https://doi.org/10.56093/ijas.v90i6.1047788

Indian Journal of Agricultural Sciences **90** (6): 1149–54, June 2020/Article

Genetic diversity study of guava (*Psidium guajava*) for pink pulp and soft seededness

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Received: 16 July 2019; Accepted: 23 September 2019

ABSTRACT

India is one of the largest guava (*Psidium guajava* L.) producing country. This exotic crop adapted very well to climatic condition of nation which results in its huge variability in the form of seedling population. In present investigation the existing genetic diversity of guava explored and analyzed on the basis of physico-chemical characters during 2015-16 at Sawai Madhopur district of Rajasthan (India). The objective was to find some pink pulped and soft seeded guava genotypes. Among 17 guava genotypes, a wide range of diversity was recorded with respect to fruit weight (74.45-357.29), fruit length (45.6-93.96 mm), fruit diameter (50.70-86.15 mm), TSS (7.9-16.3°B), acidity (0.26-0.77%), ascorbic acid (52.44-164.86 mg/100 g) and lycopene content (3.53-6.18 mg/100 g). Seed variables like hardiness (44.06-132.98 N), length (2.22-3.96 mm) and width (1.85-3.16 mm), number of seeds per fruit (99-775) and seed index (0.17-1.48) also exhibited variability. However, highest coefficient of variation was found for fruit weight (52%) and ascorbic acid content (41.1%). Fruit weight was observed to be positively and significantly correlated with fruit diameter, fruit length, peel thickness, seed core thickness, seed hardness and seed weight. Titrable acidity was positively correlated with seed length, seed width and seed index. Four genotypes, viz. BKDV, JSL2, IFA and RFJ were identified for further multiplication to establish them at the Germplasm Block for their detailed evaluation and utilization in the breeding programme.

Key words: Genotypes, GWAS, Lycopene content, Pink pulped, Seed softness, Survey.

Guava (*Psidium guajava* L.) is known as a 'super fruit' (Cardoso *et al.* 2017); although it is originated in Tropical America (Hays 1953) but it is also very well adapted to Indian conditions. In India, guava cultivation started in seventeenth century (Menzel and Paxton 1985). The prolonged cultivation of guava over the centuries resulted in huge diversity in the form of seedling population in India due to cross pollination (Rajan *et al.* 2007, Dinesh and Vasugi 2010). Pink colour of guava pulp is due to lycopene and it is considered a good source of lycopene even better than the tomato (Bramley 2000) and has more preference in processing industry. Pink pulp guavas have additional health benefits due to antioxidant activity of lycopene (Rao and Ali 2006). Some pink pulped guava varieties are Lalit, Arka Kiran, Hisar Surkha, Anakpalli, Hafsi, Red fleshed, Banarasi Surkha, very common in India (Dinesh and Vasugi 2010). Hence, proper exploration of the existing variability, its collection and evaluation are necessary step towards successful breeding programme and also for conservation.

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Survey, collection and evaluation of existing diversity proved to be an important tool for breeding programmes of various countries. In Mexico, Mondragón-Jacobo *et al.* (2010) collected guava trees of Brazilian as well as Mexican origin, which resulted in the selection of seven pink coloured guava genotypes as 23-1, 26-8, 23-5, 25-5, 21-17, 22-11 and 25-9 plus one selection of white flesh guava; 21-9. Many commercial varieties of guava like Media, and China were developed through selection in by farmers in Mexico (Mondragón-Jacobo *et al.* 2010). Similarly, Paluma and Seculo XXI in Brazil; Allahabad Safeda, L-49 (Sardar), Lalit, Shweta, Pant Prabhat, Arka Mridula in India (Dinesh and Vasugi 2010) are also the selections. The survey and collection also proved worthy to improve existing collection in other crops also (Lachenaud *et al.* 2016). So, to strengthen the guava breeding programme; the present study was planned for survey, collection and evaluation of guava diversity from Sawai Madhopur district of Rajasthan, India. The major focus of survey was to explore pink pulped and soft seeded guava genotypes.

