

MORPHOLOGICAL AND PHYSICO-CHEMICAL DIVERSITY IN SOME INDIGENOUS MANGO (*Mangifera indica* L.) GERMPLASM OF PAKISTAN

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Mango is the second major fruit crop of Pakistan. A large number of unexplored indigenous mango germplasm with great economic significance is present in the country. Hence, 425 mango accessions were studied from Azad Jammu & Kashmir (AJK) as well as Northern and Southern Punjab to explore the genetic diversity. Total 33 traits (25 qualitative and 8 quantitative) enabled the assessment of morphological and physico-chemical diversity of the studied indigenous mango germplasm. The first three principal components (PCs) contributed 68.06% variability among all mango accessions. The PCs also successfully grouped mango accessions according to their morphological and physico-chemical characteristics. Trunk height, tree circumference, crown diameter, leaf colour, leaf blade length, petiole length, inflorescence length, inflorescence width, fruit shape, fruit weight, soluble solid contents, titratable acidity, sugar:acid ratio, reducing sugars, non-reducing sugars and total sugars were found highly variable. Many of these characters are of substantial economic significance and could be used as breeding goals to increase the germplasm repository as well as fruit yield and quality. In conclusion, morphological and physico-chemical traits were highly useful for mango germplasm characterization. Several accessions also showed potentially good traits which could be used to develop new mango cultivars through future breeding schemes.

Keywords: Plant description, germplasm conservation, gene pool, geographical distribution, morphological traits

INTRODUCTION

Mango (*Mangifera indica* L.) the choicest and abundantly consumed tropical and subtropical fruit, belongs to genus *Mangifera* and family Anacardiaceae having more than 40 species around the world (Subedi *et al.*, 2009; Rajwana *et al.*, 2011). Mango has been originated in the premises of Indian-Myanmar region and distributed to various phytogeographical areas of the world (Jha *et al.*, 2010). Its cultivation in Pakistan dates back to the era of Mughal emperor 'Akbar' (Rajwana *et al.*, 2011). Mango in Pakistan is cultivated between latitude 25-32° North East, including the districts of Sindh (Hyderabad, Mirpurkhas, Tando Allah Yar) and Punjab (Multan, Rahim Yar Khan, Bahawalpur, Lodhran, Muzaffargarh, Khanewal, Vehri) province (Raza, 2011). Punjab province contributes about 67% to the total mango area having about 65% share in total fruit production in the country (MinFAL, 2010). Azad Jammu & Kashmir (AJK) and Northern Punjab (Gujrat, Sialkot) also have rich mango genetic diversity mostly in the form of wild seedling plantation which remained unexplored for decades.

Despite the growing importance of Pakistani mango in the international market, its true potential has not yet been fully utilized and its production struggles with several constraints

such as small number (10) of commercial varieties with low yield, narrow genetic base, disease and insect pest attack (Rajwana *et al.*, 2011). Presently, huge genetic diversity of mango is still waiting to be explored in Pakistan. Therefore, unexplored germplasm can successfully be utilized in the breeding programs to develop new high yielding and premium quality cultivars (Mian and Nasir, 1989; Ahmad *et al.*, 2007). Presently, development of new mango varieties with premium quality and high yield is the extreme demand of mango industry. However, its development through orthodox/conventional breeding is tedious, resource dependent and time consuming. On the other hand, selection of chance seedlings from existing indigenous germplasm is most suitable alternative (Begum *et al.*, 2012).

Mango trees showed extensive diversity due to out-breeding, allopolyploidy, continuous grafting and phenotypic differences arising under varied agro-climatic conditions in different mango growing regions (Ravishankar *et al.*, 2000). In addition, mango being highly cross pollinated, open pollination between the cultivars has resulted in new genetic combinations that have not yet been documented. Geneticists and plant breeders are mainly concerned with diversity of germplasm at the molecular level; whereas, horticulturist/pomologist characterize and evaluate the

accessions/varieties on the basis of fruit physico-chemical characteristics (Rajwana *et al.*, 2011; Jamil *et al.*, 2015; Naqvi *et al.*, 2015). Previously, genetic diversity has been characterized through morphological traits (Gonzalez *et al.*, 2002; Khan *et al.*, 2015; Azmat *et al.*, 2016) in various fruit crops such as bananas (Gibert *et al.*, 2009), figs (Aljane *et al.*, 2012), papaya (Ocampo *et al.*, 2006), plum (Aazami and Jalili, 2011), mandarins (Domingues, 1999) and cashew nut (Chipojola *et al.*, 2009). However, tree and leaf morphological characterization of mango is lacking or still in the initial phase in Pakistan (Rajwana *et al.*, 2011). On the other hand, Pakistani mango diversity is threatened by natural habitat loss due to domestication and it may become extinct if not explored, identified and conserved properly. Therefore, the objectives of the current work were (a) identification of key morphological and physico-chemical markers to characterize unexplored indigenous mango germplasm of the country and (b) identification of suitable genotypes with promising morphological and physico-chemical traits which could be used for future breeding and conservation of mango germplasm repository.

MATERIALS AND METHODS

Eco-geographical survey: A comprehensive survey was conducted during 2011-2013 in the districts of Azad Jammu & Kashmir (AJK) (Mirpur, Kotli and Bhimber), Northern Punjab (NP) (Gujrat and Sialkot) and Southern Punjab (SP) (Rahim Yar Khan, Multan, Muzzaffargarh and Khanewal) with altitudes ranging from 76-1013 m above sea level. The sample collection sites include rainfed (AJK and NP) and irrigated (SP) areas of the country. During eco-geographical survey 425 indigenous mango germplasm accessions were tagged after comprehensive interview with owners of the each mango accession to know about their previous history. Names of the accessions with their respective codes MRP (Mirpur), KTL (Kotli), BMB (Bhimber), GRT (Gujrat), SKT (Sialkot), MLT (Multan), KHW (Khanewal), MZG (Muzzaffargarh) and RYK (Rahim Yar Khan) were given to individual tagged trees. Coordinates and elevation were noted with the help of GPS device (Garmin Corporation, GPSMAP® 76 CSx, Taipei, Taiwan).

