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Morphological characterization of Indian pummelo (*Citrus maxima*)

A K DUBEY[1], R M SHARMA[2], O P AWASTHI[3], NIMISHA SHARMA[4] and ANJANA KHOLIA[5]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

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ABSTRACT

The present study was conducted with the aim to characterize different indigenous pummelo genotypes based on 19 quantitative and 9 qualitative traits. Leaf shape, leaf margin shape, leaf apex shape, petiole wing, petiole wing shape, fruit shape, fruit apex shape, fruit axis, fruit surface texture and pulp colour were the main qualitative characters of study. The higher coefficients of variation were determined for petiole area (36.33%), juice quantity (17.38%), leaf area (14.50%) and fruit weight (14.22%). Principal component analysis (PCA) for quantitative traits indicated that six PCA had Eigen-value greater than one. The cumulative variation explained by six components, viz. leaf length, fruit width, leaf length: width ratio, leaf area, petiole area and lamina wing ratio accounted over 86.12 % variability. However, the result of the PCA for qualitative traits showed that three parameters namely leaf shape, leaf margin shape and leaf apex shape among 10 Principal Component Axis (PCA) had Eigen-values greater than one, and accounted for over 70.23% of the total variability. Based on PCA and coefficient of variation, traits like fruit weight, seed content, juice content, leaf and petiole area, leaf length, leaf width and leaf length: width ratio had significant involvement in pummelo diversity, and can be used in differentiating accessions of citrus.

Key words: Coefficient of variation, Genetic diversity, Principal component axis, Pummelo

Among different citrus species, pummelo is considered to be one of the true citrus species together with citron (*Citrus medica)* and mandarin (*C. reticulata)* based on karyotype analysis (Hynniewta *et al.* 2011). This species is also the progenitor of grapefruit (C. *paradisi*) and tangelo among other modern citrus hybrids. In recent years, the demand for pummelo has increased mainly in warm areas of the world where other sweet citrus fruits cannot be grown (Paudyal, 1999). Furthermore, pummelo is now widespread in Bangladesh, Chile, Combodia, India, Indonesia, Japan, Malaysia, Thailand and Vietnam.

The vast genetic diversity of wild and semi-wild citrus germplasm has modestly been used for improvement programmes due to lack of their characterization, mainly due to wide gaps in the knowledge of useful characters of various citrus species and varieties (Sharma *et al.* 2004). The duplicates within a gene bank collection are usually unintentional and undesired and can occur due to exchange of accessions between gene banks or acquisition of the same accession, i.e. a cultivar by several gene banks or due to safety-duplication (Knupffer *et al*. 1997). Information on genetic diversity and phylogeny of cultivars can improve the efficiency of germplasm characterization and its use in breeding programmes. Moreover, genetic diversity within and among populations is the backbone of conservation of plant genetic resources for both present and future use (Quedraogo 2001). Morphological traits are among the first markers used in germplasm management (Gitonga *et al.* 2008), besides having significance of the existence in differentiating between subspecies or varieties within taxa (Paganova 2009).

Several authors have investigated and characterized morphologically different selections of citrus plants, in order to increase the number of genotypes with potential to be used in breeding programs or to be released as new varieties. In lime and lemon, Dubey *et al.* (2015) opined that traits like fruit weight, fruit length, juice volume and seed content had significant contribution in lime and lemon diversity, and could be used in differentiation among the accessions of citrus. Characters related to plants, flowers, fruits and leaves have been used to describe and characterize distinct mandarin varieties and its hybrids (Domingues *et al.* 1999). In view of the problems with respect to safety of germplasm maintained in gene banks, it is necessary to identify duplicates and to concentrate on unique accessions for easy management. Hence, present study was undertaken to study the variability existing within pummelo genotypes, using qualitative and quantitative traits.

