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Analysis of genetic variability among citrus (*Citrus* spp) genotypes using morphological traits

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ABSTRACT

Fifty citrus (*Citrus* spp) genotypes comprising cultivars, strains and hybrids were characterized by using morphological characters in this study. Twenty quantitative characters from leaves, flowers and fruits were evaluated and morphological data from fifty accessions were submitted to one-way ANOVA, principal component and cluster analysis. The matrix of average dissimilarity between genotypes was estimated using Euclidean distance. Significant variation in quantitative characteristics among genotypes was observed. The average distance ranged from 0.09 - 3.77. The clustering pattern clearly differentiates the genotypes into separate clusters based on their dissimilarity values. The first 3 principal components explained 58.72% of variation and identified seeds/fruit, juice content, pH, TSS, TSS:TA and petal size as important traits that can be used to differentiate genotypes. Among all genotypes, small fruited mandarins were highly diverse while sweet orange and grapefruit genotypes presented the lowest diversity. The high diversity observed within the genotypes points to ample possibilities of obtaining desirable trait combinations in specific cultivars.

Key words: Citrus genotypes, Dissimilarity, Euclidean distance, Traits, Variability

Citrus (*Citrus* spp) is one of the world's most important fruit crops. It is widely grown in the tropical, subtropical and borderline subtropical areas of the world. In India, it covers an area of 10.42 lakh ha with a production of 100.9 lakh tonnes (Anonymous 2011). The suitable climatic condition and long history of cultivation cause great diversity of species, cultivars and clones within citrus. Despite this vast cultivation, phylogeny and taxonomy of many citrus varieties remain uncertain which has been complicated by several factors, such as hybridization, apomixis, polyploidy and bud mutations (Araujo *et al.* 2003). Knowledge of genetic variation and genetic relationship among genotypes is an important consideration for classification, utilization of germplasm resources and breeding. At present, morphological study is still considered important and has been deployed as an initial step for cultivar identification and diversity assessment (Rodriguez *et al*. 2009, Elameen *et al*. 2010).Although many molecular techniques has been developed in studying the genetic diversity, Susandarini *et al.*(2013) emphasized the practical importance of morphological characters in horticultural plant species as well as in plant systematics for cultivars identification. Therefore, the present study was undertaken to assess the extent of diversity and relationship among citrus genotypes which could provide useful information for further improvement in citrus breeding programs.

MATERIALS AND METHODS

The experiment was carried out at the Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi which is situated at 28.40 ºN latitude and 77.10º E longitude at an altitude of 228.16 m above the mean sea level. The climate of Delhi is semi-arid with hot summers and cool winters. Fifty genotypes of citrus subjected to the present study were presented in Table 1.

Twenty quantitative traits on leaves, flowers and fruit physico-chemical characters were studied. The morphological traits, such as spine length (mm),leaf length (cm), leaf width (cm), petal length (mm),petal width (mm), pedicel length (mm), diameter of opened flower (mm), calyx diameter (mm), fruit length (mm), fruit width (mm), fruit weight (g), 100 seed weight (g), seeds/fruit, segments/fruit, peel thickness (mm) and juice content (%) were recorded by using standard methods as per citrus descriptors set out by IPGRI (1999). Juice pH was noted with a pH meter and total soluble solids was measured with the help of digital refractometer. Total acidity was assessed as per method given by Ranganna (1986).

ANOVA was performed using SAS 9.0 software while Cluster analysis and Principal Component Analysis (PCA) were carried out using NTSYS 2.1 (Rohlf 2000).To minimize the effects of different scales of measurement, quantitative data were standardized prior to cluster analysis using the STAND module of NTSYSpc. Genetic dissimilarities based

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Table 1 List of 50 citrus genotypes

Common name	Code
Karna Khatta (*C. karna* Rafin.)	SSA (Soh Sarkar), KKH (Karna Khatta)
Attani (*C. rugulosa* Hort. ex Tanaka)	AT1 (Attani-1), AT2 (Attani-2)
Rough lemon (*C. jambhiri* Lush.)	RLC3, RLC4, RLC7, RL (Rough lemon), JK (Jatti Khatti)
Rangpur lime (*C. limonia* Osbeck)	RL-3, RL-4, SYL (Sylhet lime)
Hybrid (Unknown parents)	HYB
Citrange (*C. sinensis* Osbeck × *P. trifoliata* Rafin.)	CAR (Carrizo), TRO (Troyer), MOR (Morton)
Sadaphal (*C. semperflorens* Lush.)	SAD
Sour orange (*C. aurantium* L.)	SOR
Grapefruit (*C. paradisi* Macfad)	IMP (Imperial), RBL (Redblush), FOS (Foster), SRU (Star Ruby), DUN (Duncan), MSL (Marsh Seedless)
Sweet orange (*C. sinensis* Osbeck)	MS2, MS3, MS4, MS5, MS13, MS16, MOS (Mosambi), JAF (Jaffa), PIN (Pineapple), VAL (Valencia)
Galgal (*C. pseudolimon* Tanaka)	GAL, HL6 (Hill lemon-6)
Lemon (*C. limon* Burm. f.)	LS1, LS2, KKA (Kagzi Kalan)
Acid lime (*C. aurantifolia* Swingle)	ALC1, ALC2, ALC3, ALC4, KSL (Konkan Seedless)
Sweet lemon (*C. limetta* Risso)	SL1, SL3
Pectinefera (*C. depressa* Hayata)	PEC
Yama Mikan (*C. intermedia* Hort. ex Tanaka)	YAM
Mandarin (*C. reticulata* Blanco)	MAN, NS7

on Euclidean coefficient were calculated among all possible pairs using the SIMINT option and UPGMA-based clustering was done using SAHN module of NTSYSpc. Dissimilarity matrix was then subjected to eigen value decomposition to identify orthogonal components of the original matrix and generate a PCA biplot which provide an additional representation of genetic diversity among the studied genotypes.

