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Assessment of morpho-genetic diversity of guava (*Psidium guajava*) hybrids and genotypes

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ABSTRACT

A total of 20 diverse guava genotypes, including the newly bred hybrids were characterized using the morphological, physio-biochemical parameters and microsatellite markers at ICAR-IARI, New Delhi during 2018–19. Most of the qualitative morphological and physio-biochemical parameters had a coefficient of variation value >20%, which indicated substantial diversity. The longest leaves (14.87 cm) were recorded in Lalit, which was statistically at par with Pant Prabhat (14.67 cm), while amongst the hybrids, it is in GH 2018–10 (13.93 cm). The maximum leaf area was recorded in Pant Prabhat (60.33 cm^2) while amongst hybrids it was in GH 2018-2 (53.40 cm^2). The highest stomatal conductance and net photosynthetic rate was recorded in guava genotype Shweta (9.63 µmol/m^2/s) (0.25 mol/m^2/sec) while amongst the hybrids it was in GH 2018-8 (8.73 µmol/m^2/s) (0.26 mol/m^2/sec), respectively. The mean genetic diversity indices, viz. major allelic frequency, number of alleles, gene diversity, heterozygosity and polymorphic information content of eight SSRs were 0.497, 3.75, 0.599, 0.071 and 0.542, respectively among the guava genotypes. Furthermore, the eight SSRs based Neighbour-joining (N-J) tree separated the newly bred guava hybrids into different clusters, clades and out-groups.

Keywords: Diversity analysis, Leaf lamina, Leaf shape, Neighbour-joining, Stomatal conductance, Transpiration rate

Guava (*Psidium guajava* L.) belongs to family Myrtaceae, which comprises around 150 genera and over 5650 species (Govaerts *et al*. 2008). India is currently one of the world's largest guava producers and produces 4236 thousand metric tonnes of guava from an area of 276 thousand ha (Anonymous 2018–19). In terms of the climatic and edaphic variables, the guava has wider adaptability but faces different challenges. Thus, breeders have fixed the breeding goal to develop high yielding dwarf genotypes, with fruits of uniform shape, good size, attractive peel and pulp colour, fewer and soft seeds and wilt tolerance (Rajan and Negi 2007, Dinesh and Vasugi 2010). In this connection, several introductions of guava genotypes and explorations have been made to enhance the genetic variability in the existing guava gene pool in India (Singh and Rana 1993, Solanki *et al*. 2011). The inter-varietal hybridization programme were also initiated to develop genotypes with a broader genetic base. However, for successful initiation of inter-varietal hybridization and selection of potential hybrids, parental genotypes and progenies characterization is one of the most essential steps. The genetic characterization supplemented with phenotypic evaluation is one of the efficient ways to characterize the guava germplasm (Nogueira *et al*. 2012, Shiva *et al*. 2017, Kumari *et al*. 2018). Thus, in the present investigation, 20 guava genotypes including parents and newly bred hybrids were evaluated for their morphological, physio-biochemical parameters and genetic level employing SSR markers.

MATERIALS AND METHODS

One and half year old 20 guava genotypes, including 10 varieties (Arka Kiran, Allahabad Safeda, Hisar Surkha, Lalit, Lucknow 49, Punjab Pink, Pant Prabhat, Purple Guava, Shweta and Thai Guava) grafted on L 49 rootstock and 10 newly bred hybrid [GH 2018-1 (Thai Guava× Purple Guava), GH 2018-2 (Thai Guava × Arka Kiran), GH 2018-3 (Allahabad Safeda × Lalit), GH 2018-4 (Thai Guava × Hisar Surkha), GH 2018-5 (Pant Prabhat × Lalit), GH 2018-6 (Lucknow 49 × Punjab Pink), GH 2018-7 (Lucknow-49 × Lalit), GH 2018-8 (Shweta × Punjab Pink), GH 2018-9 (Pant Prabhat × Arka Kiran) and GH 2018-10 (Shweta × Lalit)] seedlings, were selected for characterization. The

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experiment was conducted at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi during 2018–19.

