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Morphological characterization of pummelo germplasm collected from different parts of India

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ABSTRACT

Pummelo is a natural non hybrid mono-embryonic and sub-tropical fruit species with vast morphological variability. Morphological characterization of 46 accessions collected from different parts of India on the basis of 42 phenotypic characters including leaf, fruit and seed characters is presented in the paper. Pair-wise similarity analysis revealed moderate to significant variation in the 46 accessions of pummel accessions. Pairwise similarity among the pummelo accessions ranged from 0.31 to 0.66 with an average of 0.48 based on morphometric data. Maximum similarity (0.66) was observed between accessions number of PS-1 and PS-3. Based on Euclidean distance divided these accessions into five main clusters out of which, Cluster I proved the largest cluster comprising of 14 accessions while cluster II consisted of seven accessions and cluster III with ten accessions. Cluster IV was smallest one with only two accessions, while cluster V consisted of 13 accessions. Principal component analysis based on 42 morphological characters showed significant variation among all the accessions. The least variation was observed in qualitative characters, while the quantitative characters significantly differed. The two dimensional (2-D) plot generated from PCA showed three groups which were found to be more or less similar to the clustering pattern of the UPGMA dendrogram. The PCA identified traits were most significant that were responsible for grouping of certain genotypes into specific cluster and hence, revealed some aspects of interrelation among genotypes that were not discernable by the UPGMA analysis. The analysis gave 36 principal components out of which first ten principal components contributed 69.52% of the total variability. The results derived from this study indicated significant level of variability present within the pummelo group in India, which can be used as important source of genetic diversity and for future breeding programmes.

Key words: *Citrus maxima*, quantitative characters, cluster analysis, principal component analysis.

INTRODUCTION

In India, natural undisturbed populations of citrus genepool observed in North Eastern Himalayan region and the hills of the Central and Western Himalayan region, rich in diverse *Citrus* genetic resources. There are several *Citrus* species, which include 52 cultivars and 7 probable natural hybrids, reported to have originated from North Eastern region of India (Bhattacharya and Dutta, 1). Being important *Citrus* species, *C. maxima* (pummelo) is believed to be originated in Polynesia and Malaya region (Morton, 10). However, this is one of the most widely cultivated citrus fruit crop in Southeast Asia (Malik *et al*., 7). The *C. maxima* is one of the basic species which has importance in *Citrus* taxonomy and played important role in origin and evolution of various other types of *Citrus* species through hybridization for one or more generations between the three species viz., *C. maxima, C. medica* and *C. reticulata* or some subgenus or closely related genera. In India, pummelos (including grape fruit) are grown in 16,850 hectare area with an annual production of 3,90,500 tonnes, and productivity of 231.75 t/ha during 2016-17 (FAOSTAT, 4). The fruits of *C. maxima* have cryo-protective action due to the presence of antioxidants including vitamin C, phenolics, carotenoids and flavonoid (Mokbel and Hashinaga, 9) thus, possess high shelf life. Like other economic tree species in *C. maxima* also there is a great threat of declining variability due to artificial selection. As during the process of domestication there is decrease in genotypic variation leading to decline in variability due to elimination of unwanted phenotypic characters (Wen *et al*., 15). Therefore, the characterization of Citrus genetic resources is of great importance for the assessment and quantification of existing diversity within the species/ cultivars for analysing the relationship between them for efficient management of germplasm. Estimation of genetic diversity is very helpful for exploration and collection of important genotypes, for monitoring genetic erosion, selecting suitable species for breeding programmes and to develop *in situ* and *ex situ* conservation strategies.

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This would also be supportive for food industry to recognize the food and nutritional value of biochemical constituents of genotypes collected from various parts of the world. Thus, the morphological characterization data plays an important role in providing physio-chemical information in recognition of plant taxa whcih is more important than information generated from DNA barcoding techniques for particular taxa (Ceolin and Miotto, 2). Hence, in the present study an attempt was made to study the possible patterns of genetic relatedness among 46, pummelo genotypes using agro-morphological characters.

