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Diversity analysis of guava (*Psidium guajava*) germplasm collection

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ABSTRACT

Guava (*Psidium guajava* L.) is one of the commercially important cultivated fruit crops in India. Guava ranks fifth in production share of fruit crops in the country. During 2014-15, India produced 36.68 lakh tonnes of guava from an area of 2.68 lakh ha with 13.7 t/ha productivity. In the present investigation, a total 33 genotypes including 6 species of *Psidium* and 28 genotypes of *Psidium guajava* were characterized using 39 SSR markers. All accessions are being cultivated at IARI research farm, Pusa campus, New Delhi. Samples were collected from research farm to determine the overall diversity and to assess the similarity among genotypes using molecular markers. Out of 39 primers 26 were reproducible. A total of 64 alleles were produced by 26 primers and number of alleles per locus varied from 1 (mPgCIR01, mPgCIR18 and mPgCIR19) to 5 (mPgCIR256) with mean value of 2.34. Gene diversity ranged from 0.0377 to 0.6681 with a mean value of 0.2766. Highest gene diversity was observed for primer mPgCIR256 (0.6681) and lowest for primer mPgCIR24 (0.0377). SSR primer mPgCIR256 was most informative with high rate of polymorphism as well as number of alleles. Phenotypic correlation indicated the correlated traits with dependent yield trait. PCA and cluster analysis showed a significant phenotypic and genetic diversity among existing cultivars. All accessions were grouped into two major groups and under each group four sub groups. Maximum diversity was found in leaf traits, viz. leaf length, leaf breadth, petiole and petiole length. Genetic distance and the dissimilarity matrix showed three major clusters on the basis of genetic similarity. All these accessions were grouped in to three clusters, viz. A, B and C. While cluster A includes only cultivars, cluster B includes all species except *P. friedrichsthalianum*. This indicates that, the diversity among and between species is at cluster level. Genetic diversity analysis among *Psidium* species showed that *P. guajava* had more similarity with *Psidium guienensis* compared to other species. Present investigation indicated the presence of substantial diversity among guava species which can be utilized for future guava improvement programmes.

Key words: Characterization, Correlation, Diversity analysis, Morphological traits, *Psidium guajava,* Simple Sequence Repeats

Guava (*Psidium guajava* L.) is a native of tropical America, is the most valuable, economical, cultivated member of a dicot family Myrtaceae (Nakasone and Paull 1998); introduced in India by Portuguese during 17th century (Menzal 1985). Guava is one of the most important fruit crops of India which occupying 4.1 per cent of the total area under fruit cultivation. The leading guava producing states of India are Madhya Pradesh which accounts 25% of total production followed by Maharashtra, Uttar Pradesh, Bihar, West Bengal, Andhra Pradesh, Punjab, Gujarat, Chhattisgarh and Karnataka (National Horticultural Board 2014-15). Guava is ideal fruit crop for nutritional security in India because it is rich source of Vitamin- C (260-300 mg/100g). Guava is often called as "super fruit", because it is very good source of vitamins "A" and "C", pectin and dietary fibers. Besides, its nutritional benefits, several problem remains which affect the productivity and quality of guava.

Improvement of guava through conventional approaches is a long-term and cumbersome process that relies on the arbitrary rearrangement of existing genes between two closely related parent plants. The combination of morpho-agronomic characteristics along with molecular markers is considered as a novel tool to characterize guava germplasm and estimate diversity level in the germplasm. In addition, these techniques are equally important to know the parentage relationship among accessions and recommend cultivars

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and/or genotypes having breeding potential (Finger and Klank 2010). Studies on genetic diversity are not only an integral source of data for breeding programs, but also for taxonomic and evolutionary approaches. Further, molecular approaches are not only useful for determining the genetic diversity among cultivars or species and for identifying genes of interest but also for crop improvement through genetic transformation (Morand *et al*. 2002, Zeid *et al*. 2003). Nowadays, efforts are being made to develop high-yielding disease resistant and stable cultivars. Morphological descriptions of many varieties and species are insufficient and vague. Studies focus on genetic diversity and characterization of wild species and local cultivars is still lacking in Indian breeding programme, which play significant role for further crop improvement. Further, characterization of *Psidium* species and genotypes are very important for registration and plant variety protection. Keeping in view of the above requirements on guava, the present research work was carried out with the objectives to characterize 33 guava germplasm using morphological parameters and SSR markers.

MATERIALS AND METHODS

The experiment was conducted during 2013-15. A total of 33 genotypes (Table 1) including six species, viz. *Psidium friedrichsthalianum, P. pumilum, P. guienensis, P. quadrangularis, P. cattleianum, P. guajava* and 28 genotypes namely Allahabad Safeda, Arka Kiran, Arka Amulya, Behat Coconut, Black Guava, Hafsi Red, Hisar Safeda, Hisar Surkha, Hisar Surkha Variant, Lalit, Lucknow- 49, Kashipur Collection, Pusa Srijan, Pant Prabhat, Punjab Pink, Punjab Red, Red Peeled, Red Type, Yellow Type, Sasni Collection, Sasri Collection, Snow White, Shweta, Sour type, Thai guava, Thai Variant 1, Thai Variant 2 and Tamil Nadu Collection were evaluated and it was carried out at the Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi with a randomized block design with three replications over two years (2013 and 2014).