RESULTS AND DISCUSSION

Wide range of variability was observed among the genotypes with respect to different studied traits. Results of the analysis indicated the existence of wide morphological variability between the genotypes (Table 2). Fruit weight, total acidity, TSS:total acidity ratio, number of seeds/fruit, 100 seed weight and peel thickness presented higher variation($p \leq 0.05$) in the studied genotypes. Superior performance for fruit physico-chemical traits were scored by genotypes belonging to sweet orange and grapefruit. Spinelessness was observed in 40% of the genotypes while seedlessness (< 5 seeds/fruit) was noted in 11 genotypes. Comparatively, among all the traits, TSS, ascorbic acid content, segments/fruit, petal length, petal width, flower diameter, leaf length and leaf width presented lower variation among the genotypes.However, such analysis is unable to decipher the extent of genetic variability present among the genotypes. To overcome this problem and simultaneously to determine pairwise genetic relationship between genotypes or group of genotypes, Cluster analysis and Principal component analysis was conducted that separates genotypes into different groups.

The results of cluster analysis on 50 citrus genotypes suggested the presence of enough variation among the genotypes for different agro-morphological traits. Accessions with greater similarity for the traits were placed in the same cluster. Cluster analysis is of great practical significance for plant breeders as it distributes the genotypes into different clusters. Representative genotypes from each cluster can be selected for use in crop improvement programs. Cluster analysis based on 20 morphological traits divided 50 genotypes of citrus into two main groups, which was further categorized into different clusters. As depicted by the dendrogram (Fig 1) which was constructed using ward's method, the clustering of genotypes was on the basis of their relative magnitude of Euclidean distance coefficient, in such a way that intra-cluster Euclidean distance among genotypes was lower than inter-cluster Euclidean distance. Means and standard deviations for different traits that reflect the genetic differences among the clusters are presented in Table 3 and pairwise dissimilarity matrix are shown in Table 4.A higher range of variation with average Euclidean distance coefficients ranging from 0.09 -3.77 was observed between groups as well as cluster, thereby indicating that genotypes within and among clusters were highly diverse.

Cluster I comprised of small fruited mandarin, viz. Mandarin, Pectinefera, Yama Mikan and NS 7, as most diverse which was separated from the rest of the genotypes at 3.77 Euclidean distance coefficients. Except for juice content, pH, TSS, TSS:acidity ratio and ascorbic acid content, this cluster showed the lowest performance for fruit physico-chemical traits. Intra-cluster heterogeneity with Euclidean distance coefficient ranging from 0.25 - 1.8 was observed.At 2.01 level of Euclidean coefficient Cluster II was subdivided into two Sub-clusters (Sub-cluster I and II). Sub-cluster I was found most heterogeneous among all clusters (0.36 - 200 Euclidean distance) and comprised 11 genotypes, viz. ALC 1, ALC 2, ALC 3, ALC 4, Konkan Seedless, Sweet lemon 1, Sweet lemon 3, RL 3, Hybrid, RL 4 and Sylhet lime, which was represented as lowest ascorbic acid content (32.30 mg/100 ml juice) and lower in fruit length (59.11 mm), fruit width (54.04 mm), fruit weight (84.28 g), peel thickness (2.44 mm), seed number (8.85), petal width (5.46 mm), calyx diameter (4.60 mm) and pedicel length (4.17 mm).Sub-cluster II was again separated into two Minor Sub-clusters (Minor Sub-cluster I and II) which differentiated from each other at 1.25 level of