MATERIALS AND METHODS

Project community and sample collection: The survey area was Sawai Madhopur, Rajasthan, India, which is

situated at 25°58'N latitude and 76°3' longitude and a major guava growing district of Rajasthan (Pilania *et al.* 2010). This place has more than 5000 ha under commercial guava cultivation. Parallels to commercial cultivation, it has also developed diversity in guava in the form of seedling population. Survey has been conducted with the help of KVK, Sawai Madhopur during 2015-16. In total 17 guava genotypes were collected based on their pulp colour, seed softness and flavour (mainly TSS: acid ratio). The representative samples of 10 fruits from each genotype were collected. The relevant information like name of the farmer, age of tree, yield etc. were recorded at the time of survey. The trees were marked for future identity. The fruits were packed in CFB (corrugated fiber board) boxes and brought to the laboratory.

Sample analysis: The collected samples were brought to the laboratory of the division. They were cleaned with running tap water. Physical fruit variables, viz. fruit weight, diameter, length, breadth were recorded following standard procedures.TSS (Total soluble solids) was recorded with refractometer. Acidity and ascorbic acid content were estimated by the procedures given by Ranganna (1986).

Lycopene estimation

Extraction: Sample was prepared by weighing 10 g of guava fruit sample (without seeds) and sonicated it with solvent mixture of acetone: hexane (1:1) for 20 min. The sonicated sample was filtered and then further extraction was done with the help of homogenizer. The sample homogenized four to five times with the same solvent for ten minutes in each run till the pulp became colourless. After each run the samples were filtered. The whole amount of filtrate was evaporated in a rotary evaporator. The dried sample was then dissolved in TBME (Tertiary butyl methyl ether).

HPLC analysis: Extracts obtained after extraction were analyzed using HPLC method fitted with 600 quaternary pump, with auto injector (20 lL loop), and a 2998 photodiode array detector (Waters Corp., Milford, Mass., U.S.A.) and a 250 _ 4.6 mm dia and 5-lm C30 YMC column (YMC Co. Ltd., Ireland). Two milliliters of each sample solution was filtered through a 0.4-lm nylon filter before injection into HPLC (High pressure liquid chromatography). The mobile phase comprised of isocratic mixture of MTBE (methyl tert-butyl ether): methanol (80:20, v/v) and it was run at a flow rate of 1 ml per min. Carotenoids were monitored at a wavelength of 450 nm. A computer using "Empower 2" software was used to integrate the peak areas (Saha *et al.* 2015).

Seed hardness estimation: The texture of guava seeds was analyzed by using Texture analyzer (Model: *TA+HDi,* Stable Micro Systems, UK) fitted with 500 kg load cell. Single Compression (TPA) test was employed for analysis of hardness of guava seeds and it was determined by using a P75 cylindrical probe (75 mm diameter cylindrical plate) at a pre-test speed of 3mm/s, test speed of 1mm/s and post-test speed of 2 mm/s until 70% sample deformation (Pereira *et al.* 2007).Guava seeds, extracted and dried up to a constant moisture level, were used for analysis; single seed was placed under the probe for test. The hardness of seed was obtained from force-time curve and was expressed as peak force in the compression test.

Statistical analysis: Physical fruit variables were replicated ten times, chemical fruit variables were replicated three times and measurement of seed hardness replicated ten times. The cluster analysis, principal component analysis and factor analysis were done in this study. The data were analyzed utilizing SAS9.4 software available at ICAR-IASRI, New Delhi.

RESULTS AND DISCUSSIONS

Population diversity: Seventeen guava genotypes were collected and out of these only three were pink pulped and rest were white pulped. There are two possible reasons of this, farmers generally replace few guava plants having pink pulp to white pulped one so that they can easily market the fruits in bulk and second is that that farmers can get easily plants of white pulped guava varieties from government or private nurseries. This results more seedling population of white pulped ones.

