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Pummelo in Homestead Garden: Conservation through Family Farming

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The pummelo (*Citrus grandis* Merr.), the largest fruit size in citrus species, is an underutilized fruit in India. Pummelo in Bihar is mostly grown as homesteads and this fruit is to be developed as potential table fruit because of thick rind, easy to handle and transport. Genetic diversity is an important factor in any crop improvement programme for obtaining high yielding cultivars. Variation among seedling progenies is a rule in pummelo since it is monoembryonic *Citrus* spp. This study was conducted through four cell analysis in the five project communities under the UNEP-GEF project at the Pusa Site, Bihar during 2012 with aim for estimation of genetic diversity for physico-chemical traits, selection of superior clones and to draw the interrelationships between observations for precise characterization of pummelo seedling. Superior plants were also identified based on the highest yield *i.e.* number of fruits/ plant (> 300). Statistical analysis for physico-chemical parameters revealed significant differences among the selected plants for the 12 horticultural traits indicating the higher variability. Principal Component Analysis (PCA) and cluster dendrogram performed on the basis of studied parameters showed continuous variation in the fruit physico-chemical traits and many groups were formed, indicating existence of large and continuous variation. The first four PCs contributed 68.30% of the variability for fruit quality and PC1 accounted for 30.44% (acidity percentage) followed by fruit length (PC2), fruit width (PC3) etc. The maximum coefficient of variation was found in in case of rind thickness, 100 seed weight and acidity percentage. A large number of variability of pummelo with seedling origin and monoembryonic helps in identification of 4 pummelo clones for pulp colour (2 each for dark red flesh and pink flesh) with excellent eating quality, 9 superior clones were identified for profuse bearing with red fleshed and early. The above variation in the selected pummelo seedlings can be exploited for development of superior varieties, marketing and maintenance of exiting superior clone and above all grower can be ensured livelihood security through homestead farming practices in the community.

Key Words: Characterization, Citrus diversity, Evaluation, Homestead, Pummelo seedling

Introduction

Pummelo (*Citrus maxima* (J. Burman) Merrill or *Citrus grandis* Merr.) is a tropical fruit plant species originated from South East Asia. Pummelo is considered to be one of the three true *Citrus* species along with *C. medica* and *C. reticulata*. Pummelo as plant species with long history of cultivation is facing a risk of decreasing variability as a result of artificial selection. Many home gardens, as an ecosystem, contain multiple levels of diversity, including cultural, genetic and agronomic diversity (Engels, 2001) found at Pusa, Bihar and pummelo is necessarily grown there. It is mostly grown in the homesteads in India and the ripe fruit is rich in vitamin C, many important phytochemicals, consumed for its medicinal properties. The pummelo is an underutilized fruit with a potential for commercialization. Currently in India, there are several pummelo seedling types with interesting horticultural traits, but little information is available about their genetic diversity. The high phenotypic variability of the Indian pummelo resulted in the existence of various strains or landraces.

Historically, citrus cultivar improvement started when humans began domestication of this crop by preferential selection and preservation of individuals found among wild plant populations (Barrett, 1977). Clonal selection, identification of chance seedlings or bud-sport mutations has been the major force in the development of citrus cultivars as they possessed some superior trait relative to previously existing cultivars. Most of the present day cultivated citrus scion and rootstocks, originated through

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chance seedlings or bud sport mutations and only a few originated through planned breeding programmes (Hodgson, 1967).

Although genetic diversity of citrus has got immense potential to raise the quality fruit production and productivity, the conservation especially through homestead gardening has not been assessed in depth. These indigenous citrus genotypes like pummelo must be identified, conserved and utilized otherwise loss of valuable genotypes is bound to occur. Therefore, identification of elite valuable genotypes, their proper use and conservation as unique resource is essential. It helps growers to go for quality production not only for internal consumption but also for distant markets. This investigation evaluates the morphological diversity existing at Pusa Site, Bihar with exploration of the opportunities for the growers/farmers, traders and consumers, thereby reduce poverty and enhance food security in the rural areas in the country. Pummelo is a tropical fruit species with a high morphological variability for fruit characters including shape, size, and thickness of peel, flesh colour and taste (Susandarini *et al.*, 2013). Cultivated pummelos have been reported to be highly diverse based on phenotypes of their fruits (Chonchalow, 1984; Dass, 1990; Spiegel Roy and Goldschmidt, 1996).