MATERIALS AND METHODS

In the present study, the morphological characterisation studies were undertaken at cryolab of Tissue Culture and Cryopreservation (TCCU) at ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi (India) where the collected germplasm was brought. Systematic survey and collection was undertaken from different States of India and one exotic collection from USA (Table 1) during 2015-17. The leaves and fruits (n=3) of 46 accessions of pummelo were collected from farmer's field, natural habitat and field genebanks, etc.

Qualitative and quantitative data were recorded with the help of descriptor developed by International Plant Genetic Resources Institute (IPGRI), Rome, Italy (IPGRI, 5). The collected accessions were characterized on the basis of leaf, fruit and seed characters.

The fruits were cut open to extract the seeds by peeling off the transparent membrane of closely packed carpel segments and removing the mucilage

Table 1. Pummelo accessions collected and morphologically characterized.

S. No.	Collector name	Common name	Sources	Latitude (°)	Longitude (°)
1	Pusa 1	Chakotra	Bihar	25.55	85.50
2	Pusa 2	Chakotra	Bihar	25.55	85.50
3	Pusa 3	Chakotra	Bihar	26.07	85.27
4	Pusa 4	Chakotra	Bihar	26.07	85.27
5	Pusa 5	Chakotra	Bihar	26.07	85.27
6	Pusa 6	Chakotra	Bihar	25.55	85.50
7	UK- 1	Chakotra	Uttarakhand	30.25	78.13
8	UK- 2	Chakotra	Uttarakhand	30.25	78.13
9	UK- 3	Chakotra	Uttarakhand	30.15	79.13
10	UK- 4	Chakotra	Uttarakhand	30.15	79.13
11	M1	Jambura	Meghalaya	25.57	91.89
12	P1	White Pummelo	Punjab	30.14	74.19
13	P2	Red flesh	Punjab	30.14	74.19
14	EC- 145	Pummelo	USA		
15	KS-1	White flashed	Karnataka	12.37	75.83
16	KS-2	Devanahalli Pink flashed	Karnataka	12.37	75.83
17	KS-3	Royal	Karnataka	12.37	75.83
18	KS-4	Kanaoura	Karnataka	12.37	75.83
19	KS-5	Seedless selection	Karnataka	12.37	75.83
20	UK-5	Chakotra	Uttarakhand	30.29	78.07
21	AP-1	Pummelo	Arunachal Pradesh	27.56	94.20
22	UK-6	Chakotra	Uttarakhand	30.29	78.07
23	UK-7	Chakotra	Uttarakhand	30.15	79.15
24	PS-1	Pummelo	New Delhi	28.63	77.16
25	PS-2	Pummelo	New Delhi	28.63	77.16
26	PS-3	Pummelo	New Delhi	28.63	77.16
27	PS-4	Pummelo	New Delhi	28.63	77.16

S. No.	Collector name	Common name	Sources	Latitude (°)	Longitude (°)
28	PS-5	Pummelo	New Delhi	28.63	77.16
29	PS-6	Pummelo	New Delhi	28.63	77.16
30	PS-7	Pummelo	New Delhi	28.63	77.16
31	PS-8	Pummelo	New Delhi	28.63	77.16
32	PS-9	Pummelo	New Delhi	28.63	77.16
33	PS-10	Pummelo	New Delhi	28.63	77.16
34	PS-11	Pummelo	New Delhi	28.63	77.16
35	MH-1	Pummelo	Maharashtra	20.00	76.00
36	MH-2	Pummelo	Maharashtra	20.00	76.00
37	MH-3	Pummelo	Maharashtra	20.00	76.00
38	MH-4	Pummelo	Maharashtra	20.00	76.00
39	MH-5	Pummelo	Maharashtra	20.00	76.00
40	MH-6	Pummelo	Maharashtra	20.00	76.00
41	MH-7	Pummelo	Maharashtra	20.00	76.00
42	MH-8	Pummelo	Maharashtra	20.00	76.00
43	MH-9	Pummelo	Maharashtra	20.00	76.00
44	MH-10	Pummelo	Maharashtra	20.00	76.00
45	MDMS-18	Pummelo	Arunachal Pradesh	27.56	94.20
46	MDMS19	Pummelo	Arunachal Pradesh	27.56	94.20

by washing in warm water. Seeds were surface dried by spreading on the blotting sheets at room temperature to remove excess water. Number of seeds per fruits, seed shape, size (length x breadth), colour and seed weight were recorded as per the descriptor. A pairwise similarity matrix and "Unweighted Pair Group Method using Arithmetic Means" (UPGMA) dendrogram were generated based on 42 morphological characters of 46 cultivars of *C. maxima* using NTSYS software ver. 2.10e (Rohlf, 12). A pairwise similarity matrix was generated using Simple Matching coefficient and an NJ tree was constructed based on Euclidean distance method with the same software.

