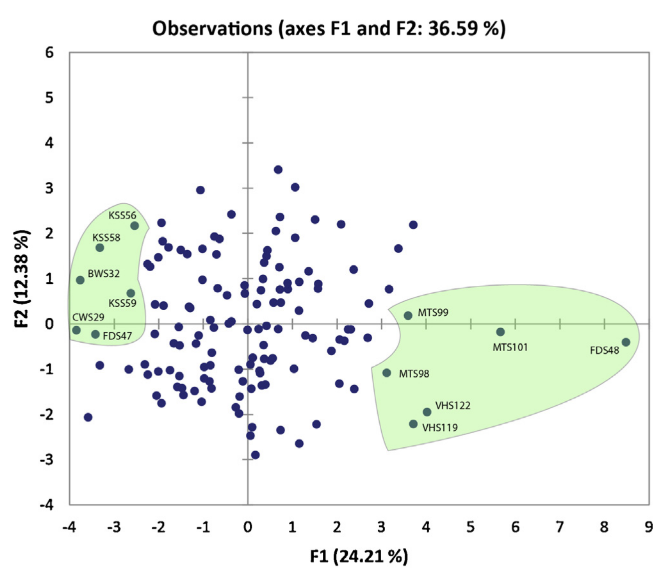

Fig. 2. Two dimensional PCA plot based on the first two components for 15 quantitative traits of 132 Pakistani guava accessions.

Table 4
First 6 components from the PCA analysis of 15 quantitative traits in 132 Pakistani guava accessions.

Traits	F1	F2	F3	F4	F5	F6
Fruit acidity	0.016	0.417	−0.138	0.003	0.046	0.372
Total soluble solid	−0.107	0.045	0.187	−0.089	−0.430	−0.030
Vitamin C	0.028	0.335	−0.135	−0.117	0.164	0.565
Total sugars	0.159	0.194	0.391	0.431	0.423	−0.071
Reducing sugars	0.075	−0.263	0.334	−0.102	0.611	0.114
Non reducing sugars	0.139	0.371	0.069	0.560	−0.117	−0.330
Number of seeds	0.202	0.099	−0.390	−0.252	0.191	−0.410
Seed weight	0.329	0.080	−0.383	−0.110	0.223	−0.071
Thickness of outer flesh	0.280	−0.350	0.198	−0.123	−0.090	−0.026
Fruit weight	0.446	−0.198	−0.107	0.015	−0.030	0.114
Diameter of cavity	0.333	0.042	−0.266	0.168	−0.048	−0.079
Fruit length	0.393	−0.027	0.158	0.088	−0.278	0.285
Fruit diameter	0.438	−0.101	0.120	0.030	−0.200	0.156
Length of leaf blade	0.127	0.467	0.325	−0.378	−0.070	−0.017
Width of leaf blade	0.193	0.243	0.305	−0.444	0.001	−0.339
Variability (%)	24.206	12.381	11.705	9.692	9.229	7.328

Fruit skin color was highly polymorphic. Most of the accessions were pale yellow–green to pale yellow, but four accessions of *P. cattleianum* (SHS_{14}, SHS_{15}, $CHSS_{82}$, VSH_{117}) were pink, VSH_{118} was red, and FDS_{52} was orange. Flesh colors ranged from white, to cream and pink. SHS_3 and BWS_{36} were pale pink and BWS_{38} was dark pink. Neck width commonly ranged from medium to broad but five accessions had narrow neck widths (SHS_9, BWS_{38}, FDS_{43}, FDS_{44} and FDS_{53}). Leaf shape ranged from obviate to oblong with only three accessions being trullate (SHS_5, FDS_{44} and BWS_{38}). Fruit shape at the stalk ranged from broad to round or necked. Juiciness for most of the accessions was in the medium range although two were dry (FDS_{48} and FDS_{50}) and seven were especially juicy (SHS_{16}, KSS_{55}, KSS_{56}, SWS_{74}, $CHSS_{91}$, VHS_{119} and $PSHS_{127}$).