The quality of pummelo is not homogenous among different seedlings, even in small village or a single house. Clonal selection is considered for pummelo an effective method for genetic improvement and the selection of new genotypes. To select a superior genotype, investigation on genetic variability of fruit characters is considered important. Grouping or classification of genotypes based on suitable scale is essential to understand the usable variability. The greatest impediment to the development of large-scale pummelo production is the lack of thin-skinned, high quality cultivars. Pummelo, like most other *Citrus*, produces seedlings that are genetically different from the mother parent. Morphological properties have been the main character used for recognition and description of plant taxa (Duminil and Michele, 2009; Dwari and Mondal, 2011).

Keeping all these points in view, the present study was taken up with pummelo natural seedlings with the main objectives to understand how households grow and conserve pummelo in home gardens in Bihar, the extent and distribution of morphological variability in pummelo at Pusa, Bihar for better conservation of pummelo, its marketability and contribution to livelihood and to identify any variability for useful attributes within the population for potential marketable traits.

Materials and Methods

This study was conducted to assess the phenotypic variation of pummelo in the unexplored areas of *Muzaffarpur* and *Samastipur* districts of Bihar in order to identify the superior phenotypes (seedling trees) with respect to fruit quality and yield. Based on Four cell analysis, survey was carried out in five communities of Pusa Site under UNEP-GEF project *viz.*, *Mahmada, Jagdishpur, Dhobgama, Murliyachak* and *Bhuskaul*, during the fruiting season of 2012. Focus group discussions, interview with individual fruit growers and *in situ* observation of trees and fruits were also carried out in each site to obtain information. The superior plants were identified based on yield as the number of fruits/plant (> 300 fruits/plant). A sample of five fruits was collected from each tree and two fruits were analyzed in the laboratory of National Research Centre on Litchi, Muzaffarpur. Fruits, fruit peel and seed weight was measured with the help of an analytical balance. Fruit length and width were measured with a graduated scale. Peel thickness was recorded using a Vernier Calliper and total soluble solids (TSS) was recorded by a hand-held digital refractometer. The acidity was measured by the titration method (AOAC, 1989). Based on phenotypic suitability, feedback from local villagers, organoleptic taste, domestic uses and its characterization, 13 superior seedlings were identified for further evaluation with respective co-ordinates and farmer's descriptors. Genetic divergence among selected trees (clones) subjected to Principal Component (PCA) and Cluster analysis was performed to examine the grouping of accessions and to assess the taxonomic affinity using the XLSTAT 2012 statistical package. Cluster analyses were carried out on the principal components using the hierarchic ascendant analysis and Euclidean average distance. Fruit quality attributes of pummelo for consumption and marketing was drawn based on feedback of growers, consumers and traders gathered in 'Citrus Biodiversity Fair' 2011 organised at the community level.

Results and Discussion

During investigation and survey it was found that there is close relationship between pummelo in home gardens and holy festival celebrated in surrounding community *i.e. Chhat Puja* (on 6th day after *Diwali* in *Kartik* month),

and in fact the two are completely interwoven. One very striking aspect related to the traditions of a family as part of a larger community, is the key role of women in managing the garden and utilizing its produce, either for *Chhat Puja* or for domestic consumption or exchange or distribution of fruits to devotees or by selling it in the market before the *Puja*. It was also found that pummelo does not have commercial orchard in the community.

Fruit Physico-chemical Characters in the Selected Pummelo Clones

Analysis of variance revealed statistically significant differences between selected plants for the 12 examined characteristics indicating the high variability in the physico-chemical properties of their fruit. Principal Component analysis and cluster dendrogram (Fig. 1) performed on the basis of studied parameters showed a distribution of selected seedling plants of pummelo, independently of their origin and continuous variation in their fruit's physical and chemical traits.