Total genomic DNA was extracted from young healthy leaves of guava by modified CTAB protocol (Doyle and Doyle 1990). Purity and concentration of isolated DNA was determined by NanoDrop and integrity by gel electrophoresis (in 0.8% agarose gel). Pure DNA samples were diluted to a final concentration of 10 ng/µl using low salt TE (10 mMTris-HCl and 1 mM EDTA) buffer. A total of 39 SSR primer pairs (Table 2) were used to study the genetic relatedness among 33 guava genotypes (Risterucci *et al*. 2005). Gradient PCR was set for each primer with selected samples to standardize the annealing temperature for amplification. PCR reaction was set in a total volume of 10µl containing 3.5µl genomic DNA (10ng/µl), 1µl of 10X buffer, 0.8µl of 25 mM $MgCl_2$, 0.4µl of 10mM dNTPs, 0.3µl of each primer (10nmol), 1U of Taq DNA polymerase and 3.9µl distilled water. Amplification was performed in a thermocycler using program of initial denaturation at (Tm-5)^{0}C min. and extension at 72^{0}C for 1 min. with a final extension at 72^{0}C for 10 min. The amplified products were analyzed on 4% metaphor agarose gel containing ethidium bromide (10 mg/ml) at a constant voltage of 120 V for 4 hr using a horizontal gel electrophoresis system. Gel pictures were recorded trans UV gel documentation system. SSR marker loci (39) were selected for initial screening. Out of 39 markers, 26 were polymorphic. A total of 26 SSR primer pairs were found to produce reproducible amplicons ranging from 100 to 300 bp. Observed allele size for each primer was almost the same and within the range as described in the literature (Risterucci *et al*. 2005). Amplicon profile were scored based on the size appeared on the gel. The major allele frequency, gene diversity, heterozygosity and

Table 1 List of *Psidium* species and genotypes used

Variety/genotype/species	Place of collection
Psidium guajava	
Allahabad Safeda	CISH, Lucknow
Arka Kiran	IIHR, Bengaluru
Arka Amulya	IIHR, Bengaluru
Behat Coconut	CISH, Lucknow
Black Guava	TNAU, Coimbatore
Hafsi Red	CISH, Lucknow
Hisar Safeda	HAU, Hisar
Hisar Surkha	HAU, Hisar
Hisar Surka Variant	HAU, Hisar
Lalit	CISH, Lucknow
Lucknow-49	CISH, Lucknow
Kashipur Collection	GBPUAT, Pantnagar
Pusa Srijan	IARI, New Delhi
Pant Prabhat	GBPUAT, Pantnagar
Punjab Pink	PAU, Ludhiana
Punjab Red	PAU, Ludhiana
Red Peeled	IARI, New Delhi
Red Type	IARI, New Delhi
Yellow Type	IARI, New Delhi
Sasni Collection	CISH, Lucknow
Sasri Collection	CISH, Lucknow
Snow White	CISH, Lucknow
Shweta	CISH, Lucknow
Sour Type	IARI, New Delhi
Thai Guava	TNAU, Coimbatore
Thai Variant 1	TNAU, Coimbatore
Thai Variant 2	TNAU, Coimbatore
Tamil Nadu Collection	TNAU, Coimbatore
P. friedrichsthalianum	IARI, New Delhi
P. pumilum	IARI, New Delhi
P. guienensis	CISH, Lucknow
P. quadrangularis	CISH, Lucknow
P. cattleianum	CISH, Lucknow

Table 2 SSR markers with allele number gene diversity, heterozyosity and pic value