Among qualitative traits, there were more negative correlations than positive ones (Table 6). Negative correlations were observed between the following traits: prominence of longitudinal ridges and longitudinal ridges (−0.609), prominence of longitudinal ridges and leaf shape on tips (−0.308), fruit shape at the stalk and neck width (−0.293), leaf base shape and fruit skin color (−0.271), fruit shape at the stalk and evenness flesh color (−0.235), prominence of longitudinal ridges and fruit neck width (−0.178), and young shoot color and flesh color (−0.177). Positive correlations include longitudinal ridges and fruit skin color (0.372), prominence of longitudinal ridges and longitudinal grooves (0.341), flesh color and evenness of flesh color (0.325), longitudinal ridges and width of fruit neck (0.297), young leaf anthocyanin color and leaf variegation (0.243), longitudinal ridges and juiciness (0.217), attitude of branches and leaf base shape (0.210), attitude of branches and leaf shape (0.195), fruit shape at stalk and prominence of longitudinal ridges (0.187), and flesh color and juiciness (0.178).

3.4. Principal component analysis (PCA) for the qualitative variables

PCA put the 18 qualitative traits into 8 components which explained 65.55% of the total variation (Table 7). The first component, which accounted for 13.81% of the total variation, included prominence of longitudinal ridges, longitudinal ridges, fruit neck width, fruit skin color, longitudinal grooves, fruit shape at the stalk, leaf shape on tips and leaf variegation. The second component, explaining 9.61% of the total variation, included leaf shape on base, leaf shape, attitude of branches, fruit shape at stalk, color of flesh, evenness of flesh color, young shoot color and young leaf anthocyanin color. The third component, accounting for 8.93% of the total variation, included evenness of flesh color, flesh color, juiciness, leaf shape on tips and fruit neck width. The fourth component, which accounted for 7.93% of the total variation, included leaf twisting, leaf midrib color, leaf variegation, young leaf anthocyanin color, juiciness, fruit shape at the stalk and fruit skin color. The fifth component, accounting for 6.82% of the total variation, included leaf shape at the tips, fruit neck width, leaf variegation, prominence of longitudinal ridges, flesh color and fruit shape at the stalk. The sixth component, accounting for 6.64% of the total variation, included longitudinal grooves, attitude of branches, juiciness, young leaf

Table 5
Statistics for 18 qualitative traits in 132 Pakistani guava accessions.

Variable	Minimum	Maximum	Mean	Std. deviation	CV
Attitude of branches	3.00	7.00	4.56	1.22	26.72
Young shoot color	1.00	3.00	2.06	0.80	38.75
Young leaf anthocyanin	1.00	9.00	7.79	2.88	36.97
Leaf shape	3.00	6.00	5.40	1.17	21.68
Leaf twisting	1.00	9.00	4.64	4.00	86.24
Leaf variegation	1.00	9.00	8.27	2.31	27.91
Color of midrib on leaf	1.00	3.00	1.13	0.36	31.74
Leaf shape on base	1.00	3.00	2.75	0.56	20.28
Leaf shape on tips	3.00	4.00	3.88	0.33	8.45
Fruit shape at stalk	1.00	5.00	3.02	1.78	58.96
Width of neck of fruit	1.00	7.00	5.64	1.17	20.71
Fruit color of skin	1.00	7.00	1.67	1.20	71.75
Longitudinal ridges	1.00	9.00	7.42	3.19	43.02
Prominence of longitudinal ridges	3.00	5.00	3.17	0.56	17.52
Longitudinal grooves	1.00	9.00	2.94	3.44	117.08
Color of flesh	1.00	5.00	2.30	0.96	41.54
Evenness of color of flesh	1.00	2.00	1.05	0.23	21.36
Juiciness	3.00	7.00	5.08	0.52	10.22

Table 6
Correlation coefficients among 18 qualitative traits for 132 Pakistani guava accessions.