Tree morphological characterization: Tagged trees were further evaluated for different morphological characteristics (tree, leaf and inflorescence) to assess the genetic variations. Data regarding eight quantitative and twenty five qualitative traits were recorded using International Plant Genetic Resources Institute (IPGRI, 2006) descriptors for mango. Twenty leaf and inflorescence samples of each accession were used for data collection.

Physico-chemical characterization: Twenty fruit from each selected accessions were collected during the sampling seasons 2011-2013. Fruit shape was evaluated by adapting IPGRI (2006) descriptors for mango. Fruit weight was

measured with a digital weighing balance (Model TK-500, Japan). Biochemical characters such as total soluble solids (TSS; °Brix) were determined with a digital refractometer (PAL-1, ATAGO Japan), titratable acidity (TA; %) was determined by titrating mango juice with 0.1N NaOH and sugars in the sampled juice were estimated by using the method as reported by Khan *et al.* (2012).

Data analysis: Means of all observations were calculated for quantitative traits and subjected to principal component analysis (PCA) to identify traits that best explained mango morphological and physico-chemical variability. The principal components with eigenvalues (>1.0) were selected as proposed by Jeffers (1967). Pearson correlations were performed to determine the association and order of importance among the morphological and physico-chemical traits. The data for qualitative characters were analyzed statistically in a factorial correspondence analysis (FCA). Cluster analysis was also performed to assess level of dissimilarity among accessions. An Unweighted Paired-Grouping Method with Arithmetic Average (UPGMA) algorithm was constructed with Euclidian distance. Statistical analysis was carried out using SPSS-20 software (SPSS, Chicago, IL, USA).

RESULTS

Morphological characteristics: Mango accessions exhibited significant variations among various quantitative and qualitative morphological traits. The most significantly explanatory traits were crown shape, leaf blade shape, leaf attitude in relation to branch, leaf venation, thickness of midrib, leaf apex shape, leaf base shape, young and mature leaf colour, inflorescence shape, type of flowers in inflorescence, inflorescence colour and anthocyanin intensity in inflorescence. Very high diversity was observed in crown shape and inflorescence colour of studied mango accessions in all three regions (Fig. 1). Quantitative morphological traits such as tree trunk circumference, crown diameter and tree height showed highest coefficient of variation (>50%) both at AJK and Southern Punjab; whereas, at Northern Punjab, petiole length exhibited highest coefficient of variation (>50%). Leaf blade length, width, inflorescence length and width in all three regions revealed lowest coefficient of variation (<35%) except petiole length which exhibited highest coefficient of variation (>50%) in Northern Punjab (Table 1).

Physico-chemical characteristics: The percent distribution of key fruit markers revealed extensive genetic diversity within AJK, Northern and Southern Punjab accessions (Fig. 2). It is worth to notice that the distinction between fruit shape remained subjective as objective measures are very complex. It differed among 425 studied accessions, as oblong fruit shape was most common in AJK (45%) and Northern

Mango germplasm characterization

Category	Characters	Description	AJK	Northern Punjab	Southern Punjab
Tree	Crown shape	Oblong	7.9	21.4	23.3
		Broadly pyramidal	7.9	3.5	37.6
		Semicircular	4.6	42.8	6.2
		Spherical	33.8	10.7	22.1
Leaf	Leafblade shape	Elliptic	26.6	14.3	12
		Oblong	25.9	53.6	15.1
		Ovate	2.2	0	3.8
		Obovate	0	0	0.8
		Lanceolate	44.6	32.1	68.1
		Ob lanceolate	0.7	0	0
		Semi-erect	28.8	35.7	20.9
	Leaf attitude in relation to branch	Horizontal	15.8	17.8	58.9
		Semi-drooping	55.4	46.4	20.2
		Narrow (<450)	2.2	17.9	4.3
	Leaf venation	Medium (450-600)	94.9	78.6	95.7
		Wide (> 600)	2.8	3.6	0
	Thickness of midrib	Thin	35.3	57.1	24.8
		Thick & tapering	64.7	42.9	75.2
	Leaf apex shape	Obtuse	5.7	0	2.7
		Acute	31.7	25	33.7
		Acuminate	62.6	75	63.6
	Leaf base shape	Acute	81.3	82.1	41.1
		Obtuse	18.7	17.7	58.5
		Round	0	0	0.4
	Leaf margin	Round	36.7	17.8	47.3
		Wavy	62.6	82.1	52.7
	Leaf fragrance	Absent	20.9	14.3	15.9
		Mild	48.9	53.6	48.8
		Strong	30.2	32.1	35.3
	Colour of young leaf	Light green	48.2	35.7	35.3
		Light green with brownish tinge	23.7	17.8	30.2
		Light brick red	28.1	42.8	20.9
		Reddish brown	0	3.6	9.7
		Deep coppery tan	0	0	3.9
	Colour of full developed leaf	Pale green	0.7	3.6	1.2
		Green	56.1	60.7	61.6
		Dark green	43.2	35.7	37.2
Inflorescence	Inflorescence shape	Conical	30.2	17.6	15.9
		Pyramidal	66.2	82.1	62.4
		Broadly pyramidal	3.5	0	18.6
	Density of flowers in inflorescence	Sparse	18.7	28.6	10.9
		Medium	63.3	67.8	34.5
		Dense	17.9	3.6	54.6
	Type of flowers	Pentamerous	75.5	78.6	62.4
		Tetramerous	0.7	14.3	0
		Pentamerous + hexamerous	18.7	0	29.1
		Pentamerous + hexamerous + hepta	4.3	7.1	4.3
		Others	0.7	0	3.9
	Inflorescence colour	Whitish	0	0	0
		Yellowish green	23	7.1	5.4
		Yellow	29.5	42.9	39.5
		Light green	12.2	14.3	0.8
		Green with red patches	5	7.1	0
		Light orange	3.6	3.6	13.6
		Pink	18.7	17.9	31
		Dark pink	0	0	0.8
	Number of stamens	Light red	2.9	0	0.8
		Red	5	7.1	7.8
		10-12 (5-6 fertile)	0	0	0
		5 (2-3 fertile)	6.5	0	4.3
		5 (1 fertile)	93.52	100	95.6
	Intensity of anthocyanin	Low	61.9	67.9	51.4
		Medium	28.8	32.2	34.9
		High	9.4	0	13.2

Figure 1. Percent distribution of qualitative morphological traits for tree, leaf and inflorescence among 425 mango accessions.

