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**AK Baswal**  
Ph.D. Scholar, Department of  
Fruit Science, Punjab  
Agricultural University,  
Ludhiana, Punjab, India

**HS Rattanpal**  
Senior Horticulturist,  
Department of Fruit Science,  
Punjab Agricultural University,  
Ludhiana, Punjab, India

**Gurtej Singh Uppal**  
Assistant Horticulturist,  
Department of Fruit Science,  
Punjab Agricultural University,  
Ludhiana, Punjab, India

**KS Gill**  
Assistant Professor of  
Horticulture, Department of  
Fruit Science, Punjab  
Agricultural University,  
Ludhiana, Punjab, India

## Studies on genetic diversity in pummelo (*Citrus grandis* Merrill) genotypes on the basis of morphological and physiochemical characters

**AK Baswal, HS Rattanpal, Gurtej Singh Uppal and KS Gill**

### Abstract

Assessment of genetic diversity in the available germplasm is the prerequisite for the development of improved genotypes through planned breeding programmes. In view of this, ten pummelo (*Citrus grandis* Merrill) genotypes maintained at College Orchard and Fruit Research Farm of Punjab Agricultural University, Ludhiana were evaluated for vegetative, floral, fruit and seed characters on the basis of IPGRI descriptor for citrus during 2013 to 2015. Morpho-physiological data was subjected to cluster analysis and the tested genotypes were grouped into four clusters, indicating that the genotypes in these clusters have dissimilarity for morphological features and performance. Cluster I was recorded with no fruiting, while cluster III had the highest mean value for fruit weight (1218.17 g), fruit diameter (175.93 mm), fruit length (151.36 mm), fruit rind thickness (21.86 mm) and number of segments per fruit (15.50) and least mean value for total soluble solids (7.72<sup>o</sup> brix) and 20 seeds weight (5.86 g). Cluster II was characterized by maximum value for titrable acidity (0.89%), 20 seeds weight (6.44 g), seed width (8.47 mm) and least mean value for fruit diameter (134.05 mm), fruit rind thickness (15.55 mm) and seed number (84.5).

**Keywords:** Cluster analysis, Morpho-physiological characters, pummelo varieties

### 1. Introduction

Pummelo is mostly grown as backyard crop in India. The ripe fruit is a rich source of vitamin C, and is considered for its medicinal properties. In India, no standard cultivar of pummelo is found except 'Nagpur Chakotra' (Singh *et al.*, 1964) [1]. Morphological study is an essential component for the assessment of diversity and classification. Even today, morphological study is being considered and has been deployed as an initial step for cultivar identification and diversity assessment with watermelon (Huh *et al* 2008) [7], sweet potato (Elameen *et al* 2010) [5] and agave (Rodríguez *et al* 2009) [10]. Furthermore, important horticultural characters are reported to be controlled by multiple genes (Campos *et al* 2005, Liu and Deng 2007) [3, 8] and are of low heritability. Thus, morphological characterization could be an essential component since most of the horticultural characters cannot be evaluated through molecular markers. A large number of citrus species/progenitors of commercial citrus fruits are believed to have originated in India. Despite of huge genetic diversity, very little work has been done in pummelo. Proper identification of trees is essential to establish trueness-to-name in commercial channels. Moreover, the testing of advanced selection and of new cultivars is an important aspect of fruit breeding (Harding 1983) [6]. Keeping this in view ten pummelo genotypes maintained at College orchard and Fruit Research Farm of Punjab Agricultural University, Ludhiana, the present study was planned to analyze variability in pummelo genotypes for different morphological characters.

### 2. Materials and Methods

The experiment was laid out during the years 2013 to 2015 on trees grown at College Orchard and Fruit Research Farm, Department of Fruit Science, Punjab Agricultural University, Ludhiana, Punjab. Characterization of pummelo genotypes was conducted on ten genotypes for one tree characters (rootstock diameter), three vegetative characters (Leaf lamina length, width and length : width ratio), seven flower characters (flower diameter, petal width, length of flower, pedicel, petal, filament and style), nine fruit characters (fruit weight, diameter, length, rind thickness, albedo thickness, number of segments per fruit and fruit axis diameter), two physico-chemical characters (total soluble solids and titrable acidity) and four seed characters (seed number, length, width and weight per 20 seeds) on the basis of IPGRI (International Plant Genetic Resources Institute) citrus descriptors (Anonymous 1999) [1].