Variables	AB	YSC	YLA	LS	LT	LV	CML	LSB	LST	FSS	WNF	FCS	LR	PLR	LG	CF	ECF	J
AB	**1**																	
YSC	−0.137	**1**																
YLA	0.004	0.116	**1**															
LS	**0.195**	−0.103	−0.005	**1**														
LT	0.031	0.005	0.004	−0.082	**1**													
LV	−0.009	−0.037	**0.234**	−0.030	−0.029	**1**												
CML	−0.069	−0.055	−0.036	−0.029	0.124	−0.123	**1**											
LSB	**0.210**	−0.009	−0.153	0.071	0.163	−0.026	0.007	**1**										
LST	−0.027	−0.030	−0.157	0.078	−0.081	−0.037	−0.074	0.065	**1**									
FSS	−0.063	−0.016	−0.024	−0.140	0.136	0.087	−0.048	−0.097	**−0.194**	**1**								
WNF	−0.031	0.091	−0.088	0.121	−0.100	**−0.247**	0.027	−0.046	0.078	**−0.293**	**1**							
FCS	−0.063	0.054	0.024	−0.036	−0.151	−0.012	−0.046	**−0.271**	0.029	−0.154	**0.250**	**1**						
LR	−0.147	0.082	−0.050	−0.083	0.031	−0.090	0.068	**−0.193**	0.108	−0.067	**0.297**	**0.372**	**1**					
PLR	0.082	−0.087	0.127	−0.024	0.165	0.095	−0.029	0.080	**−0.308**	**0.187**	**−0.178**	**−0.219**	**−0.609**	**1**				
LG	−0.140	0.001	0.091	−0.136	−0.019	0.056	0.059	0.061	−0.115	0.154	−0.121	−0.028	−0.120	**0.341**	**1**			
CF	−0.052	**−0.177**	−0.074	0.078	−0.041	0.088	0.018	0.123	−0.071	−0.080	−0.156	0.004	0.017	−0.041	0.068	**1**		
ECF	−0.018	−0.019	0.006	0.037	−0.080	−0.043	−0.088	0.033	−0.119	**−0.235**	−0.135	0.040	−0.053	0.051	0.024	**0.325**	**1**	
J	0.052	0.066	−0.019	0.003	0.041	−0.158	0.034	−0.083	−0.126	0.003	−0.022	−0.022	**0.217**	−0.148	−0.085	**0.178**	**0.229**	1

Values in bold are different from 0 with a significance level alpha = 0.05. Abbreviations: AB, attitude of branches; YSC, young shoot color; YLA, young leaf anthocyanin; LS, leaf shape; LT, leaf twisting; LV, leaf variegation; CML, color of midrib on leaf; LSB, leaf shape on base; LST, leaf shape on tips; FSS, fruit shape at stalk; WNF, width of neck of fruit; FCS, fruit color of skin; LR, longitudinal ridges; PLR, prominence of longitudinal ridges; LG, longitudinal grooves; CF, color of flesh; ECF, evenness of color of flesh; J, juiciness.

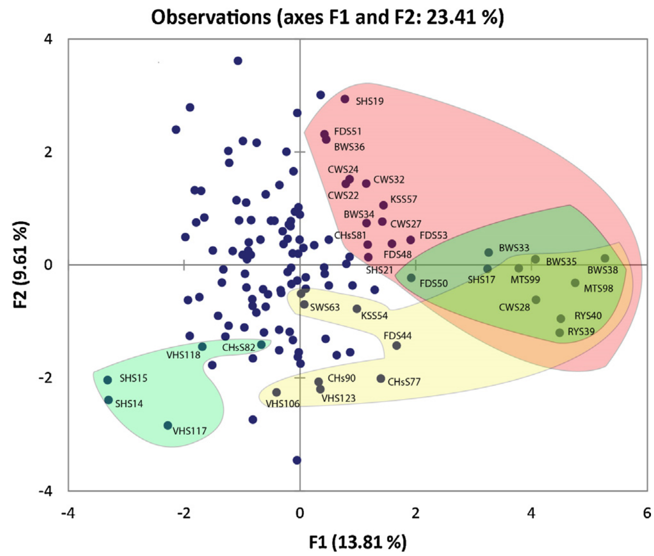

Fig. 3. Two-dimensional PCA plot based on the first 2 components for 18 qualitative traits of 132 Pakistani guava accessions.

anthocyanin color, leaf midrib color, fruit shape at the stalk and fruit neck width. The seventh component, accounting for 6.06% of the total variation, included young shoot color, leaf shape on base, leaf shape, leaf midrib color, fruit skin color and evenness of flesh color. The eighth component, which accounted for 5.74% of the total variation, included leaf midrib color, young leaf anthocyanin color, leaf twisting, fruit shape at the stalk and leaf shape on base.