Table 1. Measure of central tendency and dispersion of different quantitative traits in 425 mango accessions.

Traits	AJK			North Punjab			South Punjab		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Trunk circumference	3.6 ± 0.38	7.9	90.8	3.1 ± 0.03	0.8	26.5	1.2 ± 0.04	1.0	82.8
Crown diameter	17.8 ± 0.26	5.5	28.6	19.2 ± 0.21	4.5	23.3	7.4 ± 0.19	4.0	57.5
Tree height	45.5 ± 0.72	14.9	31.0	52.8 ± 0.69	14.4	27.2	17.8 ± 0.63	13.0	73.1
Leaf blade length	18.0 ± 0.20	4.3	21.1	16.6 ± 0.16	3.4	20.4	20.7 ± 0.21	4.5	21.7
Leaf blade width	4.9 ± 0.08	1.7	20.1	4.7 ± 0.40	0.9	19.5	5.7 ± 0.07	1.5	27.3
Petiole length	3.3 ± 0.13	2.7	32.4	3.7 ± 0.09	1.9	51.3	3.4 ± 0.05	1.2	33.2
Inflorescence length	20.2 ± 0.26	5.5	25.2	19.8 ± 0.19	4.1	20.9	27.1 ± 0.27	5.7	21.3
Inflorescence width	12.4 ± 0.20	4.2	30.1	12.2 ± 0.11	2.3	19.1	15.2 ± 0.25	5.2	34.5

AJK: Azad Jammu & Kashmir, SD: standard deviation, CV: coefficient of variation, ±: standard error

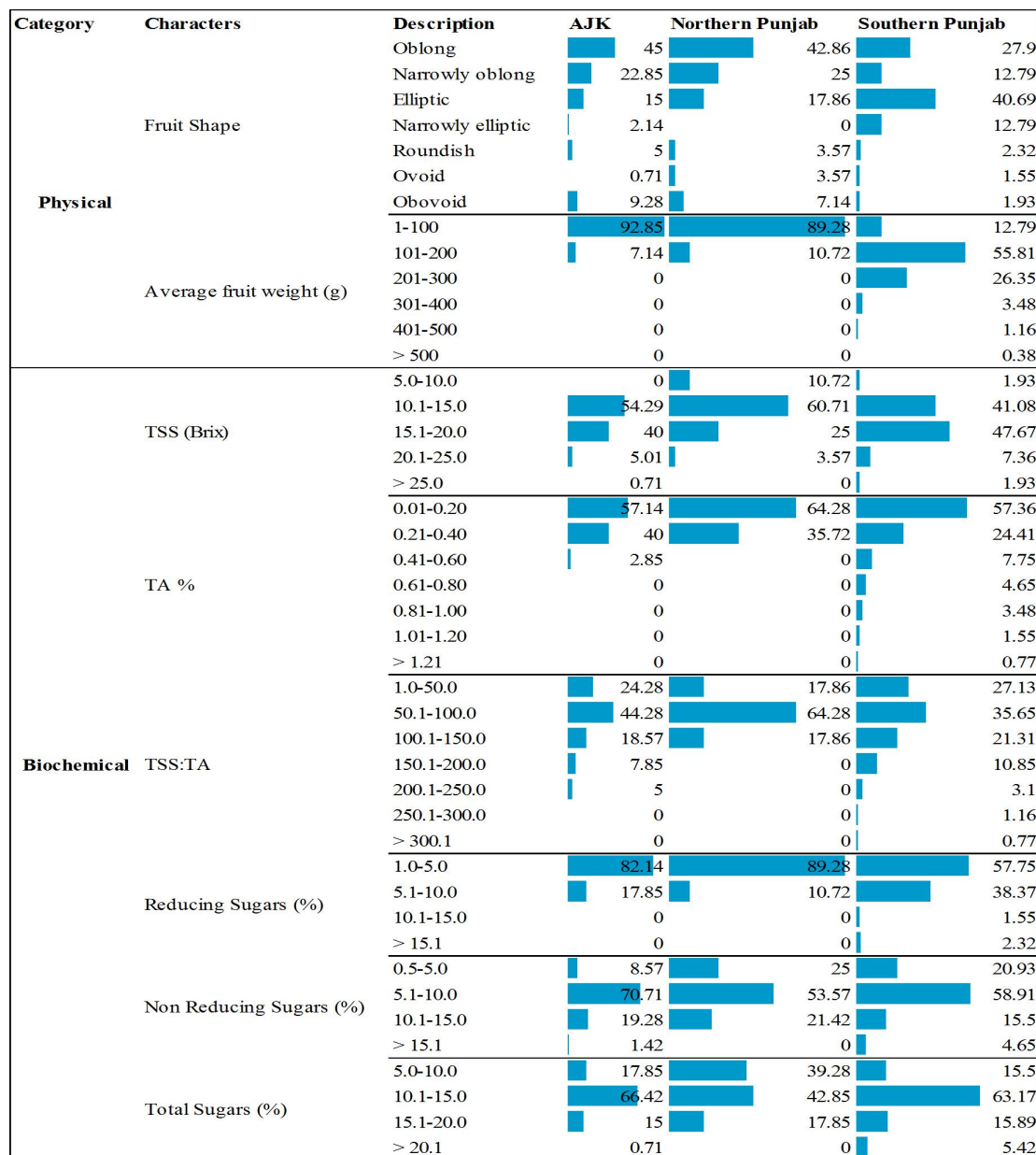
**Figure 2. Percent distribution of fruit physical and biochemical traits among 425 mango accessions.**

Table 2. Percentage of explained and cumulative variances and eigenvectors on the first three principal components for quantitative character in 425 mango accessions.