Marker code	Sequence	Allele No.	Gene diversity	Heterozygosity	PIC
mPgCIR01	5'TAGTGCTTTGGTTGCTT, 5'GCAGGTGGATATAAGGTC	1	0.0000	0.0000	0.0000
mPgCIR04	5'TTCAGGGTCTATGGCTC, 5'CAACAAGATACAGCGAACT	2	0.2854	0.3448	0.2447
mPgCIR05	5'GCCTTTGAACCACATC, 5' TCAATACGAGAGGCAATA	4	0.3314	0.1538	0.3100
mPgCIR10	5'TGAAAGACAACAAACGAG, 5'TTACACCCACCTAAATAAGA	2	0.3750	0.4375	0.3047
mPgCIR11	5'CCTTTTTCCCGACCAT, 5'TCGCACTGAGATTTTGTGCT	2	0.0644	0.0000	0.0624
mPgCIR14	5'AATACCAGCAACACCAA, 5'CATCCGTATCTAACCTC	2	0.3662	0.0690	0.2992
mPgCIR15	5'CCTTTCGTCAATATTCACTT, 5'CATTGGATGGTTGACAT	3	0.3450	0.0370	0.3183
mPgCIR16	5'TAAGCTGCATGTGTGC, 5'ATGGCTTTGGATGAAA	2	0.4819	0.4286	0.3658
mPgCIR17	5'CCTTTCGTCAATATTCACTT, 5'CATTGGATGGTTGACAT	2	0.1420	0.0769	0.1319
mPgCIR18	5'TAAGCTGCATGTGTGC, 5'ATGGCTTTGGATGAAA	1	0.0000	0.0000	0.0000
mPgCIR19	5'AAAATCCTGAAGACGAAC, 5'TATCAGAGGCTTGCATTA	3	0.4244	0.4000	0.3833
mPgCIR20	5'TATACCACACGCTGAAAC, 5'TTCCCCATAAACATCTCT	1	0.0000	0.0000	0.0000
mPgCIR23	5'CATAAGGACATTTGAGGAA, 5'AATAAGAAAGCGAGCAGA	3.	0.0961	0.1000	0.0936
mPgCIR24	5'GTCTATACCTAATGCTCTGG 5'CCCAGGAAAATCTATCAC	2.	0.0377	0.0385	0.0370
mPgCIR26	5'CTACCAAGGAGATAGCAAG 5''GAAATGGAGACTTTGGAG	1	0.0000	0.0000	0.0000
mPgCIR236	5'ACTCATATTCCGTTTGCATC 5'GAATTAACGACGAGTTCCAC	3	0.3429	0.3548	0.3022
mPgCIR249	5'TTTGTCTGGTCGTCCTAGTT, 5'CTTCAGTCCATCAGCAAAAT	4	0.6318	0.5938	0.5728
mPgCIR247	5'CTTTGAACTCAATGTGTTTG 5'CCTTGTCACTCTTATAGCATCA	2	0.4770	0.7857	0.3633
mPgCIR251	5'TTTACAGCTTCTTCCTTTGC, 5'GTCGTCTACTTCGATGGTTC	2	0.1626	0.1786	0.1494
mPgCIR255	5'TTGTTCTCTTTGTGGTTCCT, 5''GTGACAACGTCAGTGGAGA	2	0.1483	0.1613	0.1373
mPgCIR252	5'AGGAAGAAAGGGTGGTCTAT, 5'CTCCTGTTCTCGTTCTTAGG	4	0.5950	0.6333	0.5307
mPgCIR227	5'GGGATGCTCAAAACTGTAG, 5'CCTGTTACATTGACGAATCA	2	0.1896	0.2121	0.1716
mPgCIR256	5'AGGTGCATGATTACGATTT, 5'CGAGGTTCTTGATGTTGTCT	5	0.6681	0.2258	0.6070
mPgCIR253	5'TGCCCTTAGCCTATAACTCA 5'CTTGTGGTCCAAGATGCTAT	2	0.3496	0.0645	0.2885
mPgCIR228	5'CAGAACAAAGAAGAGGATG, 5'TGGATCAGTAGAATCGTTTG	2	0.3750	0.3667	0.3047
mPgCIR257	5'CGACTCATTTTCTGGTCTGT, 5''CAACCACCTTCATCAATTTC	2	0.3018	0.2222	0.2562
Mean		2.3462	0.2766	0.2263	0.2398

PIC for each locus were calculated using Power Marker 3.5. In addition, genetic distances across the genotypes and neighbour- joining tree were also calculated using Power Marker 3.5. The dissimilarity matrix generated by Power Marker was used to construct un-weighted neighbor joining tree using DARWIN software 5.0.158.

Guava genotypes were evaluated for total 12 morphological characters, viz. leaf length, leaf breadth,

petiole length, leaf area, length and breadth ratio, fruit weight, fruit length, fruit width, fruit index, core diameter, number of seeds. Average of five leaves was taken uniformly from third top opened leaf. Fruit characters were taken as average of five fruits for each accession. Leaves were measured using digital Vernier caliper and fruit weight with digital balance. The measurement of fruit length and leaf was made on Polaris, i.e. between the apex and the end of the stem. The maximum width of the fruit was measured perpendicular to the direction of polar axis is defined as width.

The experiment was conducted in a randomized block design with three replicates and five years data was analyzed to arrive average data suggested by Gomez and Gomez (1984). Correlations between fruit and leaf were estimated using SAS 9.3 software (2011). Clustering of genotypes into groups was performed using the method tree procedure PROC CLUSTER based on Euclidean distance. In order to identify the patterns of morphological variation and contribution of traits principal component analysis (PCA) was conducted as PROC PRINCOP in the SAS 9.3 software (2011).

