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Genetic diversity analysis in guava (*Psidium guajava*) on the basis of morphological and physico-chemical traits

D SINGH[1], M I S GILL[2], R S BOORA[3] and N K ARORA[4]

Punjab Agricultural University, Ludhiana, Punjab 141 004

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ABSTRACT

Assessment of genetic diversity in the available germplasm is the prerequisite for development of improved genotypes through planned breeding programmes. In view of this, thirty five guava (*Psidium guajava* L.) genotypes maintained at the Fruit Research Farm of Punjab Agricultural University, Ludhiana and its Regional Fruit Research Station, Bahadurgarh, Patiala were evaluated for tree, vegetative, reproductive, fruit and seed characters based on UPOV descriptors during years 2010 to 2013. Morpho-physiological data was subjected to Mahalanobis D^2 statistic and it grouped the tested genotypes into six clusters with Cluster III and V showing maximum inter cluster distance. Cluster I, cluster IV, cluster V and cluster VI showed intra cluster distance of 55.12, 55.40, 50.70 and 61.84, respectively, indicating that the genotypes in these clusters have dissimilarity for morphological features and performance. Cluster III had highest mean value for fruit weight (312.39 g), fruit length (109.60 mm), fruit width (72.88 mm) and fruit outer flesh thickness (17.95 mm) and least mean values for seed weight per 100 g of fruit (0.933 g). Cluster V was characterized by minimum mean value for 100-seed weight (1.017 g) and maximum value for total soluble solids (11.60 %) and titrable acidity (0.486 %). Contribution towards observed diversity was found to be 47.90, 16.73, 13.11 and 10.76 per cent for seed weight per fruit, fruit diameter, 100-seed weight and leaf width. Genotypes from Cluster III, V and IV may be chosen for intercrossing to get the desired trait combinations through high heterotic response and superior recombinants in their progenies.

Key words: Cluster analysis, D^2 statistic, Divergence analysis, Guava, Morpho-physiological

In India, at present, guava (*Psidium guajava* L.) is grown throughout the length and breadth of the country right from sea level to 1300 m altitude, and is so acclimatized that though guava is an introduced crop in India, so much genetic diversity is available that it seems like a native of India (Rajan *et al.* 2007a). Crop improvement work attempted in India has resulted in release of several superior selections or hybrids. High heterozygosity and frequent cross pollination resulted in the present day variability in seedling populations from which promising genotypes have been selected (Saxena *et al.* 2007). For incremental improvement of guava, a diverse gene pool is essential. Knowledge of the genetic diversity available and the origin of the cultivars would assist in the selection of parents for effective hybridization programmes. A careful study of the germplasm would help to eliminate duplicates in the germplasm collection, thus saving land, space and time (Prakash *et al.* 2002). Genetic diversity in the germplasm of *P. guajava* and other *Psidium* species has been screened and characterized based on morphological (Mani *et al.* 2011, Molero *et al*. 2003, Urdaneta *et al*. 2007, Santos *et al*. 2010, Dinesh and Reddy 2001) and chemical features (Sharma *et al*. 2010). Keeping in view the availability of wide range of guava germplasm at Punjab Agricultural University, Ludhiana and its Regional Fruit Research Station, Bahadurgarh, the present study was planned to analyze variability in guava germplasm for different morphological characters.

MATERIALS AND METHODS

The experiment was carried out during the years 2010 to 2013 on non-juvenile trees grown and maintained in the Fruit Research Farm, Department of Fruit Science, Punjab Agricultural University, Ludhiana and Regional Fruit Research Station, Bahadurgarh, Patiala, Punjab. Characterization of guava germplasm was conducted on 35 genotypes for two tree characters (internodal length, twig diameter), five vegetative characters (leaf chlorophyll index, leaf length, leaf width, leaf length to width ratio and petiole length), two flower characters (flower size and number of petals), seven fruit characters (Fruit weight, length, diameter, length/diameter ratio, pedicel length, calyx cavity diameter and outer flesh thickness), three physio-chemical characters (total soluble solids, titrable acidity and vitamin C content) and five seed characters (seed core diameter, seed number