Variables	Eigenvectors		
	PC1	PC2	PC3
Crown diameter (m)	-0.335	0.265	0.439
Trunk circumference (m)	-0.449	0.186	0.349
Tree height (ft)	-0.401	0.225	0.226
Leaf blade length (cm)	0.354	0.532	0.004
Leaf blade width (cm)	0.334	0.521	0.011
Petiole length (cm)	0.138	0.440	0.003
Inflorescence length (cm)	0.420	-0.231	0.447
Inflorescence width (cm)	0.305	-0.208	0.659
Eigenvalues	2.71	1.52	1.21
Proportion (%)	33.92	19.04	15.11
Cumulative (%)	33.92	52.95	68.16

Punjab (42.86%); while, in Southern Punjab (40.69%) elliptic fruit shape was most frequent (Fig. 2). In case of fruit weight, average fruit weight varied among accessions and most of them present in a range of 1-100 g in AJK and Northern Punjab and Southern Punjab fruits fall in 100-200 g range. Furthermore, heaviest fruits were found in the mango accessions of Southern Punjab compared to other studied regions of the country (Fig. 2). Among bio-chemical characters; TA (0.01-0.20 range) and TSS: TA ratio (50.1-100.0 range) were common in majority of the studied fruits of all three regions; while, TSS varied among accessions and more than 45% studied accessions of Northern Punjab and AJK were in range of 10.1-15.0 °Brix and Southern Punjab 15.1-20.0 °Brix (Fig. 2). Among 425 studied accessions majority of the all three region fruits fall in same range of sugars (reducing, non-reducing and total sugars) percentage (Fig. 2).

Principle component analysis using morphological data:

The first three principal components (PCs) accounted 68.1% variability amongst all accessions under study (Table 2). The first PC accounted for 33.9% variability, dominated by tree characteristics including crown diameter, tree circumference and tree height. The PC₁ seems to be more related to tree characters as PC₁ had high values for these traits. However, PC₂ excelled in leaf blade length, leaf blade width and petiole length and it explained 19% of total variance with negative loadings of inflorescence width and inflorescence length. However, PC₃ was dominated with inflorescence width and inflorescence length. This component explained 15.1% of total observed variability in mango accessions and no negative loadings were noted in PC₃.

The PCA of quantitative morphological traits showed a clear grouping of mango accessions. The accessions were separated in two axis and showed variation among three regions (Fig. 3). Group-I [RYK-457 (numeric number = 250)] due to highest leaf blade width and Group-II [MLT-479 and MLT-467 (numeric numbers = 296 & 290)] were placed on the top right side of the quadrant having highest

leaf blade length and petiole length, respectively; while, Group-III [GRT-200 (numeric number = 144)] in upper central position with maximum crown diameter and Group-IV [BMB-107 (numeric number = 97)] was located in upper left side of the quadrant (Fig. 3) having maximum tree circumference and crown diameter.

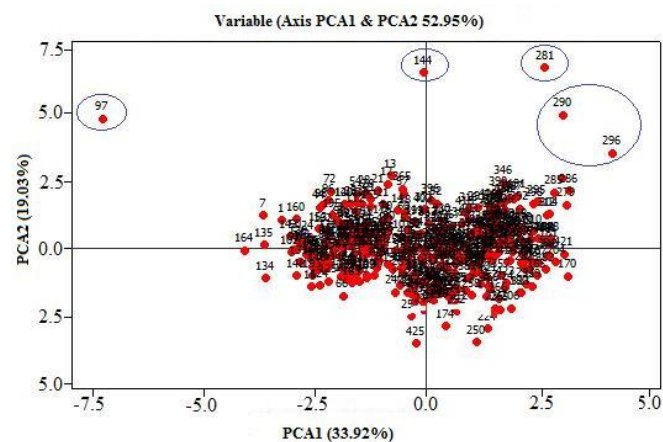


Figure 3. Projected variability score of indigenous mango accessions onto the biplot defined by the principal coordinates (1-2) of morphological traits.

Mango germplasm diversity based on cluster analysis using morphological data:

A pair wise association among indigenous mango germplasm was measured from the tree morphological characters, using Euclidean distance and revealed a clear clustering into two different cluster groups. Out of 425 studied mango accessions majority of the accessions (366) were clustered into group A and cluster group B (55) (Table 3). The main cluster A included 28 sub-clusters while, main cluster B contained 8 sub-clusters. It was not possible to present dendrogram of all the studied accessions due to large size of the dendrogram so, we only

Table 3. (a) Mean values with standard error of “cluster A” along with its sub-clusters (A1- A28) based on quantitative morphological data of 425 mango accessions.