A 2D PCA plot based on the first two components (see Fig. 3) grouped samples according to phenotypic resemblance and morphological characteristics. For example, 23 accessions with longitudinal ridges on fruits were placed as a distinct group (SHS_{17}, SHS_{19}, SHS_{21}, CWS_{22}, CWS_{24}, CWS_{27}, CWS_{28}, BWS_{32}, BWS_{33}, BWS_{34}, BWS_{35}, BWS_{36}, BWS_{38}, RYS_{39}, RYS_{40}, FDS_{48}, FDS_{50}, FDS_{51}, FDS_{53}, FDS_{57}, $CHSS_{81}$, MTS_{98} and MTS_{99}). Furthermore, 10 of these 23 accessions with medium prominence of longitudinal ridges were placed as a sub-group (SHS_{17}, CWS_{28}, BWS_{33}, BWS_{35}, BWS_{38}, RYS_{39}, RYS_{40}, FDS_{50}, MTS_{98} and MTS_{99}). Thirteen accessions with acute leaf shape on tips (BWS_{35}, BWS_{38}, RYS_{39}, RYS_{40}, FDS_{44}, KSS_{54}, SWS_{63}, $CHSS_{77}$, $CHSS_{90}$, MTS_{98}, MTS_{99}, VHS_{106} and VHS_{123}) together with accession (CWS_{28}) with obtuse leaf shape on tips were placed close together. Five accessions of *P. cattleianum* (SHS_{14}, SHS_{15}, $CHSS_{82}$, VSH_{117} and VSH_{118}) were placed in the lower left plane. These results indicate that the qualitative traits of longitudinal ridges, prominence of longitudinal ridges and leaf shape on tips were highly correlated, and led to the highest factor loadings in this analysis.

3.5. *Dendrogram using agglomerative hierarchical clustering (AHC)*

Euclidean distance was used to analyze the genetic dissimilarity of the 132 accessions based on the combined quantitative and qualitative data, and Ward's method was used for the agglomeration. The dendogram (Fig. 4, Table 8) revealed three distinct groups. The first two groups, C1 and C2, were separated with a dissimilarity result of 249. C1 contained 76 accessions, and C2 had 47 accessions. The third group included 9 samples and was separated with a dissimilarity result of 242. Among all 132 cultivars from different regions in Pakistan, there were no specific clusters based on locality. The highest genetic distance exists between C1 and C3 (84.25), followed by C1 and C2 (76.02), and C2 and C3 (46.65).

Table 7
First 8 components from the PCA analysis of 18 qualitative traits for 132 Pakistani guava accessions.

Trait	F1	F2	F3	F4	F5	F6	F7	F8
Attitude of branches	0.088	0.345	−0.200	−0.074	−0.198	−0.406	−0.175	−0.002
Young shoot color	−0.097	−0.251	0.004	0.029	−0.313	−0.079	0.663	−0.011
Young leaf anthocyanin	0.114	−0.245	0.146	−0.329	−0.286	−0.261	0.036	−0.444
Leaf shape	−0.044	0.373	−0.129	−0.220	−0.162	−0.135	−0.263	−0.036
Leaf twisting	0.145	−0.037	−0.096	0.464	−0.036	−0.212	0.063	−0.383
Leaf variegation	0.183	−0.145	0.095	−0.398	0.343	−0.213	0.019	−0.392
Color of midrib on leaf	−0.022	−0.029	−0.010	0.398	−0.101	0.243	−0.344	−0.473
Leaf shape on base	0.164	0.387	−0.203	0.174	0.045	0.129	0.365	−0.247
Leaf shape on tips	−0.224	0.138	−0.306	−0.126	0.412	0.130	0.240	−0.041
Fruit shape at stalk	0.250	−0.332	−0.013	0.236	0.257	−0.240	−0.167	0.343
Width of neck of fruit	−0.357	0.004	−0.213	−0.025	−0.392	0.220	−0.098	−0.005
Fruit color of skin	−0.331	−0.181	0.161	−0.235	−0.090	0.113	−0.216	−0.031
Longitudinal ridges	−0.467	−0.166	0.146	0.181	0.133	−0.114	−0.059	−0.173
Prominence longitudinal ridges	0.488	−0.036	−0.022	−0.059	−0.304	0.170	−0.103	0.101
Longitudinal grooves	0.251	−0.197	0.151	0.009	−0.044	0.498	−0.004	−0.031
Color of flesh	0.037	0.321	0.471	0.020	0.269	0.140	−0.067	−0.152
Evenness of color of flesh	0.020	0.309	0.515	−0.088	−0.135	0.104	0.202	0.075
Juiciness	−0.122	0.130	0.407	0.321	−0.151	−0.346	0.043	0.163
Variability (%)	13.806	9.608	8.934	7.938	6.821	6.640	6.062	5.737