[1]Assistant Professor of Horticulture (e mail: gilljhander84@gmail.com), [2]Senior Horticulturist, [4]Assistant Horticulturist, Department of Fruit Science; [3]Assistant Horticulturist, RFRS, Bahadurgarh, Patiala

per fruit, seed weight per fruit, seed weight per 100 g fruit and 100-seed weight) on the basis of UPOV (International Union for the Protection of New Varieties of Plants) descriptors. Each genotype was replicated thrice with one plant per replication. Data on tree, vegetative and floral characters was recorded from all the four directions of plant. For fruit characters, 10 fruits/plant were collected randomly and observations were recorded on each fruit separately. Parameters like internodal length, twig diameter, flower size, leaf length, leaf width, petiole length, fruit length, fruit diameter, pedicel length, outer flesh thickness and seed core diameter were recorded using Digital Vernier Caliper, Mitutoyo Inc., Japan. Total soluble solids content of fully mature fruits was recorded using Digital Hand Refractrometer. 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. For estimating Vitamin C content, a known weight of fruit pulp finely chopped and macerated in 3% metaphosphoric acid was titrated against 0.25% solution of 2,6-dichlorophenol indophenols dye and expressed as mg of vitamin C/100 g of fruit pulp. Seeds from fruits in each replication were collected by cleaning and air drying. Seed number/fruit were counted manually for each fruit and then averaged for each replication. Seed weight/fruit and seed weight/100 g fruit on the basis of fruit weight were recorded. The 100 seed weight was determined by weighing counted 100 seeds. The orchard was laid in square system of plantation (6 m × 6 m) and trees were maintained under uniform cultural operations as per PAU recommended Package of Practices for cultivation of guava.

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 613.11mm. 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 43.1°C. January is the coldest month with mean monthly maximum temperature of 2.1°C.

The genotypes used for study included: Allahabad Safeda, Apple Colour (Seedling selection of Allahabad Safeda), Arka Amulya (Allahabad Safeda × Seedless), Banarsi Surkha (Seedling selection), Selections at IIHR, Bangalore (B S 6-10, B S 6-12 and B S 17-7), chance seedling selections at CISH, Lucknow (CISH G-1 and CISH G-3), half sib selection at CISH, Lucknow (CISH G-4), Hisar Safeda (Allahabad Safeda × Seedless), Hisar Surkha (Apple Color × Banarsi Surkha), H-21 (Red Flesh × Arka Mridula), HS-1 (Portugal × L-49= F_1 × Apple Colour), H S-2 (Portugal × L-49= F_1 × Apple Colour), L-49 (Open-pollinated seedling selection from Allahabad Safeda), Malaysian guava, Pakistan guava (Thailand selection), Portugal, Punjab Pink (Portugal × L-49= F_1 × Apple Colour), Red Fleshed, Safri, one kg (Selection from Giant Thai), selections from Portugal × L-49= F_1 × Apple Colour population (6-4, 7-8 and 12-11), selections from Portugal × L-49 population (14-10, 14-12, 16-11, 17-3, 17-8, 17-16, 19-3 and 30-9) and 21-6 (Allahabad Safeda × Portugal).

To study genetic divergence, morpho-physiological data were subjected to D^2 statistic. Mahalanobis' D^2 statistic as detailed by Rao (1952) was estimated on the basis of the 'p' characters is:

$$Dp^2 = \Sigma\Sigma pW_{ij} (X_{i1} - X_{i2}) (X_{j1} - X_{j2})$$

where, W_{ij}, Variance – covariance matrix (i, j=1, 2……p); X_{i1}, sample mean for i^{th} character for first sample; X_{i2}, sample mean for i^{th} character for 2^{nd} sample. In the present study characters (P=1-24) were used to perform the above analysis. For conducting the D^2 analysis, the computer programme, WINDOSTAT 8.0 cluster analysis was used.

RESULTS AND DISCUSSION

Divergence analysis grouped the test genotypes into six clusters (Table 1) with variable number of entries in each cluster indicating the presence of genetic diversity in the genotypes. Cluster VI contained maximum number of genotypes (13) comprising BS 6-12, 30-9, Punjab Pink, 12-11, BS 17-7, Portugal, 14-12, CISH G 1, Red Fleshed, Apple Colour, L 49, Safri, CISH G 4; followed by cluster V containing 9 genotypes, viz. Hisar Safeda, 21-6, Hisar Surkha, Banarsi Surkha, 6-4, 17-8, CISH-G 3, 14-10 and 17-16 and cluster IV comprising 6 genotypes namely BS 6-10, 19-3, 16-11, HS 1, HS 2 and 7-8. Cluster I comprised 5 genotypes, viz. Allahabad Safeda, Arka Amulya, 17-3, H-21, Malaysian guava; Cluster II and III contained single genotype namely Pakistan guava and one kg, respectively.