Table 2 Means of 20 quantitative traits among the 50 citrus genotypes

Genotype	FWT (g)	JC (%)	pH	TSS (%)	TA (%)	TSS:TA	VITC	SDN	SDWT (g)	FLT (mm)	FWD (mm)	PTCK (mm)	SEGN	PTLT (mm)	PTWD (mm)	OFD (mm)	PDCL (mm)	CALD (mm)	LFLT (cm)	LFWD (cm)
SSA	215.65	28.33	2.39	5.43	5.08	1.07	56.42	20.67	16.68	78.20	87.64	9.12	12.00	18.83	6.82	39.13	7.26	6.86	8.47	4.62
KKH	221.89	30.33	2.50	5.77	5.67	1.02	55.78	21.67	17.69	85.70	99.01	10.75	11.33	18.64	7.42	37.62	7.16	6.60	7.40	4.23
AT1	226.27	28.33	2.34	5.47	5.12	1.07	50.26	32.67	19.07	106.19	95.18	11.61	10.00	19.31	5.79	36.97	5.75	6.27	9.37	5.53
AT2	234.68	30.67	2.35	6.25	5.57	1.12	47.84	27.33	18.99	102.27	88.36	10.34	10.67	21.46	5.40	34.65	6.82	6.63	9.57	5.20
RLC3	133.49	27.00	3.25	6.34	4.28	1.48	33.27	13.33	10.75	49.21	65.93	5.91	10.67	18.56	5.29	31.50	3.25	4.99	9.60	4.57
RLC4	121.84	29.33	3.34	6.52	4.65	1.40	56.02	16.33	18.53	52.02	85.10	6.60	12.33	21.28	5.96	33.79	5.95	8.45	10.13	4.73
RLC7	125.36	48.00	2.44	6.83	4.68	1.45	31.08	12.00	8.56	61.45	62.14	2.40	11.67	13.89	4.70	21.30	2.66	4.63	9.23	4.27
JK	139.21	38.33	2.61	5.88	5.11	1.15	25.27	21.67	11.54	67.25	76.56	3.83	10.00	16.07	5.94	28.71	3.05	5.16	9.37	4.54
RL	148.14	34.00	2.71	6.03	5.16	1.17	36.85	18.67	8.75	73.25	67.08	3.86	10.00	16.12	6.35	28.81	3.12	5.34	9.18	4.28
SYL	87.94	52.00	2.49	6.53	5.50	1.19	32.26	15.33	11.30	57.03	53.96	1.79	7.00	16.19	5.54	26.82	3.14	3.67	8.78	4.70
RL3	76.10	33.25	2.54	6.53	4.87	1.34	42.18	8.00	3.75	59.90	52.96	1.94	9.67	15.01	5.40	25.57	3.39	4.45	8.27	3.17
RL4	68.54	52.00	2.55	6.78	4.90	1.38	45.57	8.33	4.04	55.52	53.07	1.68	9.67	14.47	5.26	24.27	3.15	3.63	9.10	3.90
TRO	186.22	12.33	2.77	6.52	4.62	1.40	46.49	6.33	18.47	59.97	60.60	3.36	8.33	21.28	8.73	33.76	6.81	6.57	5.26	2.08
CAR	242.10	25.49	2.73	6.86	2.58	2.66	45.34	4.33	8.54	92.07	75.13	8.74	9.00	18.86	6.97	29.24	4.66	6.37	7.63	2.27
MOR	174.02	15.00	2.37	7.02	4.78	1.45	38.47	17.67	12.08	47.03	52.87	4.18	7.00	17.42	7.75	28.49	4.70	4.73	6.79	2.29
HYB	82.61	32.33	2.54	6.57	4.89	1.34	39.39	11.33	8.82	60.53	53.38	2.37	6.00	14.88	5.38	25.57	3.24	4.81	8.46	3.68
SAD	256.75	31.67	2.29	5.53	4.57	1.21	32.47	39.00	21.37	112.98	101.60	15.53	10.67	20.45	5.48	33.39	6.82	6.58	8.10	3.77
SOR	279.97	50.08	2.46	7.28	4.86	1.50	44.09	28.67	17.70	95.84	89.04	4.83	11.00	18.70	6.45	32.06	7.05	6.27	12.33	6.00
PEC	18.83	42.65	3.36	7.43	1.83	4.06	42.18	6.67	9.40	26.60	33.44	0.60	9.33	8.41	4.24	18.69	3.20	3.22	6.33	2.80
YAM	23.00	28.45	3.38	7.84	0.86	9.15	45.57	7.00	7.57	42.47	49.27	1.56	8.00	9.35	4.59	19.83	3.22	3.26	7.84	3.33
RBL	432.21	41.67	4.60	8.21	1.16	7.08	35.45	3.33	14.90	83.85	86.39	6.95	13.00	17.61	7.13	28.51	6.79	7.32	9.50	4.10
IMP	442.85	42.64	4.56	8.26	1.29	6.42	35.76	53.67	19.62	88.62	94.80	7.82	14.00	16.56	7.24	26.39	9.36	6.29	10.08	4.30
FOS	412.72	39.67	4.22	8.66	1.38	6.26	41.06	13.00	15.59	77.86	93.59	7.73	14.33	12.62	5.28	24.55	8.46	6.13	10.17	4.81
SRU	422.90	42.33	4.59	8.53	1.25	6.81	48.41	36.33	25.68	85.29	101.57	6.09	14.67	15.53	6.44	26.16	8.11	6.39	9.50	4.34
MSL	437.66	44.39	4.63	7.96	0.95	8.41	53.93	2.00	16.50	94.72	108.16	6.28	13.67	16.03	6.69	28.25	7.82	6.08	9.93	4.37
DUN	404.93	42.33	3.66	8.25	1.34	6.15	53.42	34.00	25.75	87.27	82.65	6.96	12.67	19.45	6.17	34.54	10.77	7.87	10.93	4.90
MS2	306.50	36.58	4.72	8.84	1.02	8.66	30.75	3.00	10.00	88.81	89.74	5.43	10.67	18.73	7.18	35.67	5.58	6.34	9.63	4.28
MS3	262.20	50.00	4.39	9.20	1.02	9.01	31.09	25.33	24.21	78.75	82.00	4.80	10.00	16.27	6.24	29.68	6.32	6.32	9.86	4.64
MS4	286.87	40.00	4.45	9.10	0.79	11.52	46.21	15.33	14.54	78.46	84.24	4.86	10.67	16.58	6.23	29.62	5.35	5.04	9.30	3.57
MS5	303.94	36.57	4.49	9.30	1.02	9.12	52.71	14.33	15.12	83.23	85.67	6.70	11.00	18.42	6.88	35.60	5.74	6.53	10.43	4.60
MS13	245.26	48.73	4.58	8.85	0.89	9.94	48.13	23.67	21.38	80.66	86.29	5.24	10.33	16.38	6.38	30.21	5.35	5.28	8.73	3.63
MS16	285.68	38.67	4.52	8.28	0.69	11.94	52.35	11.67	17.77	78.39	85.29	5.74	10.67	17.02	6.19	28.80	5.02	5.24	9.36	4.37
MOS	258.09	54.25	4.67	9.84	0.69	14.33	47.15	10.00	12.97	72.66	76.99	4.69	11.00	16.88	6.92	30.58	5.55	6.03	9.83	4.83

Contd.