A wide variation for fruit physico-chemical characters was observed in different selected clones of pummelo (Table 1, 2). Mean, range and coefficient of variability for various traits are presented in Table 3. The fruit weight varied between 0.60 kg to 2.50 kg, fruit length and width varied between 10-24 cm and the peel thickness between 1-4 cm. Similarly, there were large differences for number of segments and number of seeds/ fruit. The range of TSS among different pummelo clones varied between 9-13 ^{0}Brix. A large variation for 100 seed weight was recorded among the selected pummelo clones. On the basis of coefficient of variation, maximum variation was recorded for peel thickness (CV=17.032 %) followed by 100-seed weight (CV=13.728 %) and acidity (CV=12.536 %). This variation is a clear cut indication that the superior pummelo clones can be identified from the existing variation.

Desirable Attributes of Pummelo for Better Marketability and Identification of Superior Clones

While interaction with growers, traders, scientists and consumers during diversity fair on citrus at *Pusa* Site, Bihar in 2011, it was found that pummelo fruits must have appropriate shape, size (*i.e.* round in shape with fruit weight 900-1000g for better packaging and transport), dark red segments (rich in antioxidants, attractive in colour), high total soluble solids (>12 ^{0}Brix, improve taste), less acidic (to have more consumption; better sugar: acid blend attract many consumers),thin rind (as thick rind is very difficult to peel it and increase unwanted wastes, thick rind fruits are less juicy), uniformity of segments (helps in better serves or packaging in *punnets*), high

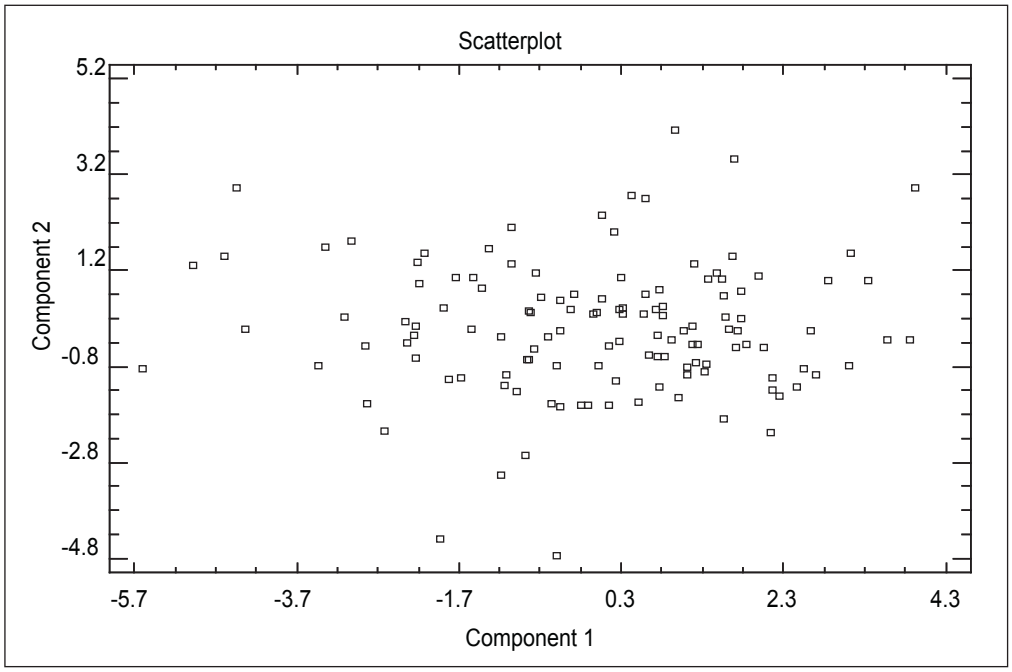

Fig. 1. Scattered distribution of 127 pummelo genotypes based on the principal component score (PC1 contributed 30.44 % and PC2 contributed 15.74 %)