RESULTS AND DISCUSSION

Pummelo trees are about 15 – 45 ft in height with irregular branches. The young branches are angular and often densely hairy; spines were present on the branches and old branches**.** The leaves were simple, dark green with evergreen vegetative life cycle. These are brevipetiolate (petiole shorter than leaf lamina). Leaf lamina shape varied from elliptic, orbicular to ovate, ovate-oblong. Leaves were 8-16 cm long and 4-8 cm wide across different accessions. The leaf apex showed variation ranging from acute to obtuse and also rounded in some accessions with the leaf margin almost entire, crenate and dentate. Leaves bear distinctly broad winged petioles which were mostly deltoid, obovate, cordiform in shape. The fruit shape varied considerably among the accessions and was mostly spheroid, ellipsoid and pyriform, whereas few fruits were oblique and obloid. Fruit base was mostly truncate, concave collard, depressed and some had convex and concave base. Fruit apex was rounded, truncate and depressed. Skin colour of most of the fruits varied from yellow to greenish yellow with few green coloured fruits also. The fruits were mostly pitted with a rough surface and few have smooth surface texture with inconspicuous, conspicuous or strongly conspicuous oil glands. The fruits were characterized by the presence of large sized oil glands. Fruit size also showed wide variation with fruit length varying from 12 to 136 cm with an average of 55.54 cm, fruit diameter from 16 to 136 cm with an average of 60.42 cm and fruit weight from 225 to 2113 g with an average of 846.43 g. Fruit rind thickness varied from 1.00 to 27.25 mm with an average of 6.73 mm and number of segments ranged from 8 to 23 with an average of 14.78. Most of the cultivars were with high juice content except few having low to medium content and TSS value ranged from 6 to 14 with an average of 9.62 (Table 2).Almost all cultivars were seeded having 4 to 174 seeds per fruit. Seeds also showed variation in seed shape ranging from fusiform, ovoid, semi-deltoid to spheroid with smooth and wrinkled seed surface.

Table 2. Quantitative leaf, fruit and seed characters of 46 cultivars of *Citrus maxima.*

Fruits	Variables (Range)	Mean
Fruity weight (g)	225 - 2113	846.43
Fruit diameter (cm)	16 - 136	60.42
Fruit length (cm)	12 - 136	55.54
Width of epicarp at equatorial area (mm)	0.1 - 7.73	1.33
Fruit rind thickness (mm)	1 - 27.25	6.73
No. of segments per fruit	8 - 23	14.78
Taste of juice TSS	6 - 14	9.62
	Seed	
No. of Seed/fruit	4 - 174	53.91
10 Seed wt. (gm)	1.56 to 5.35	3.40
Seed moisture (%)	26.43 - 46.33	34.58
	Leaf	
Leaf lamina length (cm)	8 - 16	11.64
Leaf lamina width (cm)	4 - 8	5.57
Leaf thickness (mm)	0.17 - 0.20	0.19
Petiole wing width (cm)	0.4 - 3.4	2.41

Mature seeds were whitish and creamish in colour, and cotyledon colour was white, cream and yellow with light brown and brown, chalazal spot. Ten seed weight ranged from 1.56 to 5.35 g with an average of 3.40 g. Fresh seeds showed high moisture content ranging from 26.43 to 46.33% with an average of 34.58% Table 2). All the studied cultivars were mono-embryonic in nature.