**Correspondence**  
**AK Baswal**  
Ph.D. Scholar, Department of  
Fruit Science, Punjab  
Agricultural University,  
Ludhiana, Punjab, India

Each genotype was replicated four times with one plant per replication. Data on tree, vegetative and floral characters was recorded from all the four directions of plant. For fruit characters, ten fruits/plant were collected randomly and observation were recorded on each fruit separately. Parameters like flower diameter, flower length, pedicel length, petal length, petal width, length of filament, length of style, leaf lamina length, leaf lamina width, fruit diameter, fruit length, albedo thickness, fruit rind thickness, fruit axis diameter, seed length and seed width were recorded using Digital Vernier Calipers. Total soluble solids content of fully mature fruits was recorded using Digital Hand Refractometer. Titrable acidity was estimated by titrating a known volume of pulp juice extracted against 0.1 N Sodium hydroxide (NaOH) using phenolphthalein as an indicator. Seeds from fruits in each replication were collected by cleaning and washing. Seed number/fruit was counted manually for each fruit. Seed weight was recorded on the basis of average of 20 seeds per replication.

The orchard was laid in square system of plantation (6m x 6m) and trees were maintained under uniform cultural operations as per PAU recommended Package of Practices for cultivation of citrus.

The climate is very hot in summer and cold in winter. The location is generally dry and hot, with monsoon lasting three months. Both winter and summer are severe. The average annual rainfall is 613.11 mm. On an average there are 40 rainy days. The variation in rainfall is appreciable. The month

of May is the hottest with the mean monthly maximum temperature of 2.1°C.

The genotypes used for study included: Pink pummelo, White pummelo, NRCC Pummelo-1, NRCC Pummelo-2, NRCC Pummelo-3, NRCC Pummelo-4, PTF-1, PTF-2, PTF-3 and PTF-4.

### 3. Results and discussion

Non-hierarchical cluster analysis was used to group the test genotype into four clusters (Table 1) with variable number of entries in each cluster indicating the presence of genetic diversity in the genotypes. Cluster I contained maximum number of genotypes (four) comprising NRCC Pummelo-1, NRCC Pummelo-4, PTF-1 and PTF-4 followed by cluster III containing three genotypes, viz, NRCC Pummelo-3, PTF-2 and PTF-3. Cluster II comprised two genotypes namely NRCC Pummelo-2 and Pink Pummelo, while Cluster IV contained only one genotype namely white pummelo.

In a similar studies on cluster analysis, sixteen genotypes of sweet orange (*Citrus sinensis* Obseck) were evaluated by Baswal *et al* (2016) [2]. Twenty two sweet orange cultivars were grouped into four clusters by Malik *et al* (2012) [9], thirty nine local mandarin (*Citrus sinensis*) accessions were grouped into 2 clusters by Dorji and Yapwattanaphun (2011) [4] and fifty nine mandarin (*Citrus reticulata*) cultivars were evaluated by Campos *et al* (2005) [3] and they obtained two similar groups on the basis of cluster analysis.

**Table 1:** Grouping of ten pummelo genotypes into different clusters on the basis of nonhierarchical cluster analysis.

Cluster number	Number of genotypes	Genotypes
I	4	NRCC Pummelo-1, NRCC Pummelo-4, PTF-1 and PTF-4
II	2	NRCC Pummelo-2 and Pink Pummelo
III	3	NRCC Pummelo-3, PTF-2 and PTF-3
IV	1	White pummelo

The formation of large number of clusters with variable number of entries in each cluster is indicative of diversity. Average distance cluster dendrogram depicting the dissimilarity among the clusters so formed is shown in Fig 1 which represents different genotypes which were clustered into four clusters.