Table 2 *Contd.*

Genotype	FWT (g)	JC (%)	pH	TSS (%)	TA (%)	TSS:TA	VITC	SDN	SDWT (g)	FLT (mm)	FWD (mm)	PTCK (mm)	SEGN	PTLT (mm)	PTWD (mm)	OFD (mm)	PDCL (mm)	CALD (mm)	LFLT (cm)	LFWD (cm)
PIN	293.80	40.67	4.93	8.15	0.60	13.65	52.71	5.67	15.35	76.94	82.38	6.91	13.00	17.84	6.75	30.74	6.03	6.62	9.20	4.60
JAF	264.42	40.00	4.28	8.35	0.65	12.92	45.26	12.67	16.03	72.25	78.53	5.24	13.00	16.91	6.98	29.49	6.46	6.52	10.10	4.86
VAL	247.20	56.00	4.24	8.90	0.66	13.48	44.62	10.00	11.97	58.50	63.20	2.80	12.00	16.18	6.23	28.96	5.08	5.14	8.86	3.84
GAL	177.35	48.00	2.52	6.64	5.13	2.73	41.84	12.33	15.70	85.67	96.68	5.20	7.33	17.85	7.54	34.85	6.37	6.55	9.40	4.87
HL6	180.66	43.67	3.15	6.80	3.84	1.77	40.97	20.33	24.15	108.95	86.98	4.92	12.00	23.13	7.36	37.59	6.85	6.79	11.60	5.50
LS1	146.28	32.34	2.71	6.65	4.51	1.47	26.78	3.33	3.90	71.13	69.25	3.95	12.33	19.42	7.38	32.11	7.61	6.72	9.93	4.73
LS2	126.49	32.33	2.63	7.01	4.70	1.49	22.69	2.33	9.91	76.66	70.51	3.89	11.33	21.85	7.80	36.04	9.60	7.45	10.70	5.87
KKA	131.11	28.67	2.68	6.61	3.48	1.90	41.55	2.67	9.37	69.72	64.30	2.97	13.33	23.49	6.90	32.89	7.40	7.23	6.64	5.50
ALC1	67.97	28.00	2.43	6.85	6.60	1.04	23.85	3.00	10.00	56.36	49.81	2.95	11.67	11.10	3.25	17.25	3.49	3.14	6.00	3.36
ALC2	62.47	25.67	2.44	7.14	6.53	1.09	41.24	2.67	4.87	54.32	46.90	3.34	12.00	14.29	5.12	25.74	2.25	3.12	10.47	5.03
ALC3	75.68	44.06	2.52	7.36	5.69	1.29	42.02	28.33	12.95	59.97	54.72	3.20	12.00	14.41	5.02	23.61	2.73	3.44	10.25	4.25
ALC4	67.31	26.23	2.49	7.07	6.10	1.16	41.27	9.67	6.24	68.41	56.08	4.57	12.00	11.43	3.53	20.46	4.17	3.84	7.83	4.77
KSL	72.69	32.33	2.67	6.64	4.81	1.38	33.89	4.00	17.58	58.74	50.20	2.83	11.67	25.13	7.42	42.57	7.59	7.59	8.43	4.67
SL1	126.80	47.33	6.17	7.45	0.55	13.46	46.33	1.33	23.76	57.13	58.34	2.60	11.00	22.84	7.19	39.97	5.91	6.40	9.57	4.74
SL3	135.14	54.00	5.69	7.58	0.39	20.28	46.72	5.33	20.94	62.26	65.00	1.96	10.00	21.78	6.93	36.47	6.84	6.48	8.50	4.63
MAN	25.84	28.68	3.39	7.84	0.95	7.97	44.10	6.67	7.00	43.90	49.65	1.58	8.00	10.83	4.72	20.10	3.05	3.58	7.43	3.07
NS7	28.68	40.67	3.32	7.49	0.77	6.53	44.36	7.33	4.18	46.25	48.32	1.86	9.00	11.46	5.74	19.21	3.22	3.36	8.23	3.38
Sem	8.9	0.9	0.01	0.01	0.01	0.46	1.29	6.41	1.25	8.79	11.33	0.19	0.19	0.38	0.17	0.98	0.19	0.08	0.79	0.1
LSD (5%)	8.90	2.52	0.11	0.18	0.14	1.10	1.86	6.71	3.53	4.80	5.45	0.70	0.70	0.99	0.67	0.16	0.70	0.47	0.44	0.52
CV (%)	60.85	26.52	30.36	14.88	66.27	95.48	21.03	79.39	43.36	25.88	25.03	58.88	18.00	21.49	18.18	20.14	36.83	24.77	15.37	20.33

FWT = Fruit weight; JC = juice content; pH = juice pH; TSS = total soluble solids; TA = total acidity; VITC = ascorbic acid (mg/100ml juice); SDN = seed number; SDWT = 100 seed weight; FLT = fruit length; FWD = fruit width; PLTK = peel thickness; SEGN = segment/fruit; PTLT = petal length; PTWD = petal width; OFD = opened flower diameter; PDLT = pedicel length; CALD = calyx diameter; LFLT = leaf length; LFWD = leaf width.