MATERIALS AND METHODS

Eighteen selected pummelo accessions were collected from NBPGR regional station, Bhowali, Uttrakhand and other pummelo growing areas of the country. The collected site comes under western Himalayan region, described as

[1]Principal Scientist, Division of Fruits and Horticultural Technology (akd67@rediffmail.com).

foothills of Himalaya, warm humid ecosystem with Tarai soils and Transgangetic agroclimatic zone considered hot semi-arid ecosystem with alluvium-derived soils. Experimental location has typical sub-tropical climatic conditions characterized by hot and dry summer followed by cold winter. May and June are the hottest months with the maximum temperature varying between 37.5–46.0°C. The December and January being the coolest months, with the temperature ranging between 10.4 to 21.0°C. The Sunshine hour varied from 1.2 h/day in January to 10.8 h/day in June. The budded plants of selected strains were transplanted in citrus evaluation block of Division of Fruits and Horticultural Technology, IARI at 5 m x 5 m distance. Orchard soil type was a virgin Inceptisol (alluvial soil) with a *p*H 7.3, $EC_{(1:2)}$ of 0.35 dS/m, Cation exchange capacity (CEC) of 7.60 - 10.62 cmol/kg with organic carbon 0.48%, soil N 245.43 kg/ha, P_2O_5 60.45 kg/ha and K_2O 565.22 kg/ha. Trees were maintained under recommended cultural and management practices followed in pummelo.

Pomological characterization of 18 indigenous pummelo germplasm was carried out using different qualitative traits, viz. leaf shape, leaf apex shape, leaf base shape, petiole wing shape, and leaf margin shape and quantitative traits, viz. leaf size, leaf area, lamina wing ratio, fruit physical and chemical quality. The qualitative traits were determined as per the citrus descriptors (IPGRI, 1999). Ten fully matured leaves were selected and brought to the laboratory in ice box for recording leaf area, shape, margin shape and shape of petiole wing and observations were recorded on same day. Similarly, for observing fruit shape, and fruit apex shape, 10 randomly selected fruits brought to the laboratory, washed with running tap water, and observations were recorded immediately. Fruit juice colour was recorded based on colour chart.

Ten randomly selected fruits from each germplasm were harvested at physiological maturity for recording physical fruit parameters such as fruit weight, size, juice content peel thickness, segment and number of seeds. Total soluble solids (TSS) were determined using digital refractometer (ATAGO PAL-3); titratable acidity was determined according to the method described by Ranganna (1986). The ascorbic acid content in fruit juice was estimated by Redox titration method using iodine solution and starch indicator (Silva *et al.* 1999). For both qualitative and quantitative traits, five replications per treatment were included.

The quantitative data were analysed in a one-way ANOVA to determine differences between accessions using SAS 9.3 version (SAS Institute Cary, NS, USA). Cluster analysis was done by unweight pair group method of arthematic average (UPGMA) based on 18 quantitative and 10 qualitative variables with Jaccard similarity coefficient. Data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc version 2.1) software package (Rohlf, 2000). Principal Components Analysis (PCA) of all selections using both quantitative and qualitative traits was performed by NCSS 2007 v 07.1.18 (Hintze, 2007). The principal component score with Eigen-values > 1 were used as new variable for cluster analysis.

RESULTS AND DISCUSSION

The ANOVA analysis showed differences among pummelo genotypes for the majority of the traits. High coefficient variation (CV %) was recognized for petiole area (36.33%), juice quantity (17.38%), leaf area (14.50%) and fruit weight (14.22%). While less than 10% CV was recorded for fruit length, fruit diameter, peel thickness, number of segments, TSS, titratable acidity, ascorbic acid content, leaf length, leaf width, and leaf length width ratio (Table 1).