Physical and chemical fruit variables: The range for fruit weight, fruit diameter and fruit length were 74.45-372 g, 50.70-86.15 mm and 45.6-93.96 mm, respectively (Table 1). Peel thickness and seed core thickness ranged from 4.17-20.71 mm and 28.84-62.86 mm. Maximum coefficient of variation was recorded for fruit weight (52%). High variability in any fruit characteristics indicates the high potential of genotypes in the region for choosing superior genotypes based on the objectives of the breeding program (Cosmulescu and Botu 2012). In most of the breeding programmes the fruit weight is an important criterion for selection. Consumer prefers big sized fruits. It is also believed that these bigger sized varieties are also tolerant to temperature fluctuations, hence results in slow ripening of fruit during period of sudden temperature rise. These characters are also found in Baraf Khan, commercial variety of Sawai Madhopur. Farmers informed during survey that the reason behind not selecting Allahabad Safeda for planting is its sensitivity towards high temperature due to which they faced huge losses because of sudden ripening of fruits on trees in past. Indian commercial guava varieties belong to medium group (fruit weight, diameter and length) even the Mexican guavas have small size (SAGARPA 2005). They weigh more than 100 g and less than 300 g. Only guava variety G. Vilas Pasand is a large sized guava having fruit weight of 400-800 g (Dinesh and Vasugi 2010). It is required to generate population having higher fruit weight for further improvement. Among biochemical parameters TSS ranged from 7.9- 16.3°Brix, titratable acidity from 0.26-0.77% and ascorbic acid content from 52.44-164.86 mg/100 g. Ascorbic acid content recorded 41.1% coefficient of variation. But, the range indicated lower ascorbic acid content in collected genotypes. The ascorbic acid content is another important character of guava. Higher ascorbic acid content should be criteria for selection of guava genotypes to add more health