Sub Clusters	TC	CD	TH	LBL	LBW	PL	IL	IW
A1 (26 Accessions)	1.38±0.03	8.75±0.15	7.52±0.09	21.89±0.11	6.13±0.04	3.38±0.05	29.41±0.16	19.18±0.14
A2 (7 Accessions)	0.55±0.02	4.78±0.11	10.24±0.14	22.83±0.08	6.23±0.06	3.91±0.04	33.40±0.11	17.73±0.24
A3 (8 Accessions)	0.80±0.02	4.99±0.11	14.00±0.14	21.49±0.18	5.40±0.05	3.03±0.03	27.52±0.18	23.92±0.16
A4 (9 Accessions)	1.96±0.05	8.61±0.15	9.64±0.13	20.90±0.20	5.48±0.07	3.56±0.06	37.00±0.10	24.83±0.07
A5 (15 Accessions)	0.79±0.02	5.31±0.12	19.73±0.22	23.10±0.15	6.09±0.06	3.65±0.05	24.44±0.25	12.85±0.24
A6 (14 Accessions)	2.23±0.06	12.50±0.10	10.66±0.16	21.90±0.15	6.75±0.05	4.04±0.06	23.13±0.15	11.84±0.10
A7 (7 Accessions)	1.87±0.04	10.94±0.12	10.29±0.11	20.30±0.17	5.20±0.05	3.37±0.03	17.25±0.07	9.78±0.13
A8 (12 Accessions)	1.22±0.02	6.92±0.08	7.69±0.11	21.13±0.15	5.88±0.04	3.65±0.04	22.45±0.12	8.51±0.05
A9 (10 Accessions)	0.75±0.01	4.59±0.06	7.58±0.13	21.40±0.12	6.22±0.05	3.16±0.02	23.17±0.13	11.56±0.14
A10 (14 Accessions)	1.15±0.04	4.40±0.11	13.57±0.09	20.63±0.10	5.64±0.04	3.77±0.05	26.97±0.08	14.84±0.12
A11 (12 Accessions)	0.87±0.05	3.58±0.05	10.75±0.08	16.29±0.10	4.63±0.03	3.06±0.05	23.55±0.08	11.78±0.06
A12 (14 Accessions)	0.95±0.02	6.16±0.07	7.30±0.07	18.06±0.10	5.32±0.06	3.00±0.05	27.99±0.12	13.81±0.08
A13 (12 Accessions)	1.12±0.04	6.18±0.01	17.25±0.01	15.66±0.01	4.40±0.00	2.71±0.01	25.83±0.02	11.30±0.01
A14 (9 Accessions)	2.04±0.04	11.53±0.10	12.33±0.14	15.53±0.14	4.31±0.02	2.62±0.04	29.48±0.13	14.54±0.12
A15 (6 Accessions)	1.26±0.04	7.59±0.23	6.83±0.09	24.50±0.07	6.68±0.05	4.05±0.04	13.89±0.10	7.36±0.05
A16 (9 Accessions)	0.97±0.04	12.67±0.41	11.09±0.18	18.86±0.45	4.83±0.07	3.84±0.07	29.12±0.39	15.47±0.22
A17 (12 Accessions)	1.55±0.09	7.34±0.16	41.83±0.21	23.58±0.19	6.15±0.07	3.55±0.05	31.21±0.20	16.44±0.18
A18 (9 Accessions)	0.78±0.01	6.45±0.11	24.56±0.17	15.54±0.11	4.42±0.04	2.91±0.05	29.38±0.20	18.99±0.21
A19 (24 Accessions)	0.95±0.04	6.48±0.23	29.83±0.19	24.55±0.21	6.61±0.07	4.28±0.06	27.70±0.24	16.40±0.21
A20 (9 Accessions)	2.69±0.10	14.18±0.45	32.00±0.30	20.82±0.29	7.26±0.20	3.94±0.06	30.66±0.55	22.09±0.32
A21 (13 Accessions)	2.41±0.09	13.60±0.31	35.46±0.22	18.13±0.21	5.62±0.18	2.83±0.03	24.69±0.20	14.34±0.12
A22 (14 Accessions)	2.48±0.04	15.09±0.19	33.64±0.19	18.93±0.15	5.21±0.04	3.18±0.06	27.18±0.18	16.44±0.08
A23 (10 Accessions)	3.18±0.04	22.51±0.13	36.90±0.18	19.09±0.20	5.73±0.05	2.65±0.04	22.89±0.16	11.21±0.14
A24(33 Accessions)	3.13±0.05	19.22±0.21	32.00±0.26	16.71±0.17	4.28±0.04	2.84±0.06	17.62±0.17	10.46±0.11
A25 (11 Accessions)	2.62±0.06	16.28±0.38	44.55±0.20	22.76±0.12	5.34±0.05	3.93±0.05	16.75±0.11	10.09±0.14
A26 (11 Accessions)	2.75±0.04	20.99±0.11	47.09±0.12	21.18±0.16	4.96±0.03	3.45±0.03	21.61±0.14	11.72±0.15
A27 (11 Accessions)	2.57±0.02	17.43±0.14	42.27±0.11	16.56±0.13	4.28±0.04	2.58±0.04	21.05±0.08	13.97±0.07
A28 (20 Accessions)	2.71±0.03	16.17±0.15	49.65±0.10	15.34±0.11	4.39±0.03	3.03±0.05	19.21±0.13	11.60±0.11
Average (Cluster A)	1.70±0.05	10.50±0.08	22.32±0.08	19.91±0.09	5.48±0.05	3.35±0.05	25.16±0.15	14.55±0.17

(b) Mean values with standard error of “cluster B” along with its sub- clusters (B1- B8) based on quantitative morphological data of 425 mango accessions.

Sub Clusters	TC	CD	TH	LBL	LBW	PL	IL	IW
B1 (3 Accessions)	3.65±0.05	17.62±0.11	88.00±0.08	15.33±0.08	4.50±0.03	2.83±0.01	14.20±0.20	9.90±0.05
B2 (6 Accessions)	0.38±0.01	2.41±0.10	64.50±0.36	24.50±0.29	7.20±0.11	4.25±0.05	28.07±0.22	12.99±0.14
B3 (5 Accessions)	2.50±0.04	17.26±0.19	56.60±0.31	19.54±0.06	5.20±0.02	2.94±0.04	31.58±0.10	20.74±0.12
B4 (6 Accessions)	3.86±0.05	23.35±0.28	59.83±0.22	17.30±0.15	4.52±0.05	3.23±0.04	16.28±0.12	10.93±0.18
B5 (8 Accessions)	2.11±0.03	10.89±0.19	58.13±0.12	17.75±0.15	4.69±0.04	3.54±0.04	16.61±0.15	10.50±0.09
B6 (7 Accessions)	2.54±0.03	16.23±0.19	71.86±0.12	15.49±0.15	4.59±0.04	2.44±0.04	17.60±0.15	11.17±0.09
B7 (12 Accessions)	3.09±0.04	19.35±0.16	66.67±0.18	20.68±0.25	5.23±0.06	2.86±0.04	23.03±0.12	13.81±0.11
B8 (8 Accessions)	2.55±0.03	17.53±0.07	63.13±0.12	16.53±0.08	4.30±0.03	2.94±0.03	19.14±0.13	10.41±0.06
Average (Cluster B)	2.58±0.03	15.58±0.12	66.09±0.14	18.39±0.11	5.02±0.05	3.12±0.03	20.81±0.10	12.55±0.09

TC: trunk circumference, CD: crown diameter, TH: tree height, LBL: leaf blade length, LBW: leaf blade width, PL: petiole length, IL: inflorescence length, IW: inflorescence width, ±: standard error

presented the mean values of morphological quantitative traits (Table 3). The result of cluster analysis shows that, high variation was observed in the level of similarity within the cluster group. Cluster A was characterized by more tree circumference, leaf blade length, leaf blade width, inflorescence length and width, whereas, main cluster B was predominated by maximum crown diameter, tree height and petiole length.