Table 1. Site details and morphological characters of certain pummelo seedlings

Pummelo Clones	Place of collection	Longitude	Latitude	Altitude (m)	Plant habit	Fruit shape	Pulp colour	Fruit size	Fruit colour	Nature of segment	Eating quality
Pusa Pummelo 1	*Narayanpur*, Pusa, Samastipur	N25^{0}57'55.0"	E085^{0}39'0.18"	36	Spreading	Round	Red	Medium	Yellow colour	Hard	Excellent
Pusa Pummelo 2	*Narayanpur*, Pusa, Samastipur	N25^{0}56'47.6"	E085^{0}38'44.8"	39	Spreading	Round	Dark Red	Large	Yellow	Hard	Very Good
Pusa Pummelo 3	*Malinagar*, Pusa, Samastipur	N26^{0}00'03.8"	E085^{0}41'30.0"	28	Spreading	Round	Dark Red	Large	Yellow	Soft	Very Good
Pusa Pummelo 4	*Mahamada*, Pusa, Samastipur	N25^{0}59'33.2"	E085^{0}38'46.1"	31	Spreading	Flattened	Dark Red	Large	Yellow	Soft	Very Good
Pusa Pummelo 5	*Govindpur Chhapra*, Kalyanpur, Samastipur	N26^{0}04'00.6"	E85^{0}26'52.8"	40	Spreading	Elongated	Dark Red	Medium	Dark yellow	Soft	Excellent
Pusa Pummelo 6	*Govindpur Chhapra*, Kalyanpur, Pusa Samastipur	N26^{0}04'01.3"	E085^{0}36'52.8"	28	Spreading	Flattened	Red	Large	Yellow	Soft	Very good
Pusa Pummelo 7	*Narayanpur*, Pusa, Samastipur	N25^{0}57'54.0"	E085^{0}39'01.6"	37	Spreading	Flattened	Red	Large	Dark Yellow	Hard	Good
Pusa Pummelo 8	*Mahamada*, Pusa, Samastipur	N25^{0}59'34.1"	E085^{0}38'37.7"	31	Spreading	Elongated	Red	Medium	Dark yellow	Soft	Very good
Pusa Pummelo 9	*Malinagar*, Pusa, Samastipur	N26^{0}00'01.4"	E085^{0}41'13.1"	29	Spreading	Elongated	Pink	Large	Yellow	Soft	Very Good
Pusa Pummelo 10	*Malinagar*, Pusa, Samastipur	N25^{0}59'98.4"	E085^{0}41'10.9"	28	Spreading	Round	Pink	Large	Yellow	Soft	Good
Pusa Pummelo 11	*Bhuskaul*, Pusa, Samastipur	N25^{0}58'43.2"	E085^{0}38'44.2"	27	Spreading	Flattened	Dark red	Medium	Dark yellow	Soft	Excellent
Pusa Pummelo 12	*Malinagar*, Pusa, Samastipur	N25^{0}59'33.1"	E085^{0}38'45.9"	25	Spreading	Elongated	Red	Medium	Dark yellow	Soft	Very Good
Pusa Pummelo 13	*Mahmadpur*, Muraul, Muzaffarpur	N25^{0}58'45.6"	E085^{0}39'37.6"	32	Spreading	Flattened	Dark red	Medium	Yellow	Hard	Excellent