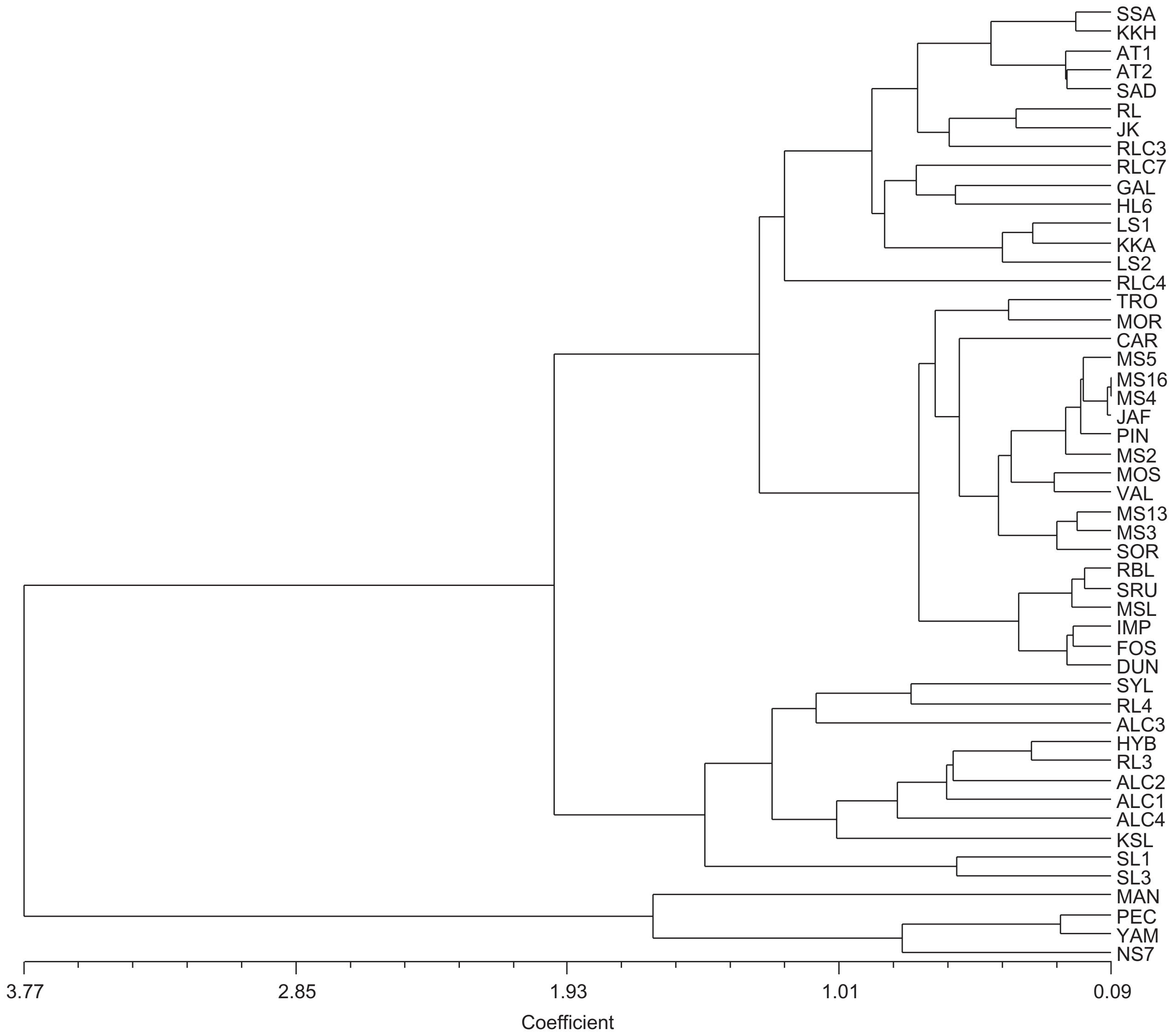

Fig 1 UPGMA dendrogram (based on Euclidean distance coefficient) of 50 genotypes generated by morphological characters

Euclidean coefficient. Minor Sub-cluster I contain the maximum genotypes (20) and presented most homogeneous clusters with Euclidean distance ranging from 0.10 – 0.90. This cluster was constituted by citranges, sweet oranges (Mosambi, Pineapple, Jaffa, Valencia, MS 2, MS 3, MS 4, MS 5, MS 13 and MS 16), grapefruit (Imperial, Marsh Seedless, Star Ruby, Red Blush, Foster, Duncan) and Sour orange and characterized by highest performance for fruit physico-chemical characters. This low genetic dissimilarity can be attributed to the presence of low variation within sweet orange and grapefruit as reported by Barrett and Rhodes (1976), Nicolosi *et al.* (2000) and Uzun *et al.* (2010). Citranges, though distantly realted to citrus, was observed to cluster closely with sweet oranges and this can be attributed to the fact that citranges shared sweet orange as one of the parents during its evolution (Savage and Gardner 1965). Minor Sub-cluster II comprised of fifteen genotypes, viz. Jatti Khatti, Rough lemon, RLC 3, RLC 4, RLC 7, Sadaphal, Attani 1, Attani 2, Karna Khatta, Soh Sarkar, Galgal, Hill lemon 6, LS 1, LS 2, Kagzi Kalan, Sweet lemon 1 and Sweet lemon 3. Except for pH, TSS and TSS:TA, this cluster was characterized by higher performance for most of the fruit physico-chemical traits.

The cophenetic analyses comparing the UPGMA cluster analysis and the dissimilarity matrix demonstrated a correlation of r= 0.9, indicating that data in the matrix was well represented by the dendrogram. Genotypes representing clusters separated by the high genetic distance should be utilized in breeding programmes as heterosis expressed in hybrids is directly related to the diversity between the parents. Similar findings were also observed in Indian jujube (Saran *et al.*2006). Based on pair-wise Euclidean distance matrix of citrus genotypes, maximum dissimilarity was noted between Pectinefera and Imperial (4.92 Euclidean distance), Pectinefera and Redblush (4.91 Euclidean distance), Pectinefera and Star Ruby (4.86 Euclidean distance), Mandarin and Imperial (4.86 Euclidean distance) and Mandarin and Redblush (4.84 Euclidean distance).As

Table 3 Means and standard deviations (SD) of clusters based on 21 quantitative traits of 50 citrus genotypes

Trait	Cluster I		Sub-cluster I		Minor Sub-cluster I		Minor Sub-cluster II		Cluster Means
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Fruit weight (g)	24.09	4.20	84.28	21.56	309.47	84.96	169.90	49.40	146.94
Juice content (%)	35.11	7.60	38.84	11.14	39.87	11.25	33.85	7.30	36.92
Juice pH	3.36	0.03	3.14	1.39	4.09	0.82	2.66	0.33	3.31
TSS (%)	7.65	0.22	6.95	0.39	8.32	0.86	6.25	0.53	7.29
Acidity (%)	1.10	0.49	4.62	2.15	1.61	1.42	4.77	0.60	3.03
TSS:Acidity	6.93	2.19	4.09	6.50	8.14	4.16	1.43	0.44	5.15
Ascorbic acid (mg/100 ml juice)	44.05	1.40	32.30	7.13	45.94	6.53	43.78	8.01	41.52
Number of seed/fruit	6.92	0.32	8.85	7.71	16.55	13.45	16.93	11.40	12.31
100 seed weight (g)	7.04	2.16	11.30	6.89	16.08	5.45	14.33	5.76	12.19
Fruit length (mm)	39.81	8.94	59.11	3.89	79.06	12.44	80.04	20.10	64.50
Fruit width (mm)	45.17	7.84	54.04	4.79	82.96	13.12	81.09	13.81	65.81
Peel thickness (mm)	1.40	0.55	2.66	0.86	5.77	1.52	6.72	3.85	4.14
Segments/fruit	8.58	0.69	10.24	2.08	11.50	2.06	11.04	1.42	10.34
Petal length (mm)	10.01	1.39	16.50	4.65	17.26	1.77	19.36	2.70	15.78
Petal width (mm)	4.82	0.64	5.46	1.34	6.74	0.71	6.41	0.98	5.86
Flower dia. (mm)	19.46	0.63	28.03	8.07	30.07	3.01	33.29	4.52	27.71
Pedicel length (mm)	3.17	0.08	4.17	1.78	6.55	1.64	5.98	2.04	4.97
Calyx dia. (mm)	3.36	0.16	4.60	1.54	6.15	0.76	6.42	1.01	5.13
Leaf length (cm)	7.46	0.82	8.70	1.22	9.37	1.49	9.25	1.23	8.69
Leaf width (cm)	3.15	0.27	4.26	0.64	4.13	0.98	4.81	0.59	4.09

presented in Table 5, maximum inter cluster distance was observed between Cluster I and Minor Sub-cluster I (4.41 Euclidean distance), which is indicative of getting more heterotic segregants if parents from these two groups were carefully chosen in citrus breeding programs.