In addition to grouping the accessions into different clusters, nonhierarchical cluster analysis was used to identify the diverse and desirable genotypes in terms of inter cluster distance and mean performance of characters, respectively. The important points considered, while selecting genotypes are: choice of the clusters that are separated by maximum inter-cluster distance and selection of particular genotypes that showed good performance in the selected clusters.

The cluster mean value for four vegetative, seven floral characters, seven fruits characters and four seed characters presented in the table 2, 3, 4 and 5, respectively indicated considerable differences for all the characters among clusters. It can be seen from the cluster means that each cluster has its uniqueness that separated it from other clusters.

Cluster I was characterized by the highest mean value for length of style (9.84 mm). There was no fruiting data was recorded in cluster I as fruiting was absent among all the genotypes of cluster I hence only vegetative and floral characters were recorded for the genotypes of cluster I.

Cluster II was characterized by the highest mean value for titrable acidity (0.89%) pedicel length (21.04 mm), leaf lamina length (124.65 mm), leaf lamina width (81.34 mm), seed weight of 20 seeds (6.44 g), seed width (8.47 mm) and

moderate mean values for all other characters.

Cluster III had the highest mean value for fruit weight (1218.17 mm), fruit diameter (175.93 mm), fruit length (151.36 mm), albedo thickness (4.23 mm), fruit rind thickness (21.86 mm), fruit axis diameter (22.86 mm), while the lowest mean values was recorded for total soluble solids (7.72<sup>0</sup>brix) and seed weight of 20 seeds (5.86 g).

Cluster IV was characterized by the highest mean value for total soluble solids (8.44<sup>0</sup> brix), fruit rind thickness (24.07 mm), seed number (130.75) and seed length (17.99 mm), while the lowest mean value was represented by fruit weight (741.25 g), fruit length (114.86 mm), albedo thickness (3.11 mm), fruit axis diameter (20.38 mm), titrable acidity (0.78%), flower diameter (37.11 mm), flower length (31.7 mm), petal length (18.24 mm) petal width (8.47 mm), leaf lamina length (105.58 mm) and leaf lamina width (65.64 mm).

In the present study, cluster I was good with respect to length of filament and length of style. However fruit characters yet to be analyzed. Cluster II was better with respect to pink pulp flesh and higher juice content but was undesirable for seed width and seed weight per 20 seeds. Cluster III was better with respect to moderate titrable acidity and low seed weight of 20 seeds but also possessed undesirable traits like high seed number and high rate of premature fruit drop due to incidence of gummosis. Cluster IV was better with respect to high total soluble solids, low titrable acidity but was found undesirable for low fruit weight, fruit length, albedo thickness, seed number and seed length. Cluster II contained two genotypes namely NRCC Pummelo-2 and Pink Pummelo possessed

higher juice content with pink flesh and lower incidence of gummosis. Cluster III had three genotypes namely NRCC Pummelo-3, PTF-2, and PTF-3 containing more number of fruits per plant with moderate juice content. Cluster IV contained only one genotype namely white pummelo which was white fleshed was better due to higher total soluble solids and low acidity.

On the basis of diversity analysis of pummelo genotypes, it could be concluded that the pummelo genotypes can be successfully used for planning future breeding programmes to obtain hybrids with desired traits. Combination with high heterotic response and superior recombinants may be obtained through hybridization between genotypes across the clusters.