Table 1 Physical, biochemical and seed parameters of collected guava genotypes

Name of genotype	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Total soluble solids (°B)	Titrable acidity (%)	Ascorbic acid content (mg/ 100g)	Peel thickness (mm)	Seed core thickness (mm)	Seed hardness (N)	Seed length (mm)	Seed width (mm)	Number of seeds per fruit	Seed index (Weight of 100 seeds g)
BKDV	219.11^{b}	82.33^{a}	81.99^{a}	11.87^{cd}	0.597^{b}	164.53^{a}	18.37^{a}	39.00^{e}	102.29^{bc}	3.34^{b}	2.77^{abc}	538.00^{ab}	1.03^{abc}
LYR	127.68^{ef}	62.10^{d}	60.65^{cd}	10.90^{fg}	0.427^{de}	59.80^{k}	12.71^{cde}	34.93^{gf}	116.94^{ab}	3.48^{ab}	2.75^{abc}	226.33^{de}	1.02^{abc}
LP	135.78^{de}	63.15^{cd}	79.21^{ab}	11.63^{de}	0.469^{cd}	53.36^{m}	12.12^{def}	31.13^{h}	81.13^{def}	3.44^{ab}	2.88^{ab}	241.00^{de}	0.88^{bcd}
BK18	227.90^{b}	78.96^{a}	72.53^{b}	10.83^{fg}	0.384^{ed}	78.20^{h}	14.73^{bc}	48.99^{c}	87.65^{cde}	3.45^{ab}	2.85^{ab}	476.67^{bc}	1.22^{a}
BKD2	367.67^{a}	82.59^{a}	85.86^{a}	8.60^{j}	0.555^{bc}	60.26^{k}	19.21^{a}	62.39^{a}	127.91^{a}	3.43^{ab}	2.83^{ab}	398.00^{bcd}	0.51^{efg}
JSL2	113.21^{efg}	62.08^{d}	52.53^{fg}	11.77^{cd}	0.341^{ef}	155.02^{b}	13.60^{bcd}	57.09^{b}	78.02^{def}	3.42^{ab}	2.56$^{b..e}$	196.00^{e}	0.59$^{d..g}$
JSL1	77.38^{i}	53.91^{g}	49.77^{efg}	10.50^{g}	0.469^{cd}	55.66^{l}	10.53^{efg}	29.78^{h}	62.65^{fg}	2.91^{cde}	2.47^{cde}	239.33^{de}	0.67^{def}
CHIJ	78.43^{hi}	53.52^{g}	52.97^{efg}	8.50^{j}	0.299^{f}	55.66^{l}	10.04^{fg}	34.40^{g}	64.80^{fg}	3.10^{bcd}	2.39^{de}	347.00^{cde}	0.44^{fg}
RFJ	108.31$^{e..h}$	57.11^{efg}	60.76^{cd}	9.40h^{i}	0.384^{def}	105.80^{e}	9.80^{fg}	39.88^{de}	67.01^{efg}	2.80^{de}	2.35^{de}	715.33^{a}	0.42^{fg}
ISKH	107.79$^{e..h}$	57.09^{fg}	63.75^{c}	13.13^{b}	0.299^{f}	78.20^{h}	13.69^{bcd}	37.39^{ef}	88.97^{cd}	3.24^{bc}	2.62$^{a..d}$	293.33^{de}	0.79^{cde}
SA	119.92^{ef}	62.60^{d}	54.11$^{d..g}$	9.87^{h}	0.725^{a}	65.79^{i}	10.10^{fg}	42.34^{d}	119.33^{ab}	3.77^{a}	2.93^{a}	511.33^{bc}	1.05^{abc}
BKJ	164.75^{cd}	67.41^{c}	61.26^{cd}	12.30^{c}	0.469^{cd}	83.26^{g}	13.64^{bcd}	46.70^{c}	94.34^{cd}	3.23^{bc}	2.85^{ab}	516.33^{bc}	1.13^{ab}
BKD1	177.84^{c}	72.93^{b}	64.61^{c}	11.27^{ef}	0.725^{a}	55.66^{l}	15.81^{b}	47.90^{c}	88.95^{cd}	3.17^{bcd}	2.58^{bcd}	487.33^{bc}	1.05^{abc}
IFA	121.53^{ef}	61.68^{de}	57.26$^{c..f}$	16.20^{a}	0.427^{de}	84.64^{f}	9.73^{fg}	34.39^{g}	55.20^{g}	2.67^{e}	2.21^{e}	549.33^{ab}	0.32^{g}
SHFA	80.59^{hi}	55.19^{fg}	49.13g	8.87^{ij}	0.427^{de}	110.86^{f}	9.63^{g}	36.35^{gf}	53.99^{g}	3.19^{bcd}	2.63$^{a..d}$	221.00^{de}	0.62$^{d..g}$
LYLLP	87.83^{ghi}	54.89^{g}	52.38^{fg}	9.80^{h}	0.469^{cd}	138.46^{c}	5.00^{h}	35.58^{gf}	93.29^{cd}	3.39^{ab}	2.85^{ab}	287.00^{de}	0.63$^{d..g}$
GR-1	103.59$^{f..i}$	59.57^{def}	49.62^{fg}	13.10^{b}	0.597^{b}	62.56^{j}	10.77^{efg}	34.65^{g}	86.12^{cde}	3.33^{b}	2.47^{cde}	292.33^{de}	0.68^{def}
CD $_{0.05}$	30.05	4.58	7.75	2.42	2.63	1.34	0.12	0.53	0.42	0.35	0.31	21.11	18.95
Range	74.45-372	50.70-86.15	45.6-93.96	7.9-16.3	0.26-0.77	52.44-164.86	4.17-20.71	28.84-62.86	44.06-132.98	2.22-3.96	1.85-3.16	99-775	0.17-1.48
Mean	142.310	63.950	61.670	11.090	0.473	86.336	12.323	40.758	86.387	3.255	2.646	384.451	0.767
Standard Deviation	73.760	9.810	12.190	1.942	0.139	35.465	3.638	9.004	23.800	0.339	0.281	173.444	0.310
CV (%)	52.00	15.00	20.00	17.5	29.4	41.1	29.5	22.1	27.6	10.4	10.6	45.1	40.5