Among 18 pummelo genotypes, PS-4, PS-5, PS-6, PS-8 and PS-9 had ovoid fruit shape., while round fruit was observed in PS-10, PS-11, PS-12, PS-14, PS-15 and PS-17. Some accessions such as PS-7, PS-16 and PS-18 have round to ovoid fruit shape. Variation also appeared in juice colour and accessions such as PS-1, PS-2 and PS-16 had deep red juice colour, while red colour was sighted in PS-6, PS-8 and PS-9. Nevertheless, accessions like PS-5, PS-11, PS-13, PS-15 and PS-17 had white juice colour. Rest of the genotypes produced pink fleshed fruits. Moreover, genotypes such as PS-6, PS-8, PS-11, PS-13, PS-15 and PS-17 had solid fruit axis, while other genotypes exhibited hollow fruit axis. Two genotypes namely PS-1 and PS-18 had depressed fruit apex, whereas other accessions had either round or flat fruit apex. Leaf qualitative characters suggested

Table 1 Analysis of variance for different quantitative traits of 18 genotypes of pummelo

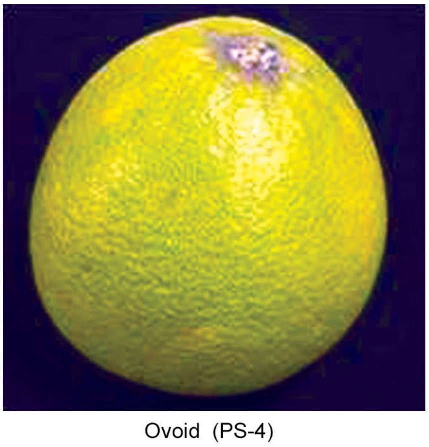
Characters	Range	Population mean	R^2	CV (%)	F value
Fruit weight (g)	325.1-1034.2	755.77	0.88	14.22	14.96
Fruit length (mm)	86.0-148.9	117.46	0.94	4.46	28.44
Fruit diameter (mm)	96.6-143.6	122.68	0.89	5.25	15.77
Peel thickness (mm)	10.5-17.8	14.28	0.80	9.06	7.91
Core diameter (mm)	8.7-34.9	21.36	0.95	11.24	37.31
Segments (number)	13.3-19.3	15.56	0.71	7.41	4.81
Seeds/fruit	32.0-152.0	103.15	0.88	13.38	14.49
Juice (ml)	51.3-273.3	154.49	0.92	17.38	21.85
Juice (%)	7.0-29.9	20.68	0.89	13.51	16.53
TSS (°B)	13.0-17.5	14.77	0.87	3.93	13.75
Acidity (%)	0.49-0.9	0.71	0.87	8.75	12.36
TSS/acidity ratio	14.5-31.3	21.74	0.82	11.66	8.51
Ascorbic acid (mg/100 ml juice)	40.2-90.1	73.21	0.91	6.74	18.98
Leaf length (cm)	6.0-13.3	9.25	0.87	7.58	13.26
Leaf width (cm)	3.8-7.5	5.58	0.90	5.92	18.04
Leaf length/width ratio	1.3-1.9	1.66	0.76	6.14	6.30
Leaf area (cm^2)	29.3-58.3	45.20	0.72	14.50	5.17
Petiole area (cm^2)	1.02-8.06	3.99	0.73	36.33	5.18
Lamina wing ratio	6.40-59.90	16.70	0.98	10.70	186.20

that genotypes, viz. PS-1, PS-7 and PS-14 had obovate to ovate leaf shape, while elliptic leaf shape was seen in PS-2, PS-5, PS-8, PS-9, PS-16 and PS-17. Remainder genotypes had ovate leaf shape. In most of the pummelo accessions, leaf margin shape was noticed crenate except in PS-2, PS-18 (entire), PS-5, PS-12, PS-13 (wavy). Leaf apex shape and petiole wing shape also varied among pummelo genotypes.

In the present study, the variability among accessions was observed for all the characters of leaves, petiole and fruits, indicating phenotypic differences among the accessions studied. The difference in individual accession could be contributed due to mutations, and cross pollination. The bud sport mutations, introduction and trials of materials in location different from its original habitat might be the possible reason for adding differences among the studied population (Dorji and Yapwattanaphun 2011). The common phenomenon of occurring zygotic twins in Himalayan mandarin varieties (Das *et al.* 2007) might be the possible cause for the variation observed in pummelo. Susandarini *et al.* (2013) also found huge variation in Malaysian pummelos. While, genetic diversity study of pummelo landraces of Indian origin, established that many superior pummelo clones are managed by local farming communities inhabiting various agro-eco-niche as on farm conservation (Singh *et al.* 2015).