Pair-wise similarity analysis of 42 morphological characters examined in the 46 accessions of *C. maxima* cultivars revealed moderate to significant variation among them. Pairwise similarity among the pummelo accessions ranged from 0.31 to 0.66 with an average of 0.48 based on morphometric data. Maximum similarity (0.66) was observed between accessions number of PS-1 and PS-3. The NJ tree (Fig. 1), based on Euclidean distance divided the accessions into five main clusters: Cluster I was the largest cluster comprising of 14 accessions while cluster II consisted of seven accessions, cluster III with ten accessions, cluster IV was smallest one with only two accessions while cluster V consisted of 13 accessions. The first cluster was further subdivided into two sub-clusters, in which accessions UK-1 and UK-2, Pusa 2 and Pusa 6, UK-6 and MH-4 were found to be closely related to each other morphologically each pair with a similarity value of 0.55. In the second cluster accessions MH-1 and MH-9 were found to be closely related to each other with a similarity value of 0.61. In the third cluster accessions M-1 and KS-1 were found to be closely related to each other with a similarity value of 0.50. Fourth cluster have only two accessions EC-145 and UK-5 which were similar to each other with a similarity value of 0.42. In the fifth cluster accessions PS-1 and PS-3 were found to be closely related to each other from all of the accessions with a similarity value of 0.66. Based on Mantel Z statistics, the correlation coefficient (r) was estimated as 0.78. A value of 0.78 is considered a good fit of the UPGMA cluster pattern to the data. The two dimensional (2-D) plot (Fig. 2) generated from PCA showed three groups which was found to be more or less similar to the clustering pattern of the UPGMA dendrogram. The analysis gave 36 principal components out of which first ten principal components contributed 69.52 % of the total variability. The first five principal components accounted for 45.17 % of the total variation and the first three accounted for 31.39 % of the variation, in which maximum variation was contributed by first component (13.30 %) followed by second component (10.14 %), and third component (7.94 %) (Table 3).

In the present study, 42 morphological characters were used for characterization of 45 indigenous accessions collected from different parts of India and 1 exotic accession (EC-145) collected from USA. The phenotypic expression has more importance for the selection of suitable genotypes for specified characters which is valuable commercially. Therefore, morphological characterization in *C.*

Table 3. Eigen values and proportion of total variability among cultivars of *Citrus maxima*.

Sr No	Eigenvalue	Percent	Cumulative
1	5.987869	13.3064	13.3064
2	4.561947	10.1377	23.444
3	3.573829	7.9418	31.3859
4	3.320912	7.3798	38.7657
5	2.880932	6.4021	45.1678
6	2.771654	6.1592	51.327
7	2.307882	5.1286	56.4556
8	2.264675	5.0326	61.4882
9	1.983335	4.4074	65.8956
10	1.634867	3.633	69.5287
11	1.509342	3.3541	72.8828
12	1.346674	2.9926	75.8754
13	1.180615	2.6236	78.499
14	1.143683	2.5415	81.0405
15	1.077855	2.3952	83.4357