Table 3 Variability in fruit and leaf characters of guava genotypes

Genotype	Leaf length	Leaf breadth	Petiole length	Leaf area	Length breadth ratio	Fruit weight	Fruit length	Fruit width	Fruit index	Core dia.	No. seeds
Sasri	10.80	5.03	0.73	36.54	2.13	138.12	6.54	6.14	1.07	3.53	326
Sasni	13.33	7.07	1.716	57.42	1.90	80.13	5.79	5.34	1.08	3.43	234
Behat Coconut	11.66	5.71	0.74	54.78	2.06	170.16	7.04	6.65	1.06	3.87	364
Swetha	14.55	5.41	0.63	59.47	2.70	183.23	6.64	6.87	0.97	4.31	283
Lalith	14.70	5.88	0.64	59.47	2.51	172.96	6.65	5.67	0.99	4.46	334
A. Safeda	13.02	5.82	0.80	56.61	2.24	106.35	6.26	5.42	1.1	2.73	367
L-49	11.48	5.27	0.87	51.35	2.18	95.26	5.93	7.49	1.1	3.67	311
Hafsi Red	10.55	6.13	0.67	36.83	1.73	217.36	6.52	5.09	0.87	3.53	151
Punjab Red	12.90	4.33	0.7	43.3	3.3	82.56	5.12	4.09	1.0	3.63	152
Punjab Pink	8.36	3.81	0.39	26.64	2.20	81.35	5.02	5.48	1.02	3.53	141
Snow White	11.92	5.87	0.60	55.12	2.04	91.36	5.56	5.57	1.01	3.63	256
Pant Praphat	14.91	7.62	0.69	59.87	1.96	177.23	6.65	6.41	1.01	3.53	423
Kaashipur	10.5	4.80	0.70	33.3	2.70	350.33	6.62	5.93	1.03	3.43	350
Red Peel	12.85	6.46	0.75	56.66	1.99	116.21	6.12	5.82	1.03	3.60	116
Red Type	14.91	6.07	0.84	60.92	2.46	112.36	6.01	4.97	1.04	3.45	133
Yellow Type	10.12	3.82	0.61	34.34	2.67	74.05	5.71	3.97	1.2	3.13	121
Sour Type	15.41	6.69	0.74	60.15	2.30	18.32	4.66	1.01	1.17	2.71	228
H. Safeda	13.28	5.31	0.69	58.83	2.63	189.23	6.44	6.21	1.03	2.41	336
H. Surkha	12.33	6.42	0.83	55.49	1.94	186.35	6.86	6.21	1.1	3.21	356
Hisar Variant	12.5	5.2	0.80	44.83	2.50	161.35	7.47	6.21	1.2	3.69	439
A. Kiran	10.50	5.29	0.40	38.27	2.10	192.36	6.86	6.47	1.1	3.57	289
A. Amulya	10.46	5.57	0.77	37.63	1.88	139.25	6.63	6.66	0.68	3.27	364
TN	9.98	6.81	0.68	32.56	1.47	173.29	7.16	7.27	1.07	3.57	125
TV-1	11.27	5.56	1.11	48.77	2.03	228.58	8.02	7.50	1.11	3.92	367
TV-2	10.5	4.80	0.80	29.57	2.80	450.26	8.21	7.27	1.09	3.61	389
Thai Guava	14.8	4.80	0.70	54.78	3.70	230.39	8.02	5.92	1.11	4.36	356
Black Guava	13.5	7.20	0.80	65.33	1.80	220.37	6.05	2.58	1.02	3.42	123
P. fredrichsthalianum	7.01	3.57	0.32	18.20	1.97	14.03	3.19	2.56	1.24	2.07	91
P. pumilum	5.07	1.60	0.31	10.93	3.16	13.25	3.12	2.58	1.21	1.87	56
P. guienensis	11.05	6.45	0.35	45.39	1.80	13.46	3.25	2.99	1.11	1.25	40
P. quadrangularis	11.90	5.20	0.80	35.87	2.50	13.32	3.15	2.75	1.01	0.98	36
P. cattlieianum	11.3	3.50	0.60	3.80	11.38	13.55	3.11	2.56	1.13	0.96	41
Pusa Srijan	11.30	3.5	0.60	11.38	3.70	14.12	3.15	2.45	1.12	1.11	42
SEm ±	0.138	0.053	0.010	0.543	0.07	7.00	0.19	0.17	0.08	0.11	10.22
CD (P=0.05)	0.390	0.151	0.027	1.539	0.23	19.00	0.53	0.47	0.22	0.33	29.19

RESULTS AND DISCUSSION

Total 33 guava genotypes were characterized on the basis of leaf blade length, leaf blade width, leaf blade length and width ratio, leaf area, petiole length, fruit weight, fruit length, fruit width, fruit index, core diameter, seeds number. The leaf size (length and width) also varied significantly among guava genotypes. In the present study, it was interesting to note that the leaf length was the maximum in Sour type (14.89 cm), whereas, the minimum was in *P. pumilum* (4.80 cm). Similarly, the leaf width was maximum in Behat Coconut and Hafsi Red (7.20 cm) and had non-significant differences with Arka Amulya (7.2 cm) and the minimum was in case of *P. pumilum*. Almost same trend has been observed for leaf area which were recorded maximum in Pant Prabhat (75.43 cm^2) followed by Arka Amulya (70.36 cm^2) and the minimum was in *P. pumilum* (8.37 cm^2) Pandey *et al*. (2017).

In general, it was observed that the leaf size was also bigger for vigorous genotypes (same age group). However, the genotypes having less plant height and stem girth showed smaller leaves. It clearly indicates that the leaf size has significantly positive correlation with the vigour of guava plants. Reduction in growth and size of leaf directly distinguish aneuploids from diploids (Sharma *et al*. 1982). For example, Pusa Srijan is a dwarfing rootstock, which was released by IARI New Delhi had smaller leaf size (length 11.3 cm, width 3.5 cm and leaf area 11.38 cm^2). It is a tetrasomic aneuploid and imparts substantial dwarfing in Allahabad Safeda in terms of plant height, plant spread and tree volume (Sharma *et al*.,1992). There were large differences among the genotypes with respect to fruit morphological parameters like fruit weight, fruit width and fruit length. The fruit weight was varies from 13.25g to 50.26g. The minimum and maximum fruit weight was recorded in *P. pumilum* and cultivar Thai variant 2, respectively. Earlier report suggested that Thai guava has maximum weight, length and width. It was recorded that Thai variant 2 (a natural variant of Thai guava) has maximum weight (450.26 g), length (8.21 cm) and width (7.27 cm) in comparison of Thai guava. Similarly, minimum fruit width and length recorded in *Psidium pumilum*, which is about 2.58 cm and 3.12 cm, respectively. This variation may be due to phenotypic and genotypic interactions among the selections. Maximum core diameter was recorded in Lalith 4.46 cm followed by 4.31 cm in Swetha.