[#]a..d indicate abcd

benefit to future guava varieties. Some ascorbic acid rich varieties like Red Fleshed having 386 mg/100 g pulp (Dinesh and Vasugi 2010) are also cultivated. The guava breeding objective of ICAR-IARI, New Delhi includes development of pink pulped and soft seeded guava varieties. The lycopene, β-carotene and zeaxanthin content of pink pulped guava genotypes (RFJ, GR-1 and LYLLP) is depicted in Fig 1. Highest lycopene content was recorded with RFJ (6.18 mg/100 g) followed by GR-1 (4.43 mg/100 g) and LYLLP (3.53 mg/100 g). β-carotene content was recorded 10.32 µg/g in RFJ and 0.943 µg/g in LYLLP. GR-1 did not have β-carotene. Zeaxanthin content was recorded 1.354 µg/g in RFJ, 0.144 µg/g in GR-1 and 0.616 µg/g in LYLLP. The lycopene content was found less as compared to commercial varieties like Arka Kiran (7.45 mg/100 g) (Dinesh and Vasugi 2010); Punjab Pink (8.163 mg/100

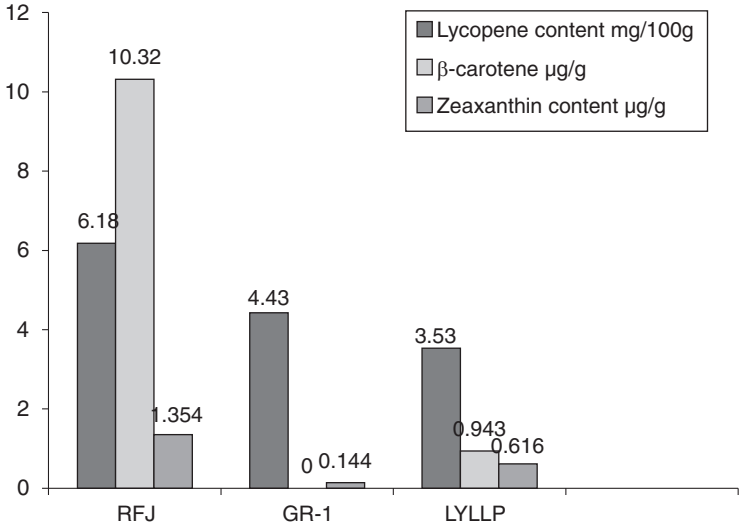

Fig 1 Lycopene, β-carotene and zeaxanthin content of the collected guava genotypes.

Table 2 Correlations among different fruit characters of collected guava genotypes

	FW	FD	FL	TSS	TA	AA	PT	SCT	SH	SL	SW	NS	SI
FW	1												
FD	0.904*	1											
FL	0.812*	0.783*	1										
TSS	-0.113	0.052	-0.014	1									
TA	0.264	0.376*	0.121	0.002	1								
AA	-0.056	0.085	-0.019	0.041	-0.140	1							
PT	0.777*	0.836*	0.703*	0.063	0.198	-0.022	1						
SCT	0.715*	0.646*	0.381	-0.18	0.128	0.165	0.612*	1					
SH	0.53*	0.458*	0.404*	-0.177	0.417*	-0.087	0.377*	0.391*	1				
SL	0.259	0.325*	0.230	-0.196	0.282*	0.016	0.230	0.295*	0.560*	1			
SW	0.275*	0.263	0.282*	-0.240	0.252	0.034	0.163	0.183	0.572*	0.404*	1		
NS	0.237	0.327*	0.168	0.102	0.208	0.062	0.197	0.173	-0.049	-0.239	-0.051	1	
SI	0.211	0.375*	0.270*	0.030	0.319*	-0.092	0.266	0.088	0.467*	0.381*	0.518*	-0.062	1

g) (Thakre *et al.* 2016). But, it is far superior than the Maxican guavas having lycopene content ranges from 0.54-3.28 mg/100 (Mondragon *et al.* 2010).The less number of pink pulped guava genotypes indicates that they needs to conserve at farmers levels. Otherwise in future we will lose its diversity.