Table 2. Fruit characters of selected superior clones of pummelo

Clone No.	Fruit weight (kg)	Fruit length (cm)	Fruit width (cm)	Number of seed/ fruit	Number of segments	Peel thickness	Acidity (%)	TSS (°Brix)	100-Seed weight (g)	Fruit length: Breath ratio	TSS: acidity ratio
Pusa Pummelo 1	0.979	12.00	13.05	78.00	14.00	1.55	0.80	11.10	46.99	0.92	20.53
Pusa Pummelo 2	1.613	16.35	17.65	152.50	14.00	1.20	0.70	12.65	51.09	0.92	17.97
Pusa Pummelo 3	1.215	14.75	14.75	81.50	13.50	1.85	0.536	12.30	41.77	1.00	22.95
Pusa Pummelo 4	1.322	14.50	16.25	94.00	17.50	1.75	0.576	12.15	42.57	0.89	22.95
Pusa Pummelo 5	1.048	14.95	14.50	80.50	12.00	0.90	0.488	11.95	29.80	1.03	24.49
Pusa Pummelo 6	0.917	11.25	15.05	91.50	15.00	1.05	0.448	12.30	33.25	0.747	27.45
Pusa Pummelo 7	1.780	15.00	18.15	128.00	18.00	1.995	0.448	10.20	44.25	0.825	20.54
Pusa Pummelo 8	0.991	14.15	12.85	70.50	14.50	1.70	0.632	11.45	41.77	1.10	18.12
Pusa Pummelo 9	1.367	17.00	15.85	90.50	15.00	2.55	0.520	11.10	57.52	1.07	221.35
Pusa Pummelo 10	1.265	14.55	14.75	52.00	16.50	2.55	0.584	10.40	50.51	0.985	17.81
Pusa Pummelo 11	1.037	11.75	13.75	97.00	13.50	1.15	0.472	12.50	34.48	0.85	26.48
Pusa Pummelo 12	0.862	13.80	13.30	72.00	13.00	1.55	0.624	12.55	51.26	1.035	20.11
Pusa Pummelo 13	1.186	16.55	17.40	45.00	13.50	2.65	0.480	11.00	39.49	0.91	22.92

Table 3. Variability parameters in pummelo for certain fruit characteristics

Traits	Range	Mean	SEm±	STDEV	CV
Fruit weight (g)	0.586-2.497	1.264	0.108	0.153	12.132
Fruit length (cm)	10.250-23.300	15.068	0.746	1.055	7.002
Fruit width (cm)	9.90-23.900	15.654	0.767	1.085	6.932
Flesh colour	1.00-6.00	4.693	0.125	0.177	3.814
Skin thickness (cm)	0.800-4.100	1.686	0.203	0.287	17.032
No. of segments/ fruit	11.50-19.50	14.827	0.808	1.143	7.711
TSS (^{0} Brix)	8.90-13.15	10.791	0.265	0.375	3.455
No. of seeds/fruit	17.50-168.00	91.256	5.804	8.208	8.994
Nature of juice sacs (soft/hard)	1.00-2.00	1.976	0.109	0.154	9.079
Acidity (%)	0.320-1.056	0.588	0.052	0.074	12.536
Fruit length: Breadth	0.700-1.275	0.965	0.043	0.061	6.325
100-seed wt. (g)	23.140-68.47	44.479	4.318	6.106	13.728

SE: Standard error of the means; CV: Coefficient of variation

juiciness, and less contents of *limonoids* (once oxidise led to throat congestion).

Amongst these selected clones of pummelo, thirteen were showing better fruit quality in terms of peel thickness, colour of segments, high TSS, number of seeds/fruit and titrable acidity etc. (Table 2). The clones having high TSS (>12.0 ^{0}Brix) in addition to other desirable attributes were finally selected for vegetative propagation and further evaluation. These clones were considered superior because of sweetness (as most of the pummelo grown in the locality is bitter in taste, reduces marketability and consumption), a very desirable trait for pummelo. The cultivation of these high TSS clones will certainly improve the consumption of pummelo in India and particularly in Bihar state.

Pusa Pummelo 5 had excellent eating quality due to dark yellow fruits, thin rind, high TSS, soft segments and small seeds (100-seed weight is 29.80 g) (Table 1, 2). It is also true that excessively thin-skinned fruits are liable to be damaged in the long distance transport and a compromise has to be made for these characters in such a way that no other character is affected. These need further studies to help selection process for increased TSS and reduced rind thickness so that improved pummelo varieties can be developed for the benefit of the growers.

Pusa Pummelo 1 is early maturing, red fleshed suited for table purposes. Pusa Pummelo 2, 3, 4 were large sized, red fleshed and have very good eating quality. Most of the pummelo clones (Pusa Pummelo 3, 4, 5, 6, 8, 9, 10. 11, 12) have soft segments mostly liked by consumers and less content of limonoids. Pusa Pummelo 5, 6, 7, 8, 9, 12 are profuse bearer, red fleshed and large sized suited for table purposes. Pusa Pummelo 9, 10 has large sized fruits, pink fleshed, with profuse bearing. Pusa Pummelo 11, 13 have dark red flesh with excellent eating quality. Various clones may be multiplied by the community and diversity of pummelo will increase. Pusa Pummelo 1, 5, 11, 13 can be encouraged for marketing in domestic *mandis*, once growers get better income, they will conserve and maintain the diversity with interest and can pass on to next generation.