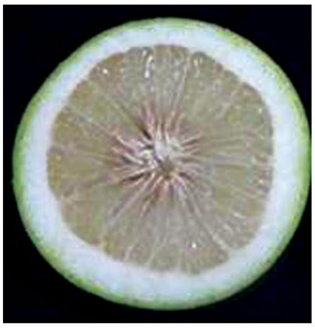
Additionally, analysis of variance of different cluster groups for different quantitative traits indicated group A Subgroup A1 characterized by highest leaf length (9.49 cm), leaf width (5.65 cm), petiole area (4.42 cm^2), fruit weight (824.25 g), fruit length (122.30 mm), fruit diameter (127.08 mm), peel thickness (14.77 mm) and seeds per fruit (111.76 seeds). However, group A sub cluster A2 described by highest lamina wing ratio (44.05) and core diameter (23.80 mm). Furthermore, group B and subcluster B1 represented highest ascorbic acid content (79.50 mg/ 100 ml juice content), whereas group B sub cluster B2 categorized by highest juice content (27.49%) and TSS/acid ratio (26.27) (Table 2). Pummelo accessions, viz. PS-1, PS-2, PS-14, PS-5, PS-16, PS-9, PS-12, PS-18, PS-6, PS-10, PS-17, PS-13, PS-7 and PS-8 falling in sub cluster A1 had superiority over other accessions in respect to fruit weight, fruit length and width, peel thickness, and segments. However, accessions falling in A2 cluster (PS-4) had highest core diameter and lamina wing ratio. Furthermore, sub cluster B2 comprising PS-11 and PS-15 had superiority over other accessions for juice content and TSS/acid ratio.

In order to see the relationship, percentage similarities and display position of genotypes used in this study, a genetic tree was constructed from the pairwise distance matrices (Fig 1). A dendrogram generated based on quantitative data grouped all the 18 pummelo accessions into two clusters A and B at similarity value of 0.20. Cluster A comprised most of the studied pummelo accessions and further subdivided into one cluster as A1 and one out group as A2 at 14% similarity value. Cluster A1 consist most of the genotypes and further subdivided into minor clusters, viz. A1.1 and A1.2. Cluster A1.2 consist only two selections PS-7 and PS-8. However, Cluster A1.1 further subdivided into clusters A1.1.1 and A1.1.2. In cluster A1.1.2, PS-10 and PS-17 genotypes were present and PS-13 was present as an out group. Selections


Ovoid (PS-4)


Round (PS-15)


Roundish to ovoid (PS-13)


White (PS-15)


Light pink (PS-3)


Red (PS-1)


Deep Red (PS-6)