Principal component analysis, as a data reduction tool plays an important role in identifying important traits responsible for differentiation between genotypes which at the same time, revealed some aspects of interrelation that were not interpretable by the cluster analysis. Hair *et al.* (1998) suggested that eigen values greater than one are significant and should only be considered so that fewer components are dealt with. Furthermore, according to Guei *et al.* (2005),first three principal components are often the most important in reflecting the variation patterns among the different genotypes and the characters associated with these are most important in differentiating various genotypes. Hence, the first three components were extracted to explain the variability existed among the 50 genotypes. Principal component analysis (Table 5) gave first 10 principal components having eigen values greater than 1, which contributed 93.38% of the total variability of the collected genotypes. The first 5 principal components accounted for 75.72% of the variation and the first three accounted for 58.72% of the variance, in which maximum variability was contributed by first component (28.67%) followed by 2nd component (16.9%) and 3rd component (13.14%). Result from Table 6 showed that PC1 was most highly influenced by characteristics of the fruit morphology, viz. juice content (3.99), pH (5.62), TSS (6.04), total acidity (–5.68), peel thickness (–3.17) and TSS:TA (5.97). In PC2, the variability was positively contributed by petal width (3.35) and negatively contributed by juice content (–3.62), total acidity (–4.89), leaf length (–3.37) and leaf width (–3.38). The third PC was mostly influenced by fruit weight (3.38), seeds/fruit (3.17), peel thickness (3.12) and petal length (3.35). The results of present studies are in accordance with those of Malik *et al.* (2012) and Shrestha *et al.*(2012) who reported fruit weight, fruit length, fruit diameter, fruit rind thickness, TSS, pH, TSS, total acidity, TSS/total acidity, leaf length and leaf width as important variables with the highest attribution to the variation contributed by the principal components.

The projection of the 50 citrus genotypes onto the graph defined by the first two principal components confirmed the same grouping pattern reflected in the dendrogram.Based on component values, the location of genotypes and their grouping were determined in the biplot. Cluster I comprised small fruited mandarin genotypes and located on the negative side of both the components was influenced by lowest performance for fruit physico-chemical and flowers characters. Sub-cluster I was located on the positive side of PC2 and distinguished by low pH, TSS, high total acidity and lower TSS:TA. Minor Sub-cluster I was located on the positive side of PC1 and was distinguished by high TSS:TA, seed number and seed weight, fruit weight, fruit size, segment per fruit, leaf length and leaf width, whereas Minor Sub-cluster II was located on both the positive side of PC1 and PC2 and distinguished by low TSS and high petal width.

In conclusion, significant variability among the genotypes was observed. The analysis of genetic distance among 50 genotypes clearly separates the genotypes into two main groups that represent a variable assembly of