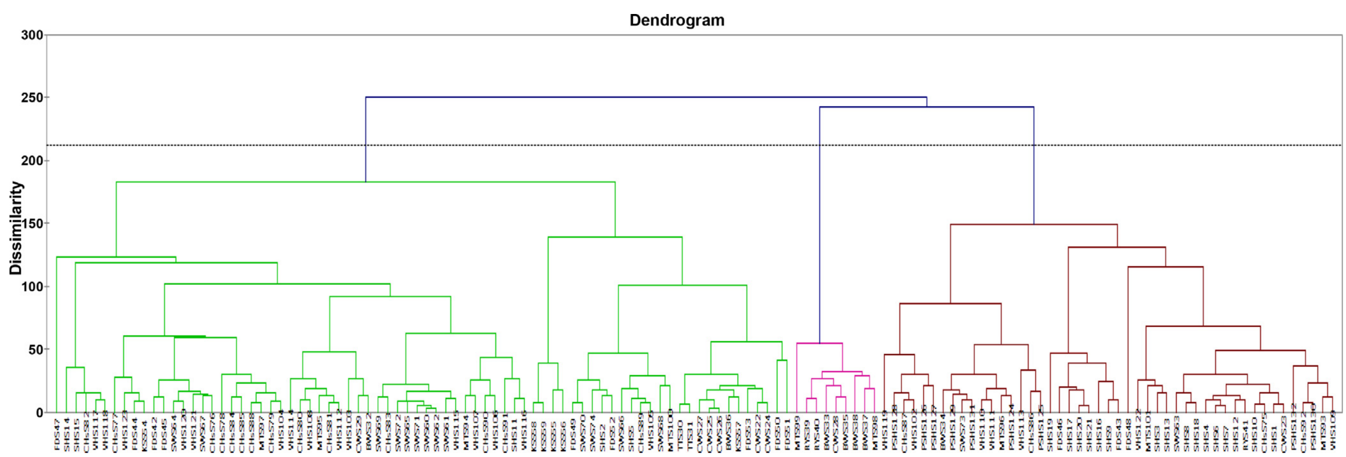

Fig. 4. Dendrogram for 132 Pakistani guava accessions based on 15 quantitative and 18 qualitative traits.

Six accessions of *P. cattleianum* were all clustered in group C1 (SHS$_{14}$, SHS$_{15}$, CWS$_{25}$, CHSS$_{82}$, VSH$_{117}$ and VSH$_{118}$). Five of these accessions except for accession CWS 25 clustered together as a small sub-group (Fig. 4, Table 8). The third group (C3) comprised 9 accessions, a common trait of which is medium prominence of longitudinal ridges (MTS$_{99}$, RYS$_{39}$, RYS$_{40}$, BWS$_{33}$, CWS$_{28}$, BWS$_{35}$, BWS$_{38}$, BWS$_{37}$ and MTS$_{98}$).

4. Discussion

4.1. Morphological characterization

Pakistani guava germplasm represented by 132 accessions was investigated by measuring 15 quantitative and 18 qualitative traits to determine if morphological traits could be useful for genotypic identification. According to Rodríguez et al. (2004, 2007, 2008) and Andrés-Agustín et al. (2006) such traits have proved useful for identifying genotypes in populations of guava and other fruits. Most of the traits studied had potential economic interest especially those related to fruit yield, fruit quality and pest resistance. They could thus serve as target traits for guava breeders and guava growers. The results from the current research support the view that morpho-pomological traits and chemical contents in fruits can be used efficiently for cultivar discrimination as well as being reliable in estimating the genetic relationships across large and diverse groups of guava genotypes. These findings are in accord with other studies indicating that both quantitative and qualitative traits are very helpful in the identification and evaluation of cultivars in guava germplasm (see Hernandez-Delgado et al., 2007; Padilla-Ramirez and Gonzalez-Gaona, 2010; Corrêa and Santos, 2012).