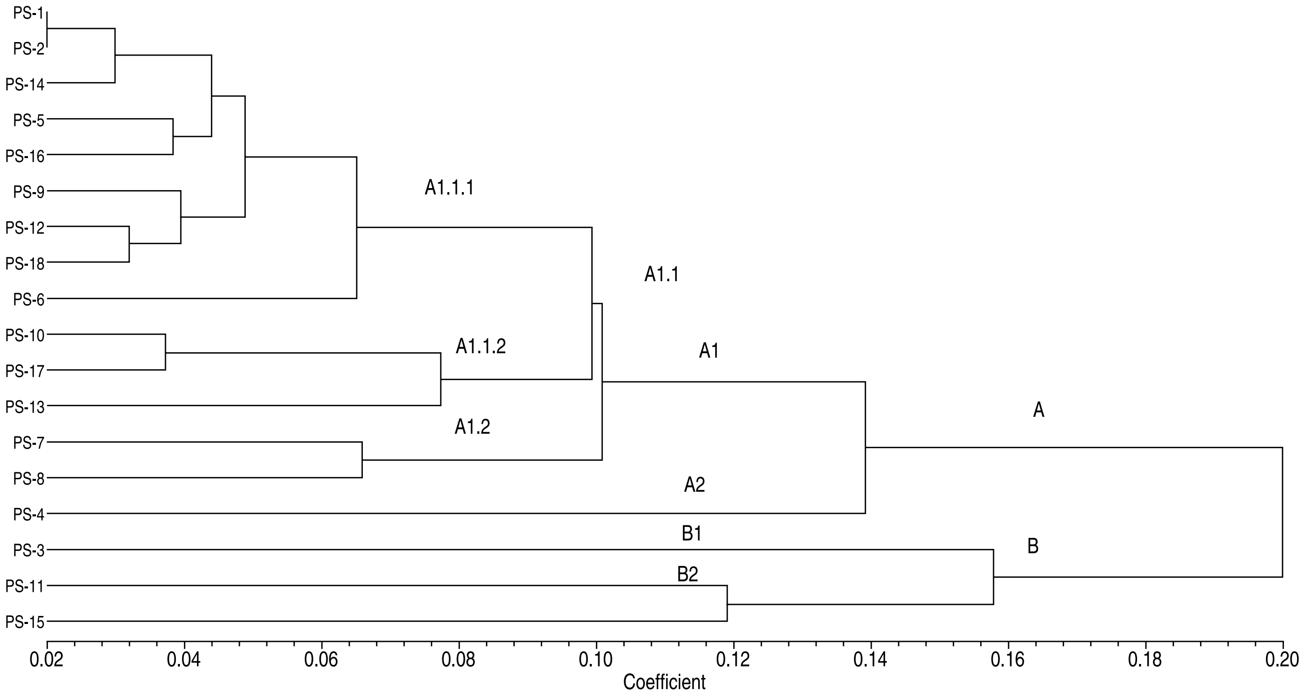


Fig 1 Dendrogram of pummelo selections based on quantitative traits using the UPGMA method.

Table 2 Characteristics of cluster groups of pummelo genotypes based on quantitative traits

Characters	Cluster A1	Cluster A2	Cluster B1	Cluster B2	LSD ($P \leq 0.05$)
Fruit weight (g)	824.25	521.93	373.23	584.57	178.31
Fruit length (mm)	122.30	104.77	97.03	100.10	8.69
Fruit diameter (mm)	127.08	107.17	101.27	110.30	10.68
Peel thickness (mm)	14.77	12.17	12.07	13.03	2.14
Core diameter (mm)	21.30	23.80	21.00	20.70	3.98
Segments (number)	15.67	14.33	13.67	16.33	1.91
Seeds/fruit	111.76	32.00	61.67	102.33	22.89
Juice content (%)	20.35	18.61	13.79	27.49	4.63
TSS (0B)	14.73	13.13	15.30	15.62	0.96
Acidity (%)	0.72	0.66	0.72	0.62	0.10
TSS/Acid ratio	21.25	19.94	21.35	26.27	4.20
Ascorbic acid (mg/ 100 ml juice)	73.24	78.33	79.50	67.27	8.18
Leaf length (cm)	9.49	8.19	8.45	8.47	1.16
Leaf width (cm)	5.65	5.03	5.52	5.42	0.54
Leaf length/width ratio	1.69	1.63	1.53	1.56	0.17
Leaf area (cm^2)	45.77	33.31	36.94	49.98	10.88
Petiole area (cm^2)	4.42	0.80	3.49	4.14	2.40
Lamina wing ratio	15.72	44.05	11.26	12.50	5.42

PS-1 and PS-2 showed 100% genetic similarity in cluster A1.1.1. Out group A2 comprised pummelo genotype, PS-4 only. Likewise, only three selections, viz. PS-3, PS-11 and PS-15 were presented in cluster B and selection PS-3 was presented separately as an out group namely B1.