Seeds are basically ripened ovule, which produce by fertilization of pollen and egg cell within mother plant. It is important character for fruit development. Seeds release auxin and gibberellins, which are essential for fruit development. Seed number were varies from minimum 40 to maximum 439, which were recorded in *Psidium guinensis* to Hisar variant, respectively. This variation differs due to genetic makeup of the plants (Shiva *et al*. 2016).

Of the total 39 primers used to characterize the 33 guava genotype, 26 primers were polymorphic and reproducible. A total of 64 alleles were produced by 26 primers and number of alleles per locus varied from 1 (mPgCIR01, mPgCIR18 and mPgCIR19) to 5(mPgCIR256) with mean value of 2.34. Single allele per locus associates to self- pollinating behaviour of guava. Amplification profiles showed more than two different alleles, agreeing with the 35-40% of out crossing reported for this crop (Nakasone and Paull, 1998). Number of alleles at a marker locus is related to genetic diversity revealed by the marker. More alleles at a particular locus revels high degree of diversity. The value of gene diversity ranged from 0.0377 to 0.6681 with mean value of 0.2766. Highest gene diversity was observed for primer mPgCIR256 (0.66810) and lowest for primer mPgCIR24 (0.0377). The pattern of allelic diversity observed in our study was low in comparison to gene diversity observed by Kanupriya *et al*. (2011), who reported mean value of 6.4 alleles per locus and Valdes *et al*. (2007) reported 4.57 alleles per locus in guava. This variation may be attributed to the different set of SSR markers used by earlier workers and differences in the guava germplasm studied by them.

Table 4 Correlation among leaf characters of guava genotypes

Parameter	Leaf length	Leaf breadth	Petiole length	Leaf area	Length and breadth ratio
Leaf length	1	0.791**	0.566**	0.957**	-0.004
Leaf breadth		1	0.664**	0.776**	-.576**
Petiole length			1	0.641**	-0.333
Leaf area				1	-0.035
Length breadth ratio					1

Table 5 Correlation among fruit characters of guava gentoytpes

Parameter	Fruit weight	Fruit length	Fruit width	Fruit index (length/ width)	Fruit core diameter	No. of Seeds/ fruit	Seed weight
Fruit weight	1	0.896**	0.941**	-0.451*	0.759**	0.571**	0.621**
Fruit length		1	0.939**	-0.371	0.797**	0.650**	0.676**
Fruit width			1	-0.583**	0.815**	0.557**	0.591**
Fruit index				1	-0.468*	-0.229	-0.254
Fruit core diameter					1	0.617**	0.611**
No. of seeds/fruit						1	0.986**
Seed weight							1

Heterozygosity is defined as the presence two alternate alleles in corresponding loci of same chromosome. The value of heterozygosity ranged between 0.038 (mPgCIR252) to 0.633 (mPgCIR24) with mean heterozygosity 0.226. The levels of observed average heterozygosity (Ho= 0.2263) in the present study was optimum but lower than that heterozygosity reported by Valdes *et al*. (2007) in open pollinated seedlings from a Cuban breeding program showed an average observed heterozygosity of 0.38. If the heterozygosity is low, then there is an immediate need for improving genetic diversity of available germplasm through controlled cross-pollination. But the current result shows optimum level of heterozygosity with an adequate genetic variation for selection of desirable commercial types. The Polymorphic Information Content (PIC) ranged from 0.0370 to 0.6070 with means value of 0.239. Botstein *et al*. (1980) described PIC as extent of polymorphism and is a function of the number of alleles and allele frequencies at any given locus. This investigation confirms mPgCIR256 (0.6070) primer as highly informative and polymorphic. The average PIC value of SSR marker is 0.239, which shows that the SSR marker is quite informative, indicating high reproducibility and reliability, evenly distributed and covering the whole guava genome and has high potentiality for multiplexing and high throughput genotyping.

Correlation studies

Significant correlations were recorded for different characters for different guava genotypes which show that leaf length, leaf breadth and petiole length were positively correlated (Table 4 and 5). All the fruit characters were positively correlated with each other except for fruit index. This clearly indicates that leaf size has significant positive correlation with the vigour of guava plants. Reduction in growth and size of leaf directly distinguish aneuploids from diploids (Sharma *et al.* 1982). For example, Pusa Srijan is a dwarfing rootstock, which was released by IARI New Delhi had smaller leaf size (length 11.3 cm, width 3.5 cm and leaf area 11.38 cm^2). It is a tetrasomic aneuploid and imparts substantial dwarfing in Allahabad Safeda in terms of plant height, plant spread and tree volume (Sharma *et al*. 1992). Mean value of morphometric characters studied are presented in Table 3. Data show large variability for a number of traits. The characters were found significant high variability existed among the guava genotypes for different traits. Some of the genotypes showed vigorous growth, however some were less vigorous. These findings are in corroboration with the findings of