Table 4 Euclidean distance matrix between genotypes using 20 morphological data

	SSA	AT1	AT2	KKH	RLC7	SYL	TRO	CAR	SAD	RL	HYB	MOR	RBL	IMP	FOS	SRU	MSL	DUN	MS5	MS16	MS4	MS13	MOS	PIN	JAF
SSA	0.00																								
AT1	0.52	0.00																							
AT2	0.45	0.24	0.00																						
KKH	0.20	0.43	0.43	0.00																					
RLC7	1.13	1.09	1.08	1.05	0.00																				
SYL	1.85	1.76	1.82	1.78	0.93	0.00																			
TRO	0.54	0.90	0.75	0.69	1.51	2.22	0.00																		
CAR	0.57	0.71	0.51	0.64	1.25	2.05	0.56	0.00																	
SAD	0.59	0.25	0.24	0.52	1.14	1.87	0.92	0.68	0.00																
RL	0.65	0.56	0.62	0.58	0.71	1.33	1.06	0.88	0.73	0.00															
HYB	1.57	1.39	1.51	1.46	0.98	0.83	1.94	1.76	1.56	0.99	0.00														
MOR	0.58	0.94	0.79	0.75	1.53	2.26	0.43	0.68	0.91	1.12	2.06	0.00													
RBL	1.22	1.46	1.20	1.33	1.81	2.63	1.04	0.95	1.32	1.66	2.55	0.88	0.00												
IMP	1.20	1.37	1.18	1.30	1.82	2.61	1.15	1.07	1.19	1.63	2.55	0.85	0.50	0.00											
FOS	1.15	1.39	1.19	1.26	1.76	2.59	1.02	0.94	1.25	1.61	2.51	0.83	0.17	0.41	0.00										
SRU	1.13	1.32	1.13	1.22	1.74	2.55	1.06	0.98	1.15	1.58	2.48	0.81	0.22	0.22	0.27	0.00									
MSL	1.08	1.31	1.11	1.17	1.66	2.50	0.97	0.83	1.18	1.52	2.40	0.84	0.23	0.55	0.20	0.37	0.00								
DUN	1.13	1.33	1.12	1.25	1.77	2.56	1.01	0.96	1.18	1.57	2.49	0.77	0.35	0.24	0.30	0.23	0.42	0.00							
MS5	0.71	0.98	0.77	0.83	1.41	2.22	0.65	0.52	0.88	1.16	2.08	0.51	0.53	0.64	0.49	0.54	0.42	0.52	0.00						
MS16	0.68	0.97	0.78	0.79	1.34	2.16	0.65	0.53	0.90	1.11	2.03	0.53	0.63	0.74	0.58	0.63	0.51	0.63	0.19	0.00					
MS4	0.68	0.97	0.78	0.79	1.34	2.15	0.68	0.55	0.89	1.10	2.03	0.52	0.62	0.70	0.57	0.60	0.52	0.60	0.17	0.08	0.00				
MS13	0.54	0.76	0.61	0.60	1.01	1.81	0.78	0.62	0.70	0.81	1.72	0.67	0.97	0.94	0.71	0.86	0.85	0.88	0.51	0.42	0.41	0.00			
MOS	0.79	1.08	0.90	0.89	1.18	1.98	0.85	0.68	1.02	1.07	1.94	0.74	0.76	0.89	0.74	0.78	0.67	0.78	0.42	0.33	0.31	0.41	0.00		
PIN	0.82	1.09	0.89	0.92	1.41	2.24	0.74	0.59	1.00	1.24	2.12	0.64	0.48	0.71	0.47	0.57	0.37	0.56	0.19	0.19	0.22	0.56	0.36	0.00	
JAF	0.71	1.01	0.81	0.82	1.33	2.15	0.69	0.57	0.92	1.13	2.03	0.57	0.61	0.71	0.56	0.60	0.49	0.59	0.18	0.10	0.09	0.42	0.28	1.62	0.00
MS2	0.71	0.98	0.77	0.81	1.36	2.19	0.65	0.43	0.90	1.12	2.02	0.61	0.59	0.79	0.58	0.67	0.46	0.66	0.19	0.26	0.27	0.56	0.43	0.21	0.26
MS3	0.65	0.89	0.71	0.74	1.16	1.95	0.79	0.67	0.79	0.98	1.89	0.63	0.82	0.78	0.75	0.70	0.72	0.71	0.42	0.34	0.32	0.20	0.35	0.46	0.32
VAL	1.01	1.32	1.12	1.13	1.35	2.12	1.02	0.90	0.24	1.31	2.16	0.85	0.67	0.83	0.67	0.74	0.65	0.70	0.53	0.49	0.47	0.62	0.28	0.45	0.43
GAL	0.95	0.85	0.88	0.81	0.63	1.29	1.33	1.11	0.90	0.73	1.08	1.42	1.71	1.74	1.67	1.63	1.53	1.68	1.29	1.26	1.28	0.99	1.20	1.32	1.26
HL6	1.10	0.77	0.82	0.99	0.87	1.36	1.39	1.15	0.84	0.77	1.00	1.53	1.85	1.84	1.83	1.76	1.69	1.77	1.44	1.43	1.44	1.66	1.43	1.50	1.44
JK	0.83	0.78	0.88	0.71	0.64	1.16	1.29	1.18	0.92	0.41	0.90	1.32	1.86	1.81	1.81	1.75	1.72	1.78	1.38	1.31	1.31	0.97	1.27	1.44	1.32
LS1	0.76	0.83	0.82	0.71	0.79	1.44	0.04	0.85	0.97	0.51	1.06	1.21	1.64	1.75	1.62	1.66	1.50	1.65	1.19	1.15	1.15	0.97	1.10	1.22	1.16
LS2	1.14	1.07	1.14	1.06	0.95	1.29	1.40	1.26	1.26	0.77	0.79	1.67	2.08	2.18	2.07	2.08	1.93	2.08	1.63	1.59	1.61	1.38	1.55	1.67	1.61
ALC1	1.89	1.69	1.79	1.75	1.24	1.14	2.20	1.99	1.83	1.38	0.63	2.39	2.76	2.82	2.74	2.73	2.60	2.75	2.34	2.28	2.29	2.01	2.21	2.36	2.29
ALC2	2.03	1.88	1.98	1.93	1.50	1.28	2.32	2.16	2.06	1.53	0.71	2.51	2.95	3.02	2.93	2.93	2.80	2.94	2.51	2.47	2.48	2.23	2.40	2.54	2.48
ALC3	1.74	1.49	1.59	1.64	0.97	0.81	2.17	1.96	1.55	1.25	0.93	2.14	2.50	2.36	2.45	2.33	2.36	2.36	2.10	2.07	2.05	1.69	1.96	2.16	2.05
ALC4	2.22	1.92	2.06	2.07	1.66	1.48	2.56	2.32	2.06	1.70	0.91	2.72	3.12	3.11	3.08	3.04	2.95	3.07	2.69	2.66	2.66	2.38	2.61	2.74	2.67
KKA	0.87	0.89	0.91	0.82	1.00	1.52	1.06	0.98	1.06	0.64	1.01	1.31	1.82	1.91	1.81	1.82	1.67	1.81	1.35	1.31	1.33	1.15	1.31	1.39	1.35
SL1	1.59	1.69	1.69	1.57	1.17	1.12	1.77	1.77	1.82	1.32	1.15	1.95	2.32	2.45	2.32	2.34	2.21	2.32	1.93	1.84	1.86	1.61	1.68	1.91	1.82
SL3	1.28	1.39	1.35	1.26	0.85	1.17	1.49	1.39	1.46	1.06	1.26	1.61	1.87	1.98	1.87	1.87	1.75	1.86	1.48	1.39	1.41	1.14	1.20	1.44	1.37
KSL	2.01	1.94	2.01	1.94	1.58	1.29	2.21	2.17	2.11	1.57	0.90	2.44	2.94	3.01	2.94	2.93	2.82	2.91	2.51	2.43	2.45	2.18	2.35	2.53	2.45
RLC3	0.54	0.87	0.88	0.55	1.02	1.59	0.90	0.99	1.00	0.64	1.35	0.94	1.61	1.61	1.54	1.53	1.47	1.55	1.12	1.04	1.04	0.85	1.04	1.17	1.07
RLC4	1.25	1.35	1.47	1.14	1.29	1.52	1.60	1.69	1.49	1.13	1.20	1.71	2.31	2.30	2.24	2.20	2.15	2.26	1.85	1.78	1.79	1.53	1.78	1.90	1.81
RL3	1.82	1.68	1.79	1.72	1.19	0.91	2.16	1.98	1.85	1.25	0.36	2.30	2.77	2.80	2.74	2.74	2.63	2.73	2.32	2.26	2.26	1.97	2.15	2.35	2.27
RL4	2.42	2.34	2.41	2.34	1.49	0.76	2.78	2.59	2.46	1.88	1.12	2.85	3.20	3.23	3.17	3.16	3.07	3.17	2.81	2.74	2.73	2.42	2.55	2.81	2.72
SOR	0.55	0.70	0.51	0.62	1.06	1.86	0.75	0.53	0.62	0.81	1.75	0.62	0.92	0.86	0.85	0.81	0.81	0.79	0.47	0.44	0.41	0.26	0.48	0.57	0.44
MAN	4.12	4.19	4.25	4.09	3.52	2.85	4.38	4.37	4.31	3.74	3.15	4.45	4.84	4.86	4.81	4.42	4.76	4.80	4.50	4.41	4.40	4.13	4.24	4.49	4.39
PEC	3.96	3.93	4.06	3.89	3.53	2.97	4.26	4.24	1.09	3.57	2.84	4.39	4.91	4.91	4.86	4.86	4.78	4.87	4.49	4.39	4.40	4.13	4.29	4.50	4.41
YAM	3.82	3.75	3.89	3.73	3.34	2.76	4.09	4.06	3.92	3.38	2.63	4.23	4.72	4.76	4.71	4.70	4.62	4.72	4.32	4.23	4.24	3.97	4.13	4.34	4.25
NS7	3.83	3.77	3.88	3.75	3.19	2.53	4.13	4.05	3.92	3.36	2.58	4.23	4.69	4.70	4.66	4.65	4.57	4.67	4.29	4.20	4.20	3.92	4.05	4.29	4.21

Contd.