Principal Component Analysis (PCA) and Grouping of Genotypes

PCA is a way of identifying the patterns in the data, and expressing the data in such a way as to highlight their similarities and differences (Winterocea *et al*., 2008). It has been used previously to establish the relationships among genotypes, *i.e.* cultivars and to study the correlation between physico-chemical and other characteristics within sets of genotypes. The PCA used in the present study showed that more than 30% variability observed in the first components (Table 4). The PC1, PC2 and PC3 accounted for 30.44, 15.74 and 11.15, respectively. Though first three (acidity, fruit weight and fruit length) are the most important, but account only for about 60 % of the variability and hence selection pressure may not be enough to get the desired type. The Eigen value of each morphological character in PCA is given in table 4, indicating the contribution in characters in the group formation. Here contribution of various morphological parameters were indicated by high Eigen value (>1.0) as criteria for marking characters with high diagnostic function (PC1, PC2, PC3 and PC4).

Plot analyses are able to create 2 or 3 dimension diagrams that each dimension of them is considered as a principal discriminator factor. In this study a two dimensional scatter plotting diagram constructed using component score 1 on X axis and component score 2 on Y axis exhibited 46.15 % of total variance. The genotypes fall into several clusters (Fig. 1) indicated most of the seedling of pummelo belongs to different source of origin. This result suggests that the factor(s) other than geographical separation is responsible for divergence, and the genotypes that have originated from the same place may have different genetic architecture or vice versa (Majumder *et al*., 2013).

These selected accessions might be important breeding material for the development of improved varieties and need further study. Selecting superior varieties became easy due to genetic characteristics of pummelo as seeds are zygotic (they contain one embryo and subjects to genetic segregation) and give rise to plants with entirely new horticultural traits); the seeds of other *Citrus* spp. tend to reproduce the characters of the mother plant. This produces a wealth of varieties to choose. Here the seedlings were evaluated in field conditions and wide range of variation in physico-chemical characters of pummelo indicated the great scope of individual plant selection based on these characters for future genetic improvement programme.

Table 4. Principal component scores for 12 morphological traits in germplasm lines of pummelo seedling

Principal component	Variates	Eigen value (%)	Variation (%)	Cumulative variation (%)
PC1	Acidity	3.65	30.44	30.44
PC2	Fruit length	1.88	15.74	46.15
PC3	Fruit width	1.43	11.91	58.11
PC4	Fruit weight	1.22	10.19	68.30
PC5	Length: Breadth	0.91	7.62	75.93
PC6	Nature of juice sacs	0.77	6.44	82.38
PC7	No. of seeds/fruit	0.74	6.19	88.57
PC8	No. of segment	0.55	4.59	93.17
PC9	Peel thickness	0.37	3.12	96.29
PC10	Pulp colour	0.33	2.81	99.11
PC11	TSS	0.09	0.77	99.88
PC12	100-seeds wt.	0.01	0.11	100.00

Eigen vectors of the most descriptive traits

The result of this study also suggested fruit morphological characters provides a practical approach in distinguishing seedling groups within pummelo and thus applicable for common people and breeder in selecting favourable clones. Moreover, Shrestha *et al*. (2012) demonstrated 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. Morphological characterization on 20 cultivars of Indonesian *Citrus nobilis* by Martasari and Reflinur (2012) highlighted contribution of morphology in recognizing major differences among cultivars. The variability of fruit characters in pummelo may arise by hybridization or selection pressure, which resulted in the occurrence of adaptive phenotypic variation. Each group is representing highly variable assembly of seedlings/cultivars and deserve attention for their conservation. Physico-chemical variability in pummelo seedlings was adequately fulfilling criteria as gene-pools, as they represented accessions from different areas. They warrant consideration for conservation properly in order to protect loss of potential plant resources.

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