Majority of the accessions in main cluster A were from Southern Punjab except sub-clusters 23, 24, 25, 26, 27 and 28 of main cluster A in which maximum number of accessions were from AJK; while, in main cluster B majority of the mango accessions were from AJK and Northern

Punjab origin. While, one accession in cluster A and three accessions from cluster B were in outer layer (Table 4).

Correlation analysis of morphological and physico-chemical characteristics: Significant variations were observed between morphological and physico-chemical traits in Pearson correlation matrix. Significant positive correlation was found in crown diameter and tree height; whereas, inflorescence length and fruit weight exhibited significant negative correlation with trunk circumference. Inflorescence length showed significant positive correlation with inflorescence width and fruit weight. Moreover, TSS showed significant positive correlation with TSS/TA ratio and total sugars as well as non-reducing sugars (Table 5).

Table 4. Dendrogram grouping of 425 indigenous Pakistani mango accessions.

Main cluster	Sub-cluster	Accessions
A	A1	MZG-563, MZG-564, MLT-514, RYK-594, MLT-358, RYK-426, RYK-440, MZG-536, MZG-566, RYK-576, RYK-577, RYK-581, RYK-593, RYK-598, RYK-599, RYK-602, MZG-548, MZG-558, MZG-565, MZG-568, RYK-612, RYK-619, MLT-637, MLT-638 and MLT-642.
	A2	MLT-248, KHW-250, KHW-253, MLT-349, MLT-526, RYK-592 and MLT-634.
	A3	MLT-233, MLT-234, MLT-355, RYK-425, KHW-488, MLT-516, RYK-623 and MLT-639.
	A4	RYK-591, RYK-608, MLT-384, RYK-423, RYK-427, RYK-587, RYK-590, RYK-595 and RYK-615.
	A5	MLT-232, MLT-236, MLT-239, MLT-240, MLT-244, MZG-255, MZG-259, MLT-353, RYK-410, KHW-493, KHW-494, KHW-495, KHW-502, KHW-331 and RYK-439.
	A6	KTL-19, KHW-335, RYK-573, RYK-574, RYK-580, RYK-597, RYK-600, RYK-606, RYK-607, RYK-609, RYK-610, RYK-616, MLT-635 and MLT-636.
	A7	KTL-21, RYK-409, RYK-416, RYK-569, RYK-578, RYK-603 and RYK-613.
	A8	KHW-507, KHW-509, MZG-534, MZG-539, MZG-542, MZG-545, MZG-567, RYK-571, RYK-579, RYK-584, RYK-596 and RYK-601.
	A9	MLT-364, MLT-405, KHW-508, MLT-525, MZG-527, MZG-538, MZG-547, MZG-554, MLT-633 and MLT-640.
	A10	MLT-238, MLT-242, MLT-246, KHW-332, MLT-403, MLT-407, KHW-490, KHW-499, RYK-583, RYK-585, RYK-586, RYK-611, RYK-617 and RYK-618.
	A11	MLT-243, MLT-344, MLT-361, MLT-369, MLT-370, MLT-373, MLT-377, MLT-378, MLT-380, MLT-381, MLT-400 and MLT-401.
	A12	MLT-371, MLT-372, MLT-374, MLT-383, KHW-503, KHW-506, MLT-515, MZG-535, MZG-541, MZG-549, MZG-556, RYK-582, RYK-589 and RYK-604.
	A13	MLT-343, MLT-393, RYK-418, RYK-435, MLT-375, MLT-376, MLT-382, MLT-385, RYK-412, RYK-422, RYK-429 and RYK-442.
	A14	RYK-421, RYK-424, RYK-437, RYK-438, RYK-588, RYK-605, RYK-621, RYK-421, RYK-424, RYK-437, RYK-438, RYK-588, RYK-605 and RYK-621.
	A15	KHW-504, KHW-511, MZG-543, MZG-544, MZG-555 and RYK-572.
	A16	KTL-20, MLT-241, RYK-433, MLT-463, KHW-513, RYK-570, RYK-575, KHW-643 and RYK-644.
	A17	RYK-445, RYK-448, RYK-452, MLT-461, MLT-462, KHW-487, KHW-498, MLT-524, BMB-39, RYK-456, RYK-419 and MLT-522.
	A18	MZG-260, MLT-395, MLT-396, MLT-399, RYK-408, RYK-420, RYK-430, RYK-453 and RYK-446.
	A19	RYK-265, MLT-245, KHW-251, KHW-252, MZG-256, RYK-264, KHW-333, KHW-337, RYK-454, MLT-464, MLT-472, MLT-478, MLT-479, KHW-483, KHW-486, KHW-489, KHW-492, KHW-496, MLT-518, MLT-520, MLT-521, MLT-523, KHW-481 and MLT-473.
	A20	BMB-65, BMB-80, MLT-231, RYK-265, RYK-431, RYK-458, KHW-480, MLT-519 and RYK-457.
	A21	BMB-38, BMB-67, BMB-73, BMB-119, BMB-133, BMB-138, MZG-262, RYK-413, RYK-444, RYK-447, RYK-450, RYK-451 and RYK-457.
	A22	BMB-72, BMB-84, BMB-86, BMB-120, GRT-196, KHW-336, RYK-428, RYK-432, RYK-449, BMB-55, BMB-91, BMB-212, RYK-443 and MLT-517.
	A23	KTL-26, KTL-34, BMB-54, BMB-56, BMB-60, BMB-75, BMB-81, GRT-96, GRT-105 and GRT-195.
	A24	KTL-30, BMB-36, BMB-70, BMB-87, MRP-271, RYK-434, BMB-127, BMB-177, GRT-192, KTL-18, KTL-33, BMB-43, BMB-69, BMB-85, KTL-18, KTL-33, BMB-43, BMB-69, BMB-78, BMB-85, BMB-95, BMB-131, BMB-132, MZG-261, KTL-27, KTL-29, BMB-40, BMB-124, BMB-150, BMB-151, GRT-184, GRT-193 and MRP-273.
	A25	MRP-16, KTL-23, KTL-25, KTL-28, BMB-46, BMB-63, BMB-74, BMB-135, BMB-139, BMB-222 and KHW-497.
	A26	MRP-07, KTL-32, KTL-35, BMB-41, BMB-37, BMB-61, BMB-88, BMB-128, BMB-134, BMB-178 and GRT-194.
	A27	GRT-103, GRT-104, BMB-122, BMB-125, BMB-126, BMB-129, BMB-174, BMB-175, BMB-176, SKT-206 and BMB-223.
	A28	MRP-09, KTL-17, KTL-31, SKT-202, SKT-203, SKT-210, BMB-224, RYK-414, BMB-130, BMB-136, BMB-152, BMB-123, BMB-137, BMB-227, MRP-327, BMB-42, BMB-45, BMB-90, BMB-94 and GRT-98.
B	B1	BMB-53, MRP-08 and GRT-189.
	B2	MLT-465, MLT-466, MLT-467, MLT-469, MLT-471 and KHW-485.
	B3	MRP-11, BMB-66, BMB-68, BMB-62 and KTL-22.
	B4	MRP-02, KTL-24, BMB-108, GRT-199, BMB-226 and BMB-230.
	B5	MRP-06, BMB-47, BMB-82, BMB-179, GRT-191, BMB-213, BMB-220 and BMB-228.
	B6	MRP-03, MRP-05, BMB-48, BMB-52, GRT-102, GRT-186 and GRT-188.
	B7	MRP-10, MRP-12, MRP-13, MRP-15, BMB-57, BMB-58, GRT-99, GRT-101, BMB-180, GRT-190, BMB-214 and BMB-229.
	B8	MRP-04, BMB-59, BMB-83, BMB-93, GRT-100, BMB-109, BMB-121 and GRT-185.
Outer layer		BMB-106, BMB-107 and GRT-200.