Table 8
Dendrogram grouping of 132 Pakistani guava accessions.

Cluster	Genotypes
C1	SHS$_{1}$,SHS$_{3}$,SHS$_{4}$,SHS$_{6}$,SHS$_{7}$,SHS$_{8}$,SHS$_{9}$,SHS$_{10}$,SHS$_{12}$,SHS$_{13}$,SHS$_{16}$,SHS$_{17}$,SHS$_{18}$,SHS$_{19}$,SHS$_{20}$,SHS$_{21}$,CWS$_{23}$,BWS$_{34}$,RYS$_{41}$,FDS$_{4}$,FDS$_{46}$,FDS$_{48}$, SWS$_{63}$,SWS$_{73}$, CHSS$_{75}$,CHSS$_{86}$,CHSS$_{87}$,CHSS$_{92}$,MTS$_{93}$,MTS$_{96}$,MTS$_{101}$,VHS$_{102}$,VHS$_{109}$,VHS$_{110}$, VHS$_{111}$,VHS$_{113}$,VHS$_{119}$,VHS$_{122}$,PSHS$_{124}$,PSHS$_{125}$,PSHS$_{126}$,PSHS$_{127}$ PSHS$_{128}$, PSHS$_{129}$, PSHS$_{130}$,PSHS$_{131}$,PSHS$_{132}$
C2	SHS$_{2}$,SHS$_{5}$,SHS$_{11}$,SHS$_{14}$,SHS$_{15}$,CWS$_{22}$,CWS$_{24}$,CWS$_{25}$,CWS$_{26}$,CWS$_{27}$,CWS$_{29}$,TTS$_{30}$,TTS$_{31}$,BWS$_{32}$,BWS$_{36}$,FDS$_{42}$,FDS$_{44}$,FDS$_{45}$,FDS$_{47}$,FDS$_{49}$,FDS$_{50}$,FDS$_{51}$,FDS$_{52}$, FDS$_{53}$,KSS$_{54}$,KSS$_{55}$,KSS$_{56}$,KSS$_{57}$,KSS$_{58}$,KSS$_{59}$,SWS$_{60}$,SWS$_{61}$,SWS$_{62}$,SWS$_{64}$,SWS$_{65}$,SWS$_{66}$,SWS$_{67}$,SWS$_{68}$,SWS$_{69}$,SWS$_{70}$,SWS$_{71}$,SWS$_{72}$,SWS$_{74}$,CHSS$_{76}$, CHSS$_{77}$,CHSS$_{78}$,CHSS$_{79}$,CHSS$_{80}$,CHSS$_{81}$,CHSS$_{82}$,CHSS$_{83}$,CHSS$_{84}$,CHSS$_{85}$,CHSS$_{88}$,CHSS$_{89}$,CHSS$_{90}$,CHSS$_{91}$,MTS$_{94}$,MTS$_{95}$,MTS$_{97}$,MTS$_{100}$,VHS$_{103}$,VHS$_{104}$, VHS$_{105}$,VHS$_{106}$,VHS$_{107}$,VHS$_{108}$,VHS$_{112}$,VHS$_{114}$,VHS$_{115}$,VHS$_{116}$,VHS$_{117}$,VHS$_{118}$,VHS$_{120}$,VHS$_{121}$,VHS$_{123}$
C3	CWS$_{28}$,BWS$_{33}$,BWS$_{35}$,BWS$_{37}$,BWS$_{38}$,RYS$_{39}$,RYS$_{40}$,MTS$_{98}$,MTS$_{99}$

This study revealed an extensive morphological diversity within Pakistani guava germplasm. Many of the traits analyzed are of particular economic interest. These conclusions come from the significant differences amongst the quantitative traits and the presence of two or more phenotypic classes per trait for qualitative traits. Whilst other researchers have reported phenotypic variability in various guava populations around the world [e.g. Cuba (Valdés-Infante et al., 2003), Mexico (Hernandez-Delgado et al., 2007; Padilla-Ramirez and Gonzalez-Gaona, 2010), Brazil (Santos et al., 2011) and Venezuela (Aranguren et al., 2010)], their studies were not extensive. By analyzing both quantitative and qualitative traits, our study was sufficiently powerful to discriminate genetically between many individual accessions. This opens up a whole area of potential genetic research on guava including selective breeding of particular traits for commercial purposes.