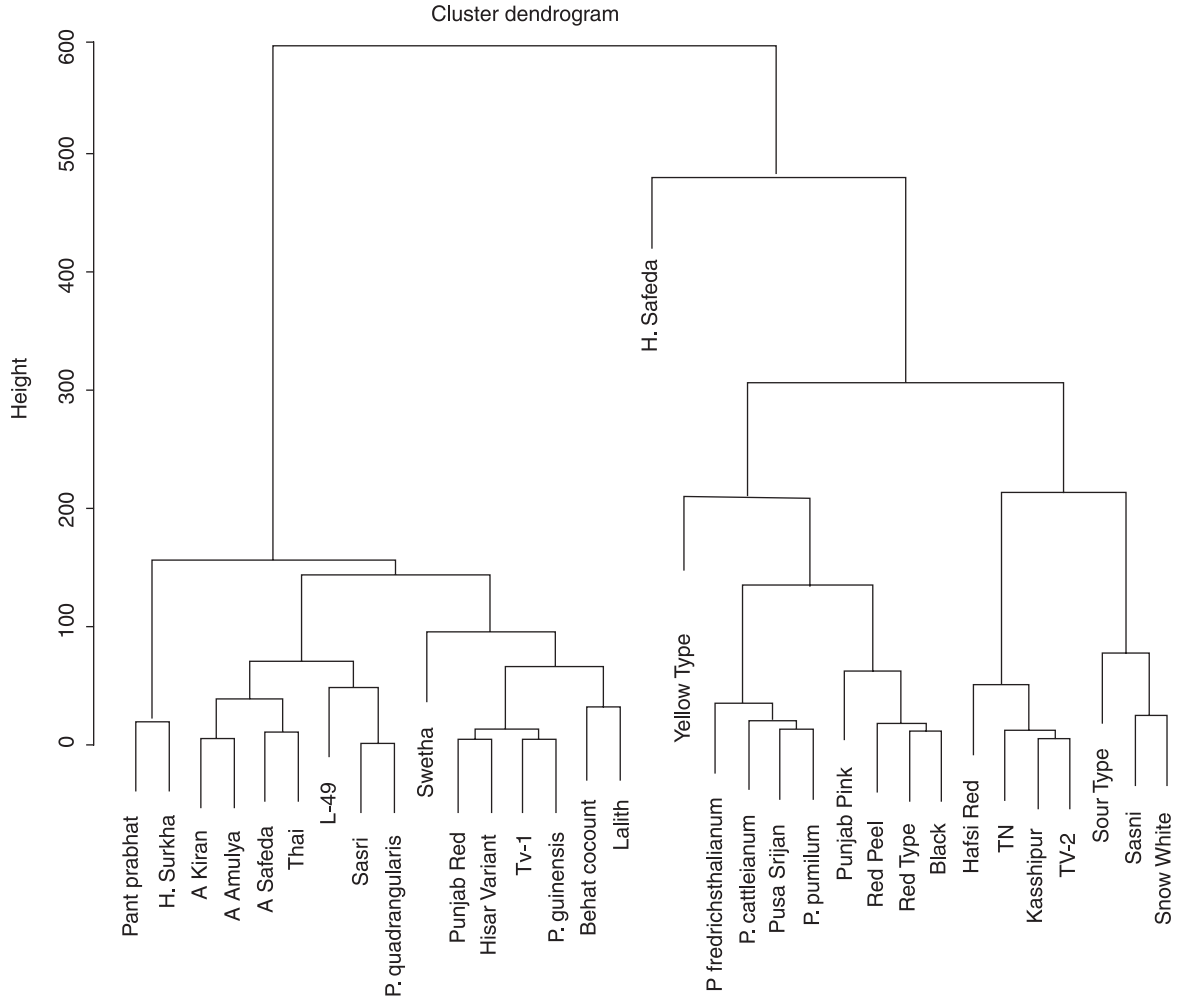

Fig 1 Cluster analysis of 33 genotypes of *Psidium guajava* based on morphological parameter.

Mehmooda *et al.* (2014).

Cluster analysis

The dendrogram based on Euclidean as shown in Fig 1 were grouped into two major groups and further divided in four groups in each cluster based on the major characters like fruit characters and leaf characters. Most of the species is grouped in the second major cluster. However, the species *Psidium quadrangularis* fall under the first group along with Sasri, which is dwarf in nature.

Genetic distance and the dissimilarity matrix which was used for cluster development using the neighbour joining method showed three major clusters on the basis of genetic similarity shown in Fig 2. Horizontal tree developed by neighbour joining method reflected variability among guava species and different genotypes of *Psidium, P. guienensis , P. cattleianum , P. quadrangularis* and *P. pumilum* were grouped in cluster B of the tree, whereas, *P. friedrichsthalianum* was grouped into Cluster C. Furthermore, the variability observed in terms of morphological traits also indicates and justify the similarity observed at molecular level. *Psidium quadrangularis, P. guienensis* and *P. pumilum* had oblong leaf shape and also grouped together in cluster B. Similarly, the leaf pubescence was present in *Psidium quadrangularis, P. guienensis, P. pumilum, P. cattleianum.* These four species also grouped together in cluster B. Thai Guava and Thai Variant 2 were grouped in cluster A; whereas, Thai Variant 1 got grouped into cluster B. Thai Variant 1 grouped into different cluster which may be attributed to natural mutation occurred at any point of time. Arka Amulya was in cluster A and Arka Kiran was into cluster B. The reason for grouping of Arka Amulya and Arka Kiran in different groups might be due to the fact that Arka Amulya is the hybrid of Allahabad Safeda and Seedless; and Arka Kiran is the hybrid of Kamsari and Purple Local. Arka Amulya and Pusa Srijan group into same group might be they are hybrid of same parent Allahabad Safeda and Seedless.