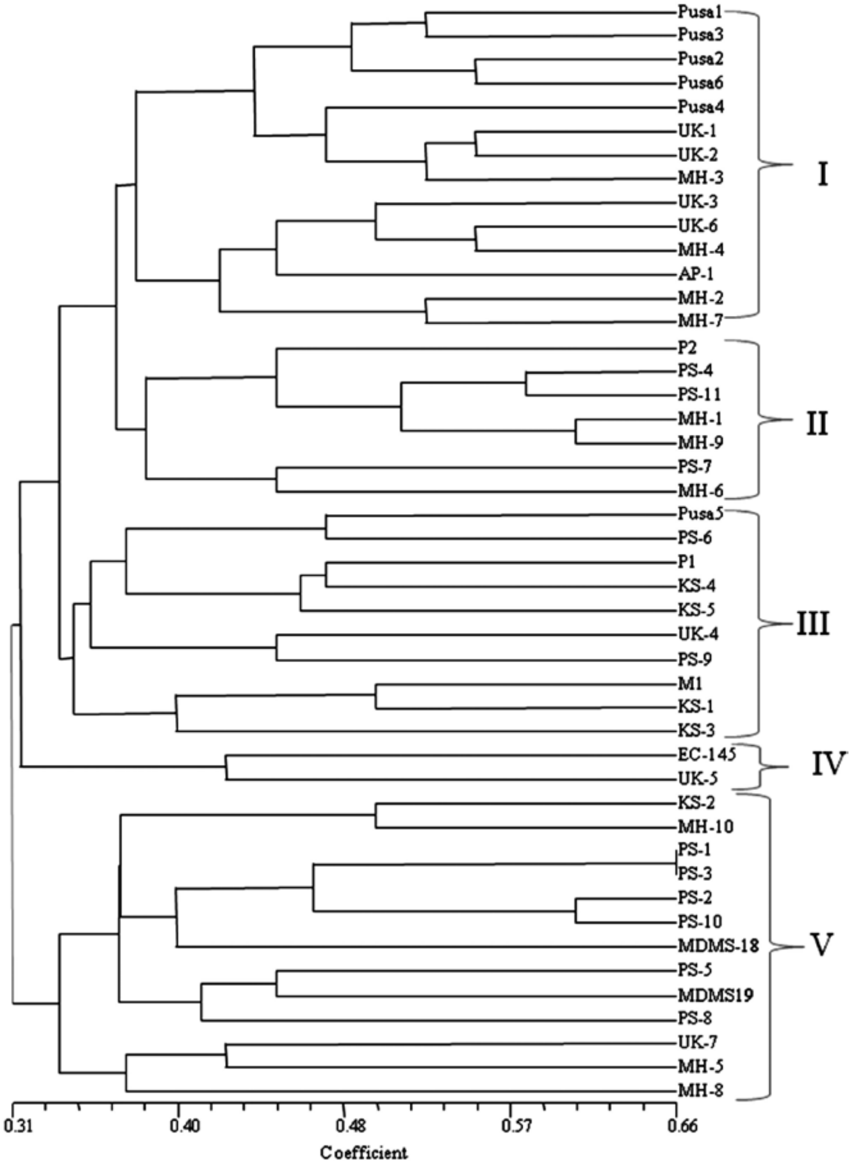

Fig. 1. UPGMA dendogram of 46 accessions of *C. maxima* based on morphological analysis.

maxima provides useful information for selecting commercially important and superior genotypes from the germplasm collection. From the results, it is clear that the Pummelo phenotypic variability did not show any correlation pattern to geographical origin of accessions. Thus, indicated that phenotypic variability was not influenced by their habitat. Similar results were presented in Indian mandarin cultivars (Pal *et al.*, 11). The results suggested that leaves and fruit morphological characters provide efficient and effective use of genetic resources for practical knowledge in identification and characterization within pummelo cultivars. This could be helpful for the citrus industry and the breeders for the identification of cultivars in horticultural plant species. Shrestha *et al.* (14) studied the role of morphological characters in distinguishing five landraces of *Citrus aurantifolia* and subsequently, used those characters as the main basis in genotype selection for breeding programs. Malik *et al.* (7) also studied morphological characterization of sweet orange [*Citrus sinensis* (L.) Osbeck] cultivars of India. Sayed *et al.*(13) characterized 10 cultivars using 31 morphological characters of leaves, inflorescences, fruits and seeds of Lime and Lemon Accessions. Yahata *et al.* (16) studied the morphological characterization and evaluation of reproductive function in haploid pummelo (*Citrus maxima* (Burm.). The size of the