A tree generated based on qualitative data grouped all the 18 pummelo genotypes into two clusters A and B at similarity value of 0.72. Both cluster A and cluster B comprised nine pummelo selections each. Cluster A further subdivided into cluster A1 and A2 at similarity value of 0.62 (Fig 3). Only 2 selections were present in cluster A2, viz. PS-5 and PS-18. Whereas, cluster B broadly divided into one out group B1 that consisted only PS-2 pummelo accession and one cluster B2 which consisted rest of the eight pummelo accessions. In cluster B2, PS-10 and PS-12 showed 100% genetic similarity; PS13 and PS-17 also showed 100% similarity.

The principal component analysis (PCA) was used to determine the extent of the variation and percentage similarity within the accession. Eigen-values and factor scores obtained from PCA were used to determine the relative discriminative power of the axis, and their associated characters. In PCA Eigen-value criterion is one of the most commonly used criteria for solving the number-of-components problem (Kaiser, 1960). With this approach, any component with an Eigen-value > 1.00 is considered for interpretation. In the present study, the result of the PCA for quantitative traits showed that six of the twenty principal component Axis had Eigen-values > 1 and all together accounted for over 86.11% of the total variability (Table 3). The first PCA accounted for 34.46% of the total variation while PCA 2 accounted for 18.65% of the total variation. The cumulative percent of variance varied from 34.46% to 86.11% for the PCA, having Eigen-value > 1. The PCA had successfully attributed linear combinations of the different physico-chemical parameters, separated out from the different clusters of pummelo accessions, which were

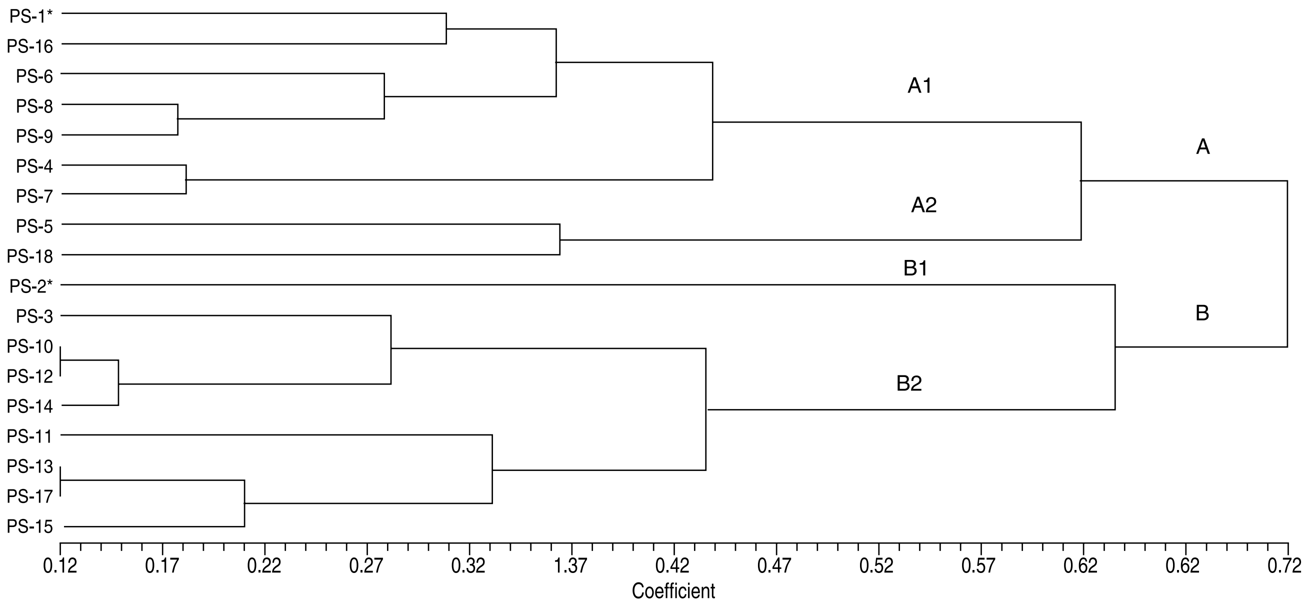


Fig 3 Dendrogram of pummelo selections based on qualitative traits using the UPGMA method.

classified into four distinct cluster groups. The comparison of the cluster analysis (CA) for quantitative traits, exposed the similarity to some extent. However, both the analysis grouped the selections in different ways.