In order to study the genetic diversity among six *Psidium* species, *P. guajava* (Allahabad Safeda) formed a cluster with *Psidium guienensis (*Fig 3). In second cluster, *Psidium freidrichsthalianum* and *P. quadrangularis* showed affinity and formed the group. However, third group represents *P. pumilum* clustering with *P. cattelianum* which shown in Fig 3. The result obtained from molecular marker analyses are quite similar to result obtained by Prakash *et al*. (2002), which revels the same trend of genetic similarity among *Psidium* species like *P. guajava* showed genetic similarity with *P. guienensis* and *P. friedrichsthalianum* showed genetic similarity with *P. quadrangularis* and also reported that all the cultivated species of local importance might have shared common gene pool before their geographical separation, which could be one of the reason for close association observed between them. Both *P. friedrichsthalianum* and *P. quadrangularis* are erect growing and have narrow, elliptic leaves with short petiole and obtuse tip. *Psidium guajava* and *P. guienensis* have leathery elliptic oblong to ovate leaves and this association revealed by molecular markers also. SSR data

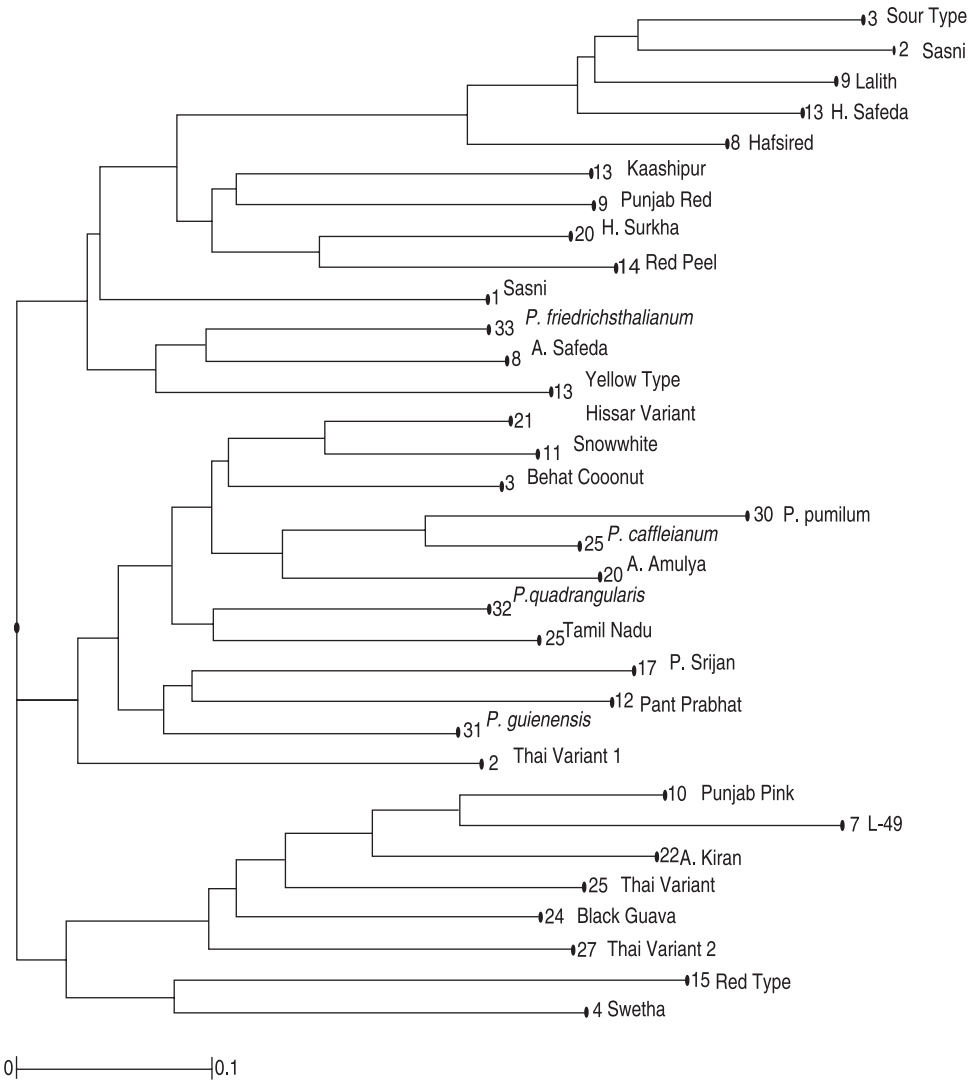

Fig 2 Horizontal tree depicting the genetic relationship of 33 guava genotypes based on SSR markers.