The extensive range of morphological variation observed in this study revealed many traits related to fruit yield and quality which could operate as breeding targets. For strategic breeding, it is important to note the limits to morphological variation. For example, among the 33 traits we studied, fruit weight is one of the most important components of yield. In this study, fruit weight values ranged from 34.6 to 390 g. Accessions FDS_{48}, VHS_{122} and MTS_{101} had the heaviest fruits at 390, 187.43 and 182.45 g, respectively, whereas in Mexico by comparison, fruit mean weights range from 5.7 to 120 g with a mean value of 54.1 g (Hernandez-Delgado et al., 2007). Considerable variability was also recorded in Pakistan guava germplasm for other fruit yield- and quality-related traits including diameter of cavity, seed weight, thickness of outer flesh, longitudinal ridges, prominence of longitudinal ridges, neck width and skin color. Skin color, seed weight and thickness of outer flesh are consumer preference traits (Mondragon-Jacobo et al., 2010; Padilla-Ramirez et al., 2012). Our results also revealed significant variability in fruit dimensions (diameter and length) and these are important in packing and transportation decisions (Padilla-Ramirez et al., 2012).

The intra-accession variability of both quantitative and qualitative traits studied indicated that these traits are suitable for cultivar identification. In Pakistan, guava growers normally name cultivars according to fruit shape and locality, and the most common names are Gola (round fruit) and Surahi (pear shape fruit). Gola and Surahi even from different localities are commonly grouped together indicating their respective phenotypic similarities. All this indicates that the local naming practices do not reflect the genetic relatedness. Thus, in this study the revealed phenotypic identity for individual accession among these Pakistani guava germplasm is more accurate than that of the common names from the farmers.

4.2. Morphological correlations

Correlations among the traits studied revealed interesting relationships. For instance, there were more negative correlations among chemical contents in fruit and fruit size-related traits, but more positive correlations among chemical contents and leaf-related traits. This could be due to large fruit size being often accompanied by poor accumulation of chemical contents leading to an inferior fruit quality. For example, accession FDS_{48} had the largest fruit size but the poorest quality, being dry, with high seed weight and low sweetness. On the other hand, strong leaf traits might enhance the chemical contents in fruits giving superior quality. On the basis of the strong positive correlations among the fruit yield and quality traits including fruit weight, fruit width, fruit length, diameter of cavity on fruit, longitudinal ridges and prominence of longitudinal ridges, it could be concluded that these characters have positive effects on determination of cultivar cropping potential. These results generally agree with those of Aulakh (2005) and Babu et al. (2007).

PCA showed that some traits had the highest loadings in the first two components. These traits included fruit weight, fruit diameter, fruit length, fruit cavity diameter, seed weight, outer flesh thickness, longitudinal ridges, prominence of longitudinal ridges, fruit neck width, leaf blade length, fruit skin color, total sugar and non-reducing sugar contents. These results indicate that such traits are not only useful for the assessment of diversity, but also for characterization of guava germplasm.

4.3. Implications for gene bank conservation

The International Guava Symposium regards *Psidium* as an important fruit, yet a number of challenges exist for it as a crop. There are several pests and diseases to which guava is susceptible. *Meloidogyne mayaguensis*, *Fusarium solani* and *Fusarium oxysporium* f. *sp*. Psidii (cause of guava wilt) are reported as major pests/pathogens causing significant losses in guava diversity (Dwivedi et al., 1990; Schoeman et al., 1997; Gomes et al., 2011). In addition, agricultural practices and farmer selection have narrowed the genetic base of cultivated guava, therefore, the need to search for new sources of variation and the estimation of genetic diversity among collections have specific implications for guava improvement or germplasm management. Further evaluation and characterization of all the collections through collaborative relationships will provide a common platform for information on global *Psidium* germplasm.