The results of the PCA for qualitative traits showed that three of the ten Principal Component Axis had Eigen-values >1, and all together accounted for over 70.23% of the total variability (Table 4). The first PCA accounted for 37.64% of the total variation while the PCA 2 accounted for 16.94% of the total variation. The cumulative percent of variance varied from 37.64% to 70.23% for the PCA having Eigen value > 1. The PCA had efficaciously showed linear combinations of the different qualitative parameters, which separated out different clusters of pummelo selections, which were classified into three distinct cluster groups. Principal component analysis identified that the cumulative variation explained by first four components accounted over 74.57 % variations, revealing a great quantitative traits variability, a high genetic diversity between pummelo accessions. This suggested that these traits had significant contribution in pummelo diversity. Similar findings have also been reported in lime (Dubey *et al.* 2015), cornelian cherry (Ercisli *et al.* 2011; Mratinic *et al.* 2015), pummelo (Paudyal and Haq, 2008) and sweet orange (Debbabi *et al.* 2013).

When the cluster analysis (CA) and PCA were compared for qualitative traits, some similarity was found. PS-10 and PS-12, PS-13 and PS -17 showed maximum similarity for both type of the analysis. When we compared the cluster analysis and principal component analysis for both qualitative and quantitative traits very low similarity was found. The selected pummelo population exhibited a high level of diversity in several quantitative traits. This is clearly explained by the grouping of trees into cluster, PCA and ANOVA where most of the quantitative traits differed significantly. This might be due to heterozygosity and

Table 3 Principle Component Analysis among 18 selections of pummelo using quantitative traits.

Character	Eigen-Value	% Variance	% Cumulative Variance
LL	6.203505	34.46	34.46
LW	3.357466	18.65	53.12
LLW	2.355803	13.09	66.20
LA	1.359930	7.56	73.76
PA	1.142451	6.35	80.11
LWR	1.081061	6.01	86.11
FWT	0.730970	4.06	90.17

Leaf length (LL); leaf width (LW); leaf length (LLW); width ratio leaf area, (LA); petiole area (PA); lamina wing ratio (LWR); Fruit weight (FWT).

Table 4 Principle Component Analysis among 18 selections of pummelo using qualitative traits.

Character	Eigen-Value	% Variance	% Cumulative Variance
LS	3.76	37.64	37.64
LMS	1.69	16.94	54.58
LAS	1.56	15.65	70.23
PW	0.91	9.15	79.38
PWS	0.70	7.09	86.47
FS	0.52	5.28	91.75
FA	0.33	3.34	95.10
FAX	0.20	2.06	97.16
FST	0.19	1.91	99.07
FC	0.09	0.93	100.00

Leaf shape (LS), Leaf margin shape (LMS), Leaf apex shape (LAS), Petiole wing (PW), Petiole wing shape (PWS), Fruit shape (FS), Fruit Apex (FA); Fruit Axis (FAX); Fruit surface texture (FST); Leaf length (LL); Leaf width (LW); Pulp colour (FC).

seedling population which overall showed wider genetic diversity as reported by Arora (1998).

The involvement of leaf length, leaf weight, leaf length width ratio, leaf area, petiole area and lamina wing ratio in the PCA leads to the conclusion that these traits contributed more to the total variation observed in the 18 pummelo accessions, and therefore, the natural gene pool of this species in India, suggesting its high genetic potential which could be used to find valuable well adapted genotypes of intended traits.

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