Seed variables: The ranges for seed hardiness, length and width were 44.06-132.98 N, 2.22-3.96 mm and 1.85-3.16 mm, whereas, number of seeds per fruit ranged from 99-775 and seed index from 0.17-1.48 (Table 1). Highest coefficient of variation was recorded for number of seeds (45.1%) followed by seed index (40.5%). BKD2 (127.91 N) followed by SA (119.33 N) and LYR (116.94 N) were having the hardest seed. Seed index was maximum with BK18 (1.22 g) and minimum with IFA (0.32 g). More number of seeds and seed index are present in most of the Indian guavas. Collected genotypes also exhibited higher coefficient of variation for these characters. It indicated that the locality didn't have more population of guava genotypes having small sized and less number of seeds. IFA and SHFA had the softest seeds having seed hardness 55.20 N and 53.99 N, respectively. There is moderate diversity for seed hardness.

Correlation between different fruit characters: Correlation coefficients among different variables are presented in Table 2. Fruit weight is positively and significantly correlated with fruit diameter, fruit length, peel thickness, seed core thickness, seed hardness and seed weight. Positive correlation between fruit weight and seed core thickness, seed hardness and seed weight is not desirable. Fruit diameter is positively correlated with fruit length, titratable acidity, peel thickness, seed core thickness, seed hardness, seed length, number of seeds and seed index. Titratable acidity is positively correlated with seed length, seed width and seed index. Peel thickness had significant positive correlation with seed core thickness and seed hardness. Seed hardness was significantly and positively correlated with seed length, seed width and seed index. In most of the cases one good and desired trait is associated with negative one. Since, fruit plants are highly heterozygous in nature; one can get the desired guava genotype where the association of good trait with negative trait is absent.

Principal component analysis: The first two principal components (PC1 and PC2) contribute the maximum variability of the total variance (Table 3). The PC scores identified the traits that mostly define these PCs on the PC1 are fruit weight, fruit diameter, fruit length, peel thickness and seed hardness; on the PC2 seed length, seed width and number of seeds per fruit. Therefore, it can be inferred that fruit weight, fruit diameter, fruit length, peel thickness, seed

Table 3 Loadings of components (having $\lambda<1$) extracted through principal component analysis

Trait	Loading				
	PC1	PC2	PC3	PC4	PC5
Fruit weight	0.380	0.251	-0.086	-0.162	-0.058
Fruit diameter	0.386	0.257	0.065	0.077	-0.026
Fruit length	0.329	0.217	-0.011	-0.064	-0.264
Total soluble solids	-0.057	0.178	0.539	0.480	-0.411
Titrable acidity	0.201	-0.140	0.460	-0.050	0.445
Ascorbic acid	-0.004	0.092	-0.379	0.816	0.233
Peel thickness	0.338	0.280	0.006	-0.026	-0.225
Seed core thickness	0.287	0.225	-0.351	-0.003	0.136
Seed hardness	0.336	-0.275	0.001	-0.037	0.062
Seed length	0.241	-0.322	-0.178	0.070	-0.043
Seed width	0.234	-0.316	-0.053	0.084	0.211
Number of seeds per fruit	0.062	0.378	0.310	0.032	0.603
Seed index	0.236	-0.293	0.297	0.184	-0.106