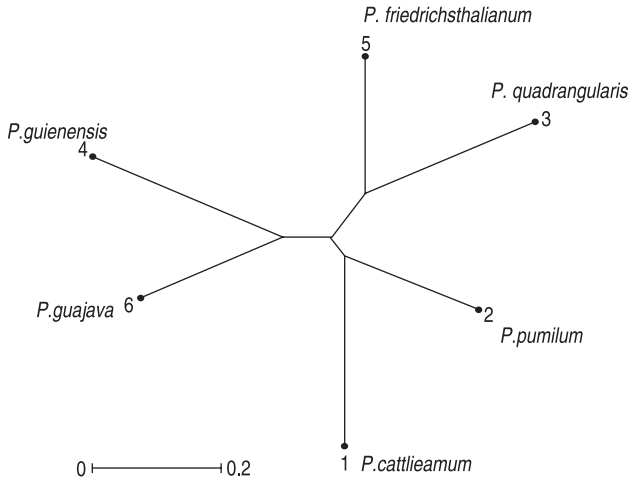

Fig 3 Unrooted tree depicting the genetic relationship among *Psidium* species (6) based on SSR markers.

Principal component analyses

The principal component analyses were carried out and plotted in a graph, which is shown in Fig 4. Fruit index and leaf index were positively correlated, which indicate that when the leaf size is bigger the fruit size is also big in size. It is also correlated with fruit weight and fruit length, which is correlated to the fruit weight. The leaf parameters like leaf length, leaf breadth and leaf area were positively correlated. The results obtained in the present investigation were in agreement with the findings of Angelica *et al*. (2012). They found that variation in growth characters amongst guava genotypes could be due to variation in genetic makeup under the present set of environmental and edaphic conditions. Similar results have also been reported by Babu *et al*. (2007).

Conclusion

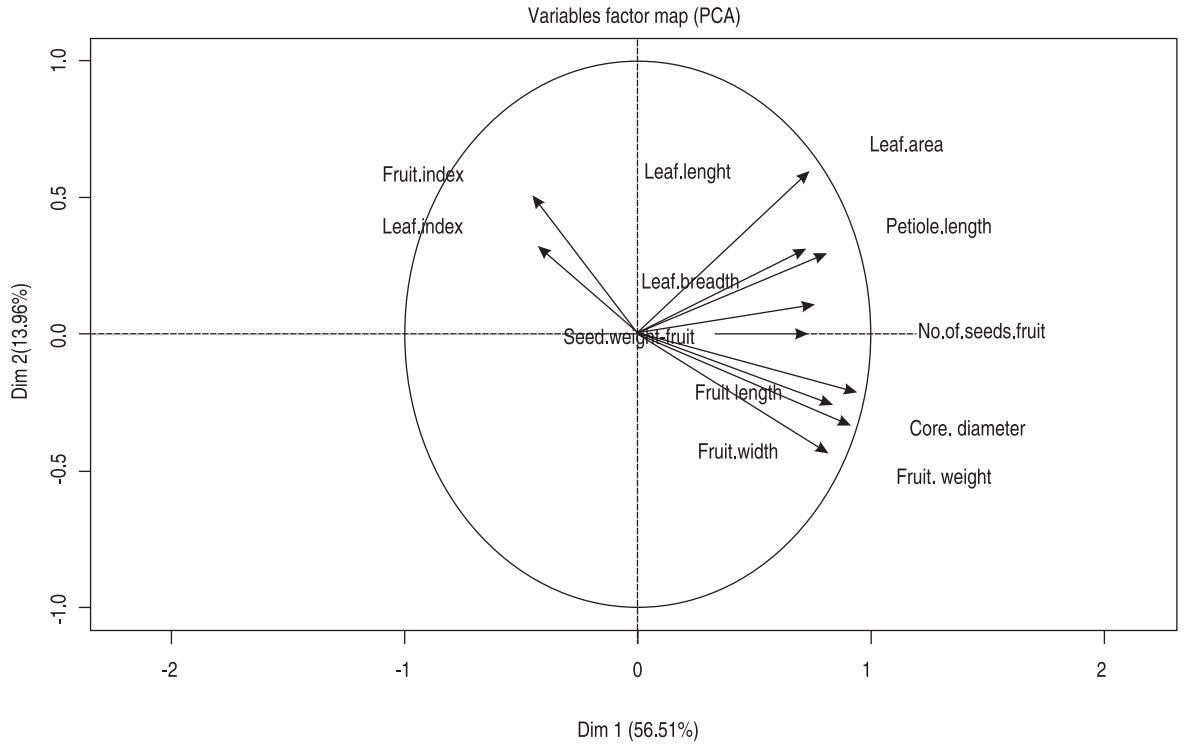

Fig 4 Principle Component Analysis of 33 guava genotypes based on morphological data.

indicated that *Psidium guajava* has more similarity with *P. guienensis* compared to other species. Based on the results obtained from the present study, it is concluded that the conserved guava germplasm had significant variability. The most informative SSR primer in the present study was mPgCIR256 which showed high rate of polymorphism. The morphological and molecular analysis indicated the existing guava germplasm is an important source of genetic diversity that can be used in the guava improvement programmes. The results suggested that SSR is useful for the discrimination of conserved *Psidium* species and genotypes of *P. guajava* for high economy.

Based on the results obtained from the present study, it is concluded that the conserved guava germplasm had significant variability. SSR data also indicated that *Psidium guajava* has more similarity with *P. guienensis* compared to other species. The most informative SSR primer in the present study was mPgCIR256, which showed high rate of polymorphism. The morphological and molecular analysis indicated that the existing guava germplasm is an important source of genetic diversity that can be used in the guava improvement programmes. The results suggested that SSR is useful for the discrimination of conserved *Psidium* species and genotypes of *P. guajava* for high economy.

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