Genetic diversity provides a certain degree of strength against destruction of plant populations by natural disasters. Extremely low levels of genetic diversity may lead to complete elimination of some species and result in a loss of overall biological diversity (Subudhi et al., 2007). In this study, the average coefficient of variation was 34.25% which indicates the strength and potential of the collected germplasm. Results from this research also showed that *P. guajava* is phenotypically very close to *P. cattleianum*, so further research particularly on molecular markers is needed for clarification. The main goal of germplasm management is to collect and to characterize diverse forms at national and regional levels. The first step starts with the evaluation of morphological and agronomic traits of interest. Plant breeders then routinely use morphological characterization for the initial description and classification of the germplasm under consideration (Pommer, 2012).

4.4. Implications for breeding

In this study, there were significant correlations between traits contributing to fruit yield and quality which is helpful for plant improvement. For example, to achieve high yield and superior quality varieties, cross combinations could be performed between accessions with large fruit size (FDS_{48}, VHS_{122}, MTS_{101}, VHS_{119}), low seed weight (BWS_{32}, BWS_{37}, CWS_{29}, FDS_{47}, RYS_{40}, TTS_{30}, FDS_{42}, $PSHS_{132}$), juiciness (SHS_{16}, KSS_{55}, KSS_{56}, SWS_{74}, $CHSS_{91}$, VHS_{119}, $PSHS_{127}$) and high total soluble solids (Brix) (FDS_{47}, FDS_{53}, $PSHS_{130}$, CWS_{27} and TTS_{31}).

Except for large fruit size, low seed weight, juiciness and sweetness, breeding goals should also target other traits including fruit skin color, flesh color and outer flesh thickness, which are critical for consumer preferences (Corrêa and Santos, 2012). A full range of colors could be developed since fruit skin color varies from pale yellow–green to pale yellow and even red (SHS_{14}, SHS_{15}, $CHSS_{82}$, VSH_{117}, VSH_{118}) and orange (FDS_{52}). Furthermore, whilst flesh color ranged from white to cream and pale pink to pink, there was one dark pink cultivar (BWS_{38}) which could be used to breed for dark pink flesh which is preferred by the processing industry (Mondragon-Jacobo et al., 2010).

In this study there was no definite correlation between geographic origin and genetic diversity of Pakistani guava, indicating that parental selection should be made on the basis of a systematic assessment of genetic distance in any specific population rather than geography. According to Dias et al. (2003), divergence between any two parents is caused by the allelic differences between them. Genotypes in the same cluster diverge less from one another and as expected, hybrids between them result in fewer desirable segregates. Thus crosses should be conducted with parents from different clusters. Crosses with maximum parental divergence are likely to produce higher heterosis and desirable genetic recombination (Roy et al., 2013). Consequently, it would be in the interest of Pakistani breeders to introduce elite international guava varieties.

It needs to be kept in mind that the composition of genotypes used for breeding can be greatly affected by environment, the lower the environmental effects, the lower, the effect on plant genotype composition (Perfectti and Camacho, 1999). In this Pakistani project, well-defined guava cultivars adapted to local environments were selected, and in their natural environment, these cultivars were genetically diverse and contributed significantly to variation of the morphological traits studied. Environmental effects can be minimized by studying the interaction between genotype and environment in targeted cultivars so as to select those with higher stability. Genetic analysis using a molecular marker system could indicate the extent of genetic variation as suggested by Pommer and Murkami (2009), Valdes-Infante et al. (2010) and Asim et al. (2013). We suggest that for future research, some reliable genetic marker systems should be used to analyze the germplasm studied in this project.

5. Conclusions

The vast and distinctive range in the morphological traits studied across the 132 accessions in this project meant that each accession could be distinguished individually. The elite selections in particular would be useful for association genetic studies and breeding of new cultivars. These would be the important first steps to research enabling utilization of the genetic resources within the Pakistani guava germplasm. The detected broad phenotypic diversity existing within this gene pool could be utilized in breeding programs which particularly target the genetic potential underpinning fruit yield and fruit quality as well as those which could alleviate biotic and abiotic stress factors. The extensive genetic potential detected in this Pakistani guava germplasm contained many traits indicative of significant economic importance. Targeted selection for these traits by guava growers and breeders in Pakistan would not be difficult. Such work could even extend the guava genebank at the international level so that a large representative collection covering many guava producing countries could be established thus enabling further genetic and breeding programs. To fully exploit the genetic potential increased clonal propagation is required.

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