Table 5. Correlation coefficients of quantitative morphological and physico-chemical traits for each accession.

	TC	CD	TH	LBL	LBW	PL	IL	IW	TSS	TA	TSS/TA	RS	TS	NRS
CD	0.523**													
TH	0.349**	0.526**												
LBL	-0.110*	-0.272**	-0.164**											
LBW	-0.077	-0.224**	-0.183**	0.653**										
PL	-0.022	-0.041	-0.037	0.290**	0.216**									
IL	-0.235**	-0.377**	-0.361**	0.200**	0.190**	0.040								
IW	-0.092	-0.158**	-0.194**	0.129**	0.111*	0.022	0.618**							
TSS	-0.109*	-0.176**	-0.114*	0.033	-0.008	0.077	0.043	0.061						
TA	0.052	0.033	-0.134**	0.066	0.006	0.030	0.034	0.026	-0.025					
TSS/TA	-0.123*	-0.177**	-0.043	0.035	0.069	0.006	0.004	0.022	0.475**	-0.634**				
RS	-0.162**	-0.225**	-0.245**	0.028	0.034	0.008	0.173**	0.126**	0.120*	0.105*	0.024			
TS	-0.100*	-0.166**	-0.089	0.022	0.001	0.071	0.002	0.025	0.925**	-0.053	0.460**	0.117*		
NRS	-0.003	-0.029	0.052	0.005	-0.018	0.061	-0.092	-0.046	0.774**	-0.104*	0.404**	-0.436**	0.843**	
FW	-0.288**	-0.464**	-0.503**	0.243**	0.241**	0.068	0.401**	0.299**	0.068	0.096*	0.074	0.287**	0.026	-0.133**

** Correlation is significant at 0.01; * correlation is significant at 0.05; TC: trunk circumference, CD: crown diameter, TH: tree height, LBL: leaf blade length, LBW: leaf blade width, PL: petiole length, IL: inflorescence length, IW: inflorescence width, TSS: Total Soluble Solids, TA: Titratable acidity, RS: Reducing sugars, TS: Total sugars, NRS: Non-reducing sugars; FW: Fruit weight

DISCUSSION

The studied mango accessions showed extensive variations which could be attributed to differences in geographical origin, ecological adaptation to sites and selection by humankind in indigenous mango germplasm of Pakistan from three regions (AJK, Northern Punjab and Southern Punjab). Moreover, most of these traits are of economic interest and consequently can be used as target characters for selection by growers and breeders. Pakistan being closer to primary center of origin of mango has very high diversity as optimum conditions are present in the country for recombination among various mango cultivars due to cross pollination. From the data it was revealed that broadly pyramidal crown shape was most common in Southern Punjab mango accession; whereas, semicircular crown shape was most prominent mango accession in AJK and Northern Punjab (Fig. 1). Morton (1987) and Mussane (2010) have also reported similar types of crown shapes in mango. Most of the germplasm exhibited extensive diversity in case of leaf blade shape, size and leaf attitude in relation to branch (Fig. 1; Table 1). Lanceolate leaf shape was abundantly found in AJK and Southern Punjab accessions; while, Northern Punjab accessions revealed oblong leaf shape (Fig. 1). Mussane (2010) and Rajwana *et al.* (2011) found significant differences and reported that mango leaf shape is a good trait for varietal differentiation. But, Human (2008) described that mango growth habit may differ with cultural practices, density of plantation, eco-geographical conditions and type of genotypes. Moreover, in case of midrib thickness and leaf apex shape extensive variations were observed (Fig. 1). Thickness and tapering midrib was most prevalent in AJK and Southern Punjab in contrast, Northern Punjab germplasm exhibited thin midrib of leaves. As far as leaf apex shapes are concerned, most of the accessions revealed acuminate leaf apex shape (Fig. 1). Similarly, variations in

leaf apex have been found to be related with genotype and eco-geographical locations of mango germplasm (Galvez-Lopez *et al.*, 2010; Rajwana *et al.*, 2011).