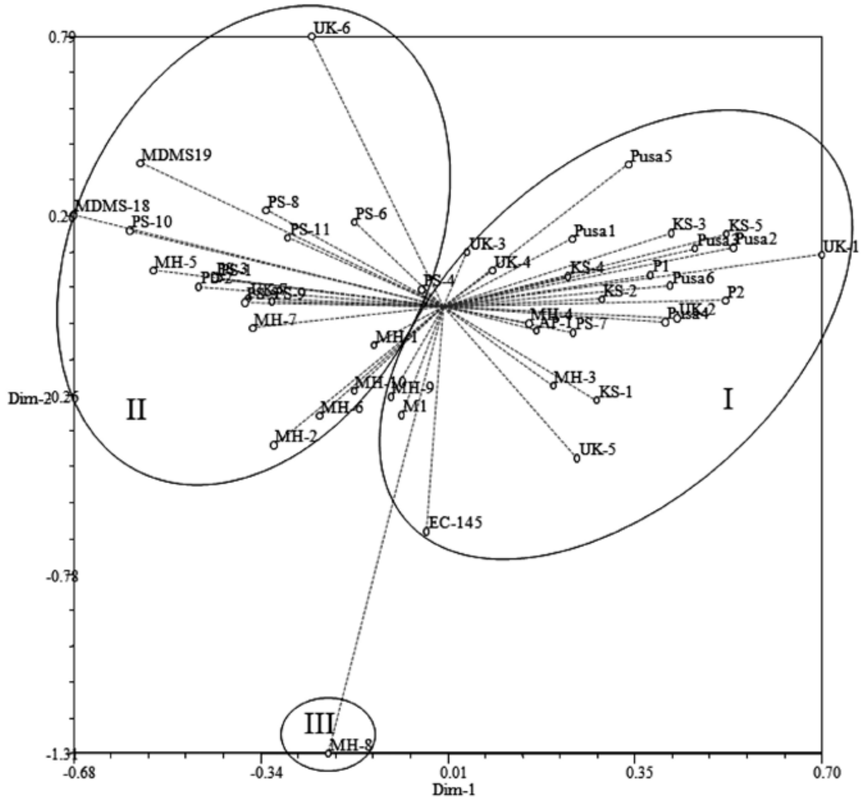

Fig. 2. A 2D plot of 46 *C. maxima* accessions based on morphological traits.

leaf blades and wing leaves a well as leaf weight and the size of the stomata of fully expanded leaves were measured. The leaf morphology plays an important role in plant taxonomy and especially in Citrus species. The leaf morphology also plays an important role in plant taxonomy and especially in Citrus species. Malik *et al*. (7) studied the leaf morphology in *C. sinensis* which were elliptic to ovate leaf lamina and narrowly winged petiole, while Pal *et al*. (11) described the leaf characters in *C. reticulata*. Malik *et al*. (6) also studied the morphological characterization on *C. macroptera* and *C. indica.* In the present study, the results of cluster analysis on 46 genotypes of Pummelo suggested that the maximum similarity was (0.66) among the genotypes for different agro-morphological traits. Accessions with greater similarity for agro-morphological 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. The information of relationships achieved from these studies may be useful in exploitation of the available germplasm resources. Pairwise similarity among the pummelo accessions ranged from 0.31 to 0.66 with an average of 0.48 as maximum similarity (0.66) was observed between the accessions number PS-1 and PS-3 collected from Delhi state of India. Similarity among genotypes was detected mainly on the basis of leaf shape, leaf apex, leaf margin, fruit characters and seeds characters. Similarly, pair-wise similarity values among the different cultivars of *C. sinensis* ranged from 0.18 to 0.64 with an average of 0.39 (Malik *et al*., 7), while in *C. reticulata* varied from 0.15 to 0.73 with an average of 0.44 based on morpho-metric data (Pal *et al*., 11). Based on Euclidean distance divided the accessions into five main clusters and principal component analysis based on 42 morphological characters showed significant variation among cultivars in this study. Dorji and Yapwattanaphun (3) conducted a study for assessment of morphological diversity among 39 local mandarin *Citrus reticulata* accessions from Bhutan. The two dimensional (2-D) plot generated from PCA showed three groups which was found to be more or less similar to the clustering pattern of the UPGMA dendrogram. The PCA identified most significant traits which are responsible for grouping of certain genotypes into specific cluster and hence revealed some aspects of interrelation among genotypes that were not discernable by the UPGMA analysis (Marak and Laskar, 8). The analysis gave 36 principal components out of which first ten principal components contributed 69.52 % of the total variability in the present study. *C. maxima* being a mono-embryonic and cross pollinated true species of Citrus revealed vast variation in pool of 46 genotypes studied here, promising accessions for desired characters may be useful in future genetic improvement programme.

Citrus maxima cultivars showed wide range of morphological variability. This variability can be attributed to the fact that Pummelo itself is a natural non-hybrid species. This characterization study using 42 morphological descriptors shows a wide range of variation for both quantitative and qualitative characters among the 46 studied genotypes. The results indicated that phenotypic variability was not influenced by their habitat. However, this study will form an important basis for selection of variability will help citrus breeder regarding the choice of parents, hence, have significant implications in citrus breeding programmes for crop improvement.

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