In similar studies on divergence analysis, twenty eight guava genotypes were grouped into 4 clusters by Coser *et al.* (2012), twenty two guava genotypes were grouped into 2 clusters by Sharma *et al.* (2010), fifty three Colombian guava accessions were grouped into 3 groups by Sanabria *et al*. (2005), nineteen guava (*Psidium guajava*) genotypes obtained from the FCAV-UNESP breeding programme were evaluated by Lima *et al.* (1999) and they obtained four similarity groups on the basis of cluster analysis.

The formation of large number of clusters with variable number of entries in each cluster is indicative of diversity.

Table 1 Grouping of 35 guava genotypes into different clusters on the basis of D^2 analysis.

Cluster number	Number of genotypes	Genotypes
I	5	Allahabad Safeda, Arka Amulya, 17-3, H-21, Malaysian guava
II	1	Pakistan guava
III	1	One kg
IV	6	BS 6-10, 19-3, 16-11, HS-1, HS-2, 7-8
V	9	Hisar Safeda, 21-6, Hisar Surkha, Banarsi Surkha, 6-4, 17-8,CISH-G 3, 14-10, 17-16
VI	13	BS 6-12, 30-9, Punjab Pink, 12-11, BS 17-7, Portugal, 14-12, CISH-G 1, Red Fleshed, Apple Colour, L-49, Safri, CISH-G 4

Inter and intra-cluster distances among the genotypes are presented in Fig 1. The Euclidian distances dendrogram depicting the dissimilarity among the clusters so formed is shown in Fig 2 which also represents different genotypes which were clustered into 6 clusters.

In addition to grouping of accessions into different clusters, non-hierarchical cluster analysis was also 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. For this purpose intra and inter cluster distances and character wise clusters means (Table 2, 3 and 4) were considered. Pictorially intra and inter cluster distances are represented in Fig 1 in a dendrogram.

The intra cluster distances were observed to be zero for cluster II and III because of single genotype each contained in these clusters. Cluster I, cluster IV, cluster V and cluster VI showed intra cluster distance of 55.12, 55.40, 50.70 and 61.18, respectively, indicating that the genotypes in these clusters have dissimilarity for morphological features and performance (Fig 1). The members of cluster V and III exhibited maximum divergence as indicated by their inter-cluster distance (196.25) followed by the members of cluster IV and III (195.78), cluster VI and III (169.25), cluster IV and II (157.71), cluster V and II (155.06), cluster I and III (144.36) and cluster II and III (127.97). The members of cluster V and VI were least divergent as revealed by their inter-cluster distance (65.97). All the inter cluster distances were larger than the intra cluster distances, indicating wider genetic diversity between genotypes of clusters with respect to the trait considered.

The cluster mean value for 9 vegetative and floral characters, 10 fruit characters and 5 seed characters presented in Table 2, 3 and 4, 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. For example, cluster I was characterized by lowest mean value for petiole length (6.24 mm), number of petals (5.80) and pedicel length (16.86 mm) and moderate mean values for all other characters. Low mean values for leaf length (92.19 mm), leaf length to width ratio (1.68), seed number/fruit (179.30) and seed weight/fruit (1.83 g) and highest value for 100-seed weight (1.56 g) were represented by cluster II with moderate mean values for rest of the traits under study. Cluster III had highest mean value for internode length (39.54 mm), young twig diameter (3.83 mm), leaf width (55.83 mm), petiole length (11.94 mm), fruit weight (312.39 g), fruit length (109.60mm), fruit diameter (72.88 mm),

Table 2 Mean performance of different clusters for vegetative and reproductive traits of 35 guava genotypes