**Table 2:** Mean performance of different clusters for different vegetative traits of pummelo genotypes.

Cluster No	Leaf lamina length (mm)	Leaf lamina width (mm)	Leaf lamina length : width ratio	Rootstock diameter (mm)
Cluster I	119.08	78.59	1.55	54.31
Cluster II	124.65	81.34	1.54	86.34
Cluster III	114.47	73.14	1.58	64.65
Cluster IV	105.58	65.64	1.63	102.43

**Table 3:** Mean performance of different clusters for different flower traits of pummelo genotypes.

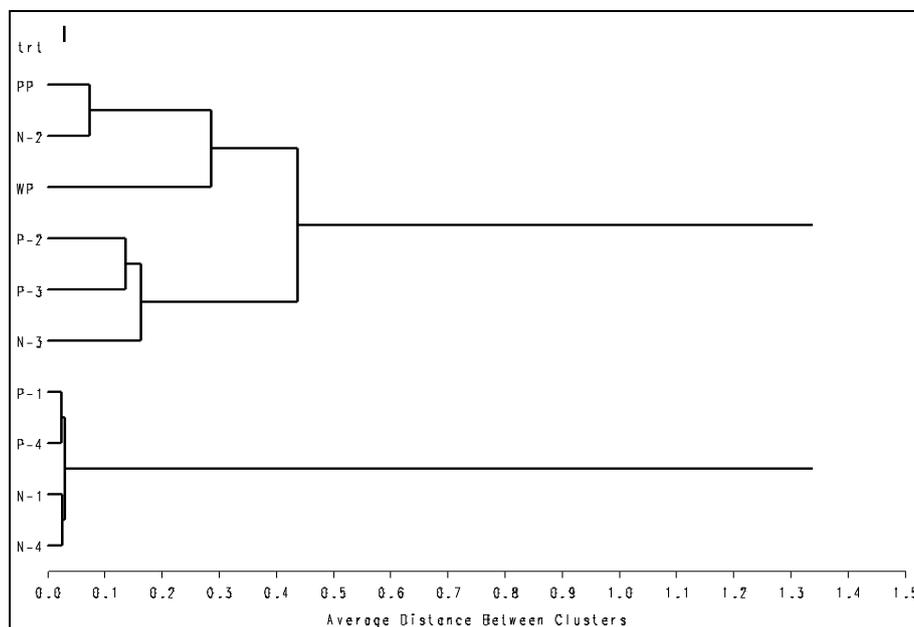
Cluster No	Flower diameter (mm)	Flower length (mm)	Pedical length (mm)	Petal length (mm)	Petal width (mm)	Length of filament (mm)	Length of style (mm)
Cluster I	34.93	22.86	7.95	18.83	7.05	9.80	5.81
Cluster II	33.64	22.54	7.36	18.26	7.94	9.10	5.78
Cluster III	32.10	19.36	6.55	16.51	7.62	8.11	5.17
Cluster IV	31.61	21.22	7.22	17.26	6.85	9.33	5.76

**Table 4:** Mean performance of different clusters for different fruit traits of pummelo genotypes.

Cluster No	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Albedo thickness (mm)	Fruit rind thickness (mm)	Number of segments	Fruit axis diameter (mm)	TSS ( <sup>o</sup> brix)	Titration acidity (%)
Cluster I	-	-	-	-	-	-	-	-	-
Cluster II	966.25	134.05	123.94	3.25	15.55	14.37	20.41	7.94	0.89
Cluster III	1218.17	175.93	151.36	4.23	21.86	15.50	22.86	7.72	0.85
Cluster IV	741.25	144.28	114.86	3.11	24.07	14.50	20.38	8.44	0.78

**Table 5:** Mean performance of different clusters for different seed traits of pummelo genotypes.

Cluster No	Seed number	Seed length (mm)	Seed width (mm)	Seed weight of 20 seeds (g)
Cluster I	-	-	-	-
Cluster II	84.50	15.89	8.47	6.44
Cluster III	119.33	16.85	7.84	5.86
Cluster IV	130.75	17.99	7.76	6.37



**Fig 1:** Dendrogram showing average distance between clusters based on morphological traits among ten pummelo genotypes.

Where N-1, N-2, N-3, N-4, P-1, P-2, P-3, P-4, PP and WP tends to NRCC Pummelo-1, NRCC Pummelo-2, NRCC Pummelo-3, NRCC Pummelo-4, PTF-1, PTF-2, PTF-3, PTF-4, Pink Pummelo and White pummelo in the dendrogram, respectively.

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