As far as leaf fragrance is concerned, most of the germplasm depicted mild fragrance with the exceptions of few accessions as fragrance was absent completely, whereas, few accessions also produced strong fragrance (Fig. 1). These findings were similar according to IPGRI (2006) descriptors, based on genetic make-up of accessions. Huge diversity was also observed for young and mature leaf colour in all three regions (Fig. 1). Ali (2013) reported that young leaves are specific in nature based on cultivar and can be used for the identification of different mango varieties. According to Singh (1960) mature leaf colour may be light to dark green. Our findings confirmed huge diversity in mango germplasm being grown in three different geographic locations of Pakistan regarding inflorescence colour. Light green, green with brownish tinge, light brick red, reddish brown and deep coppery tan coloured inflorescence were most common (Fig. 1). Rajwana *et al.* (2011) and Kobra *et al.* (2012) also reported similar results regarding colour of mango inflorescence among various mango cultivars.

Floral characters are very valuable traits for the classification of mango varieties (Khan *et al.*, 2015). Besides flower colour and stalk of flower, special emphasis has been reported to lie particularly on the panicle length and varieties have been clustered as (a) short panicked (4-8 inches), (b) medium panicked (8-10 inches) and (c) long panicked (more than 16 inches) (Table 1). Pyramidal inflorescence shape was most abundant in all three regions (Fig. 1) and our results were in accordance to Rajwana *et al.* (2011), Kobra *et al.* (2012) and Ali (2013) as they reported that inflorescence shape may be conical, pyramidal or broadly pyramidal. Flower density in inflorescence was medium in AJK and North Punjab, while, in Southern Punjab dense flowering was mostly observed (Fig. 1). According to

Kulkarni (2004) flowering is generally correlated with local environmental conditions, hereditary characteristics, and nutritious as well as hormonal aspects. Moreover, cultivation practices and cultural operations may also affect flowering time and total number of flowers per panicle (Chadha and Pal, 1986).

Recombination has resulted in very high morphologic mango diversity as numerous morphological traits previously not reported in ceremonial mango descriptors both (IBPGR, 1989 and IPGRI, 2006) were observed in native Pakistani mango germplasm. For instance, various mango accessions exhibited pentamerous, hexamerous and heptamerous flowers; whereas, some mango accessions also showed combination of pentamerous, hexamerous, heptamerous and octamerous flowers in the same accessions. These flower types have not been found in descriptors but tetra, penta and hexamerous flowers were reported by Galvez-Lopez *et al.* (2010) in Mexico. Moreover, in *Mangifera laurina* pentamerous flowers are common, *Mangifera casturi* consists of tetramerous and pentamerous, both *Mangifera torquenda* and *Mangifera quadrifida* contain only tetramerous flowers (Galvez-Lopez *et al.*, 2010). As far as anthocyanin intensity is concerned, AJK and Northern Punjab mango accessions depicted low contents of anthocyanin; however, medium to high anthocyanin was observed in Southern Punjab mango accessions (Fig. 1).

In case of fruit physical traits, fruit shape and weight are characteristic features and these usually strongly correlate with the genetic make-up of particular cultivars. Beside genetics, to some extent these also vary with production locality, cultural practices and particular environmental conditions (Ali, 2013; Jamil *et al.*, 2015). Similarly, we also found significant variations in both fruit shapes and weight (Fig. 2). Moreover, fruit shape and weight are very pivotal characters and useful for the identification and characterization of different mango accessions or varieties (Rajwana *et al.*, 2011; Khan *et al.*, 2015). Several Thai mango cultivars have also been characterized based on physical and biochemical fruit quality characteristics (Jintanawong *et al.*, 1992). Likewise, Benor *et al.* (2012) also used capsule shape and weight characters in the genetic diversity analysis and characterization of *Corchorus olitorius* successfully. Recently, diversity in some other crops such as water yam, luffa and *Corchorus* spp have been reported based on different morphological characters (Benor *et al.*, 2012; Prakash *et al.*, 2013; Siqueira *et al.*, 2014). Therefore, based on literature and our findings both fruit shapes and weight are very imperative traits that can successfully be used for the diversity assessment and characterization of mango germplasm. Besides, fruit physical traits, biochemical markers especially TSS, TA, TSS: TA ratio and sugars are also very important characteristics for identification and ultimately characterization of mango germplasm. Moreover, these are

strongly correlated with genotype and cultivation location (Ali, 2013). After characterization based on TSS, TA and TSS: TA ratio these traits could be used for future mango crop improvement programs effectively because all of the available germplasm is valuable in one or other way (Rajwana *et al.*, 2011).

Conclusion: Extensive genetic diversity was detected in the indigenous mango germplasm resources of Pakistan. The extensive and distinct range in morphological and physico-chemical traits studied across the 425 indigenous mango accessions in this study meant that each accession can be distinguished individually. The potential and elite accessions could be suitable for the association genetic exploration and future breeding of the new cultivars. This would be the first imperative step to enabling the inherent resources within the Pakistani mango germplasm. The detected extensive morphological and physico-chemical diversity existing in the studied mango gene pool could be considered useful in the future breeding schemes which predominantly focus the hidden genetic potential underpinning the objectives of high fruit yield and quality. The detected high genetic potential of the studied Pakistani mango germplasm contained numerous traits of substantial economic significance. The targeted selection of these anticipated characters by the mango breeders would not be difficult and such work could even be extended up to the international mango gene banks. However, molecular characterization is required with suitable markers to identify specific traits or genes which could be used in future breeding programs.

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