Cluster No.	Inter node length (mm)	Young twig diameter (mm)	Leaf chlorophyll index	Leaf length (mm)	Leaf width (mm)	Leaf length to width ratio	Petiole length (mm)	Flower size (mm)	Number of petals
Cluster I	28.87	3.50	51.39	114.78	49.90	2.30	6.24	38.20	5.80
Cluster II	32.79	3.71	51.47	92.19	54.83	1.68	7.21	37.63	6.33
Cluster III	39.54	3.83	45.82	97.56	55.83	1.75	11.94	34.19	6.00
Cluster IV	35.72	3.42	51.69	131.68	49.48	2.70	7.70	45.30	6.67
Cluster V	31.86	3.57	50.05	123.35	52.87	2.34	7.34	42.51	6.04
Cluster VI	31.11	3.74	48.92	121.50	52.52	2.33	7.13	42.12	7.08

Table 3 Mean performance of different clusters for different fruit traits of 35 guava genotypes.

Cluster No.	Fruit weight (gm)	Fruit length (mm)	Fruit diameter (mm)	Fruit length/ width ratio	Pedicel length (mm)	Calyx cavity diameter (mm)	Outer flesh thickness (mm)	TSS (%)	Titrable acidity (%)	Vitamin C (mg/100 g fruit)
Cluster I	140.54	67.60	62.74	1.08	16.86	9.62	13.51	11.28	0.38	185.52
Cluster II	185.60	71.59	68.35	1.05	17.77	9.12	16.20	11.07	0.45	188.99
Cluster III	312.39	109.60	72.88	1.50	28.07	7.97	17.95	9.76	0.38	131.75
Cluster IV	109.59	68.63	56.32	1.22	26.37	8.03	11.37	10.56	0.42	185.89
Cluster V	145.06	66.01	64.06	1.03	22.97	9.48	11.52	11.60	0.49	153.44
Cluster VI	139.84	68.57	62.42	1.10	22.66	9.76	12.01	11.03	0.43	215.41

Table 4 Mean performance of different clusters for different seed traits of 35 guava genotypes.

Cluster No.	Seed core diameter (mm)	Seed number fruit-1	Seed weight fruit-1 (gm)	Seed weight/ 100 g fruit	100 seed weight (gm)
Cluster I	37.21	213.00	2.04	1.45	1.05
Cluster II	38.56	179.30	1.83	0.98	1.56
Cluster III	40.95	210.40	2.91	0.93	1.34
Cluster IV	35.01	260.37	3.41	3.12	1.31
Cluster V	42.40	432.57	4.42	3.09	1.02
Cluster VI	39.96	302.35	3.33	2.40	1.09

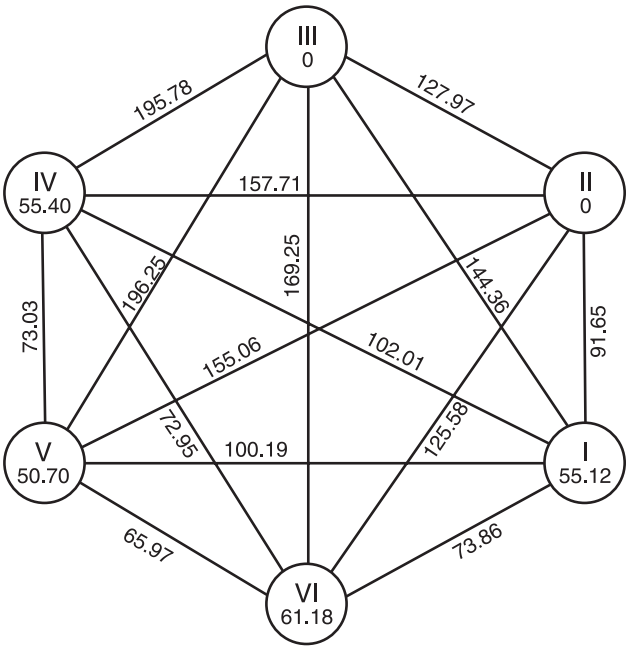

Fig 1 Dendrogram of genetic divergence $\sqrt{D^2}$ among 35 guava genotypes showing inter and intra cluster distances