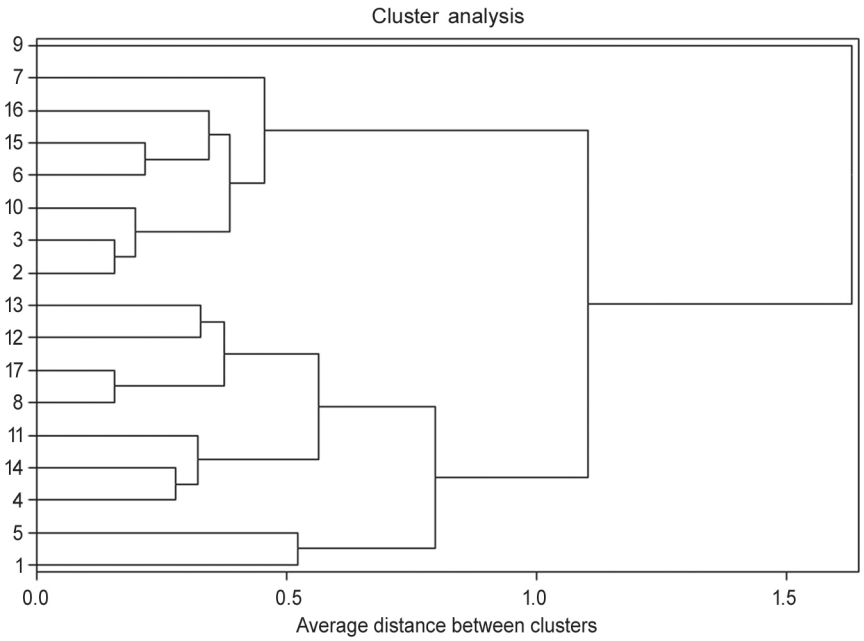

Fig 2 Dendrogram of collected guava genotypes based on fruit and seed characters (1: BKDV, 2: LYR, 3: LP, 4: BK18, 5: BKD2, 6: JSL2, 7: JSL1, 8: CHIJ, 9: RFJ, 10: ISKH, 11: SA, 12: BKJ, 13: BKD1, 14: IFA, 15: SHFA, 16: LYLLP, 17: GR-1).

hardness, seed length, seed width and number of seeds per fruit are the important traits responsible for diversity among the 17 guava genotypes.

Diversity among collected genotypes: Dendrogram, a tree diagram is typically used to show the cluster arrangements in hierarchical data produced by SAS9.4 software using data for all parameters (except lycopene content) of 17 guava genotypes (Fig 2). All 17 guava genotypes can be divided into two groups. In group one, only one genotype RFJ was present, whereas the second group comprised of 16 genotypes. Based on average Euclidian distance between the genotypes, four clusters can be formed. BKDV and BKD2 are similar. BK18, SA and IFA are similar. CHIJ, BKJ BKD1 and GR-1 are similar. LYR, LP, JSL2, JSL1, ISKH, SHFA and LYLLP are similar. Only RFJ is distinguishing from all other. This is an off type and might have come to this area from the purchase of market fruits. The maximum distance is between BKDV and BKD2 genotype (0.55 Euclidean distance). The minimum distance is between LYR and LP genotype (0.2 euclidean distance). Since, guava is not originated in India; it is expected not to have much diversity at one locality. Some of the Mexican workers also reported narrow diversity of guava in Mexico, although it is believed to have been originated in Mexico.

Grouping and identification of superior guava genotypes: Seventeen guava genotypes were grouped into three groups for different characters (S Table 4, 5). This grouping has been done to score these genotypes for preferred characters. For each character the range of limit decided in such a way that it will help scoring. In scoring, only nine most important characters were considered. The acceptable range for different characters were different [fruit weight (>200 g), TSS (10-14 °B and >14°B), ascorbic acid content (>150 mg/100g), peel thickness (>15 mm), seed hardiness (60-100 N and >100 N), Number of seeds per fruit (<250), seed index (<0.5), lycopene content (>5 mg/100 g) and zeaxanthin and β carotene content (present)].The scoring indicates that BKDV, JSL2 and IFA were best among the white pulped and can be collected for quality characters and soft seededness. Among pink pulped guava genotypes RFJ was the best.

Fruit weight and ascorbic acid content are two important characters for guava and lots of variability was present. However, the collection also had undesirable variability with respect to seed characters shows that there are more chances for selection having less and soft seeds. Under white pulped category, BKDV, JSL2 and IFA were found best. Among pink pulped guava genotypes RFJ was the best. They can be collected for their further utilization in breeding programme as parent. Pink pulped guavas are needed to be conserved.

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