Table 4 *Contd.*

	MS2	MS3	VAL	GAL	HL6	JK	LS1	LS2	ALC1	ALC2	ALC3	ALC4	KKA	SL1	SL3	KSL	RLC3	RLC4	RL3	RL4	SOR	MAN	PEC	YAM	NS7
MS2	0.00																								
MS3	0.52	0.00																							
VAL	0.59	0.51	0.00																						
GAL	1.22	1.15	1.44	0.00																					
HL6	1.38	1.30	1.64	0.61	0.00																				
JK	1.35	1.15	1.48	0.65	0.86	0.00																			
LS1	1.08	1.13	1.34	0.71	0.86	0.68	0.00																		
LS2	1.53	1.55	1.78	0.81	0.83	0.82	0.51	0.00																	
ALC1	2.25	2.18	2.43	1.28	1.20	1.23	1.32	0.97	0.00																
ALC2	2.41	2.41	2.61	1.52	1.45	1.46	1.40	1.00	0.65	0.00															
ALC3	2.10	1.83	2.10	1.18	1.09	1.08	1.50	1.49	1.28	1.50	0.00														
ALC4	2.61	2.55	2.84	1.62	1.44	1.61	1.70	1.33	0.66	0.78	1.41	0.00													
KKA	1.25	1.30	1.54	0.85	0.86	0.81	0.35	0.39	1.26	1.32	1.58	1.62	0.00												
SL1	1.86	1.73	1.83	1.28	1.40	1.25	1.22	1.08	1.39	1.45	1.58	1.88	1.17	0.00											
SL3	1.42	1.27	1.35	0.98	1.19	1.03	1.01	1.08	1.52	1.68	1.47	2.00	1.08	0.61	0.00										
KSL	2.44	2.32	2.54	1.69	1.59	1.49	1.51	1.13	0.97	0.98	1.67	1.34	1.33	1.18	1.55	0.00									
RLC3	1.10	0.99	1.25	0.95	1.22	0.62	0.69	0.99	1.71	1.78	1.64	2.08	0.78	1.32	1.12	1.71	0.00								
RLC4	1.82	1.69	1.99	1.11	1.41	0.83	0.15	1.09	1.46	1.56	1.52	1.78	1.12	1.35	1.38	1.47	0.86	0.00							
RL3	2.24	2.14	2.36	1.33	1.30	1.15	1.22	0.88	0.65	0.53	1.20	0.87	1.19	1.26	1.42	0.87	1.55	1.36	0.00						
RL4	2.74	2.57	2.69	1.83	1.90	1.70	1.89	1.64	1.21	1.21	1.36	1.44	1.92	1.49	1.68	1.33	2.11	1.89	0.94	0.00					
SOR	0.52	0.27	0.67	1.04	1.14	1.03	0.98	1.40	2.04	2.24	1.71	2.38	1.16	1.73	1.30	2.22	0.92	1.64	2.00	2.47	0.00				
MAN	4.47	4.24	4.29	3.85	3.96	3.55	3.78	3.58	3.19	3.13	3.38	3.37	3.77	3.08	3.41	2.85	3.47	3.42	2.97	2.38	4.23	0.00			
PEC	4.43	4.29	4.47	3.66	3.70	3.38	3.58	3.27	2.77	2.65	3.25	2.72	3.50	3.12	3.47	2.55	3.62	3.11	2.62	2.46	4.23	1.76	0.00		
YAM	4.27	4.12	4.31	3.47	3.50	3.19	3.39	3.06	2.56	2.43	3.13	2.50	3.30	2.94	3.29	2.34	3.44	2.93	2.40	2.26	4.05	1.79	0.25	0.00	
NS7	4.24	4.07	4.21	3.43	3.47	3.17	3.37	3.07	2.53	2.42	2.97	2.51	3.32	2.87	3.19	2.34	3.46	3.03	2.33	1.93	4.00	1.35	0.84	0.75	0.00

Table 5 Eigen values and proportion of total variability among 50 genotypes of citrus based on quantitative traits as explained by the first 8 Principal Components

	Eigen values	Explained variation	Cumulative variation
1	195.23	28.67	28.67
2	115.11	16.90	45.57
3	89.51	13.14	58.72
4	70.09	10.29	69.01
5	45.68	6.71	75.72
6	36.37	5.34	81.06
7	30.99	4.55	85.61
8	23.62	3.47	89.08
7	16.21	2.38	91.46
8	13.09	1.92	93.38

Table 6 Eigenvectors of quantitative variables explained by first three principal components

Trait	PC1	PC2	PC3
Fruit weight	1.12	1.08	3.39
Juice content	3.99	–3.62	–1.16
Juice pH	5.61	0.83	–0.67
TSS	6.04	–0.99	0.11
Acidity	–5.68	–4.89	–2.08
TSS:Acidity	5.97	0.61	–1.36
Ascorbic acid	0.43	2.67	–0.86
Number of seed/fruit	–1.86	–2.22	3.17
100 seed weight	–0.07	1.21	1.17
Fruit length	–2.34	0.22	2.39
Fruit width	–1.13	1.01	2.89
Peel thickness	3.17	0.78	3.12
Segments/fruit	0.57	–2.25	1.47
Petal length	–2.91	2.02	–3.35
Petal width	–0.46	3.35	–2.57
Flower diameter	–2.40	2.44	–2.48
Pedicel length	–0.89	1.85	0.63
Calyx diameter	–1.87	2.65	–0.87
Leaf length	0.48	–3.37	–0.41
Leaf width	–1.42	–3.39	–1.82

genotypes. Principal component analysis identified seeds/fruit, peel thickness, juice content, pH, TSS, TSS:TA and petal size as important variables that can be used to differentiate genotypes. Such knowledge on the range of genetic diversity present among the genotypes will facilitate its use in management and conservation and in selection of cross-parents in breeding programs.

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