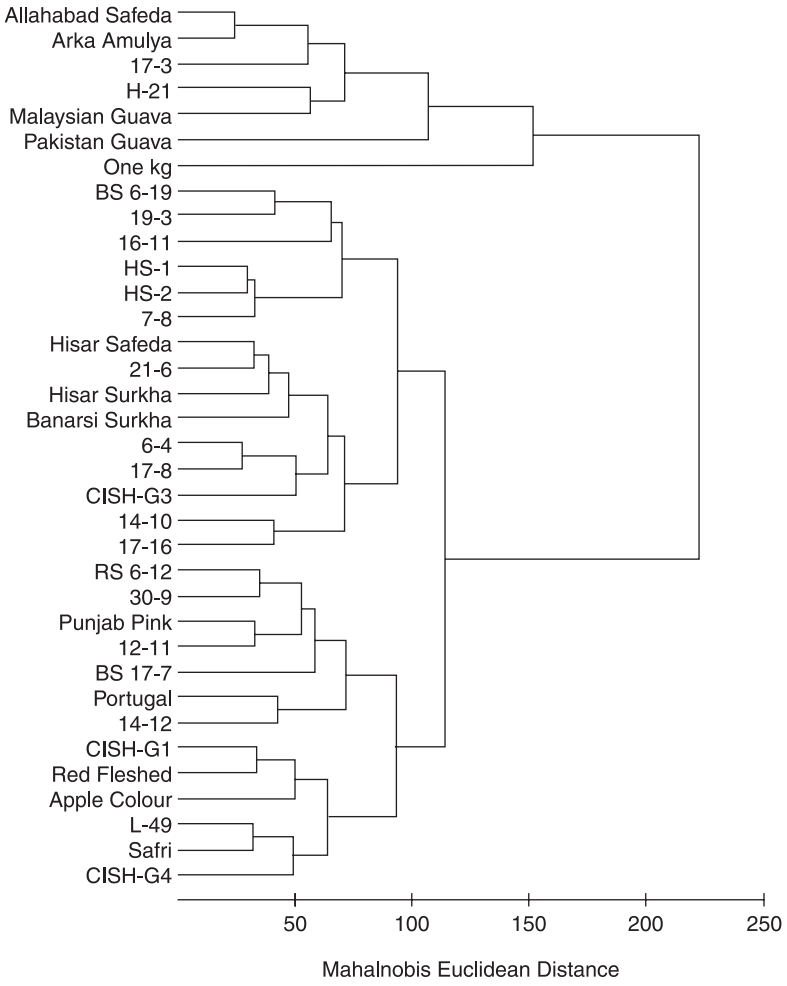

Fig 2 Dendrogram showing Euclidean Distances based on morphological traits among 35 guava genotypes

fruit length width ratio (1.50), pedicel length (28.07 mm) and fruit outer flesh thickness (17.95 mm) and least mean values for leaf chlorophyll index (45.82), flower size (34.19 mm), calyx cavity diameter (7.97 mm), total soluble solids (9.76%), Titrable acidity (0.37%) and seed weight/100 g of fruit (0.93 g).

Cluster IV was characterized by highest mean value for leaf chlorophyll index (51.69), leaf length (131.68 mm), leaf length to width ratio (2.70), flower size (45.30 mm) and seed weight/100 g of fruit (3.12 g) and lowest mean value for leaf blade width (49.48 mm), fruit weight (109.59 g), fruit diameter (56.32 mm), outer flesh thickness (11.37 mm) and core diameter (35.01 mm) medium value for other parameters.

Cluster V was characterized by minimum mean value for fruit length (66.01 mm), fruit length to width ratio (1.03), 100-seed weight (1.02 g) and maximum value for seed core diameter (42.40 mm), total soluble solids (11.60%), Titrable acidity (0.49%), seed number/fruit (432.57), seed weight/fruit (4.42 g). Cluster VI had moderate mean value for all the vegetative, reproductive, fruit and seed characters.

In the present study, cluster III was good with respect to fruit weight, fruit length, fruit diameter and seed weight/100 g of fruit but undesirable for total soluble solids and seed size. Cluster II was better with respect to seed number/fruit having least seed number/fruit. Cluster IV was better due to pink flesh colour. Cluster V was better due to high soluble solids, titrable acidity, flesh colour in some genotypes and lower seed size, but it also possess undesirable traits like thin outer flesh, larger seed core, high number and weight of seed per fruit. Cluster I was better for total soluble solids, seed number and weight per fruit and seed size (low). There existed a few good genotypes in a cluster that can be further used in hybridization for a special trait, e.g. cluster I contained genotype H 21 which possessed thick pink flesh along with smaller seed core, Malaysian guava with purple coloured flesh and Allahabad Safeda having better fruit quality with smaller seeds. Cluster II contained Pakistan guava with thick white flesh and few seeds. Cluster III had only genotype one kg having higher fruit weight and size with thick outer flesh and smaller seed core. Cluster IV had all the genotypes having pink fleshed fruits. Similarly cluster V had Hisar Safeda which had least 100-seed weight, CISH G 3 had better fruit quality with pink flesh colour. Cluster VI had genotypes CISH G 1 and Apple Colour having attractive red skin, Punjab Pink with better fruit quality along with attractive pink flesh, Red Fleshed with high vitamin C content. L 49 had medium sized fruit with thick flesh and better fruit quality, Safri with larger fruits combined with

Table 5 Per cent contribution of the traits towards genetic divergence

Source	Times ranked 1st	Contribution %
Inter node length		
Young twig diameter	1	0.17
Leaf chlorophyll index		
Leaf length	1	0.17
Leaf width	64	10.76
Leaf length to width ratio	3	0.50
Petiole length	15	2.52
Flower size	1	0.17
Number of petals		
Fruit weight	8	1.25
Fruit length		
Fruit diameter	99	16.73
Fruit length/diameter ratio		
Pedicel length		
Calyx cavity diameter		
Outer flesh thickness		
Total soluble solids		
Titrable acidity		
Vitamin C content		
Seed core diameter	13	2.18
Seed number/fruit		
Seed weight/fruit	285	47.90
Seed weight/100g fruit	27	4.54
100-seed weight	78	13.11

thick white flesh and CISH G4 with better fruit quality and attractive fruit shape.

The contribution of various characters towards the genetic divergence is presented in Table 5. Out of the 24 traits studied, seed weight/fruit contributed the maximum (47.90%) towards the observed diversity, followed by fruit diameter (16.73%), 100-seed weight (13.11%), leaf width (10.76%), seed weight/100 g fruit (4.54%), petiole length (2.52%), seed core diameter (2.18%) and fruit weight (1.25%) contributed very little to overall genetic diversity. characters like flower size, young twig diameter and leaf length had very little (0.17% each) contribution towards the divergence. Other remaining characters contributed null towards the diversity of guava genotypes. Rajan *et al.* (2007b) also observed maximum contribution (32.00%) by seed weight/fruit. This was followed by 21.55% (for number of seeds/fruit), 20.98% (fruit weight) and 21.55% (100-seed weight) contribution towards the divergence for fruit weight and seed characters among 68 guava accessions maintained at Central Institute for Sub-Tropical Horticulture, Lucknow.

On the basis of diversity analysis of guava genotypes, it could be concluded that the guava germplasm at Punjab Agricultural University can be successfully used for planning future breeding programmes to obtain hybrids with desired traits. Combinations with high heterotic response and superior recombinants may be obtained through hybridization between genotypes across the clusters. For creating wide spectrum of variability and for isolating desirable recombinants in advanced generations, the genotypes of clusters V and IV could be crossed with one kg (cluster III) and Pakistan guava (cluster II), as cluster II and III show high to moderate cluster distances. Also genotypes like One kg and Pakistan guava lack pink flesh colour, high soluble solids content, small seed size, these traits could be introgressed from cluster IV and V. Cluster V possess undesirable traits like thin outer flesh, larger seed core, high number and weight of seed/fruit, these traits could be improved by intercrossing the genotypes of this cluster with that of cluster II and III. It could be concluded that traits like large fruit size, thick outer flesh and low seed number can be combined with traits like pink flesh colour, high TSS and small seed size by choosing the parents from cluster III, IV and V to obtain the desired trait combinations in their progenies. Red skin colour could be combined with pink flesh colour by crossing the genotypes like Apple colour and CISH-G 1 with genotypes like Red Fleshed, Punjab Pink, Hisar Surkha. Genotype H-21 seems quite promising to be used as a parent as it possess the rare combination of pink flesh, high proportion of outer flesh, good fruit quality and smaller seed core.

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