For morphological parameters, fully developed 3rd and 4th position leaves from each genotype were selected from the terminal section and characterized as per the Guava Descriptor developed by the Protection of Plant Varieties and Farmers' Rights Authority (Rajan *et al*. 2011). The leaf area was measured using a leaf-area meter (Li-Cor Model 3100 area meter), and represented in sq cm^2. The enzymes, catalase (CAT, EC: 1.11.1.6), peroxidase (POD, EC 1.11.1.7) and superoxide dismutase (SOD, EC: 1.15.1.1) activities were assayed as method suggested by Aebi (1984), Castillo *et al*. (1984) and Dhindsa *et al.* (1981), respectively. Furthermore, the photosynthetic rate (*A*), stomatal conductance (*gs*), and transpiration rate (*E*) was measured using LCiSD Ultra-Compact Photosynthesis System (ADC BioScientific Ltd, Global House, Hoddesdon, UK). For genotyping using SSR markers, the genomic DNA of guava genotypes was isolated using the CTAB method (Doyle and Doyle 1990). A total of 25 SSR loci were selected for the genotyping and assessing the genetic diversity among the guava genotypes (Risterucci *et al.* 2005). The PCR reaction was set in a total volume of 10 µl containing 3.5 µl genomic DNA (10 ng/µl), 1 µl of 10X buffer, 0.8 µl of 25 mM $MgCl_2$, 0.4 µl of 10 mMdNTPs, 0.3 µl of each primer (10 nmol), 1U of *Taq* DNA polymerase (Fermentas, Life Sciences, USA) and 3.5 µl distilled water. Amplification was performed in a thermos cycler using initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 45 and extension at 72°C for 1 min with a final extension at 72°C for 10 min. Each SSR marker's amplified products were separated electrophoresis system (Biorad, USA) and gel images were captured using a gel documentation system (Alpha Imager®, USA). The allelic size of each SSR was scored among the guava genotypes using the programme PyElph 1.4 (Pavel *et al*. 2012). The analysis of variance (ANOVA) of each parameter were analysed using the programme OPSTAT. The SSRs diversity statistics and Neighbour-Joining tree of the guava genotypes were calculated using the Power Marker V3.5 software (Liu and Muse 2005).

RESULTS AND DISCUSSION

Qualitative leaf parameters: The studied guava genotypes had shown substantial variability for their qualitative leaf morphological parameters (Table 1). The six different leaf shapes were observed among the guava genotypes including hybrids. The highest frequency (05) was recorded for the obovate leaf shape, while it was lowest (01) for the round shape. Similarly, the guava genotypes were grouped into five different leaf apex shapes. The obtuse leaf shape had maximum frequency (07 No.), while minimum (02 No.) had attenuate shape. Guava genotypes also showed the substantial variability for leaf base shape, and three different leaf base shapes were recorded, viz. cordate, obtuse and round. The highest frequency for

Table 1 Variability in leaf and physio-biochemical parameters of the guava genotypes and hybrids

Genotype	Leaf shape	Leaf base shape	Leaf apex shape	Pubes-cence	Colour of leaf lamina (adaxial)	Colour of lamina (abaxial)	Petiole orienta-tion	Lamina thick-ness	Leaf length (cm)	Leaf width (cm)	Leaf length/ width (ratio)	Petiole length (cm)	Leaf area (cm^2)	Catalase activity (µmol/ mg protein/ min)	Perox-idase activity (µmol/mg protein/ min)	SOD activity (unit/mg protein/ min)	A (µmol/ m^2/s)	gs (mmol/ m^2/ sec)	E (mmol/ m^2/s)
Arka Kiran	Round	Cordate	Rounded	Present	Green	Light Green	Straight	Interme-diate	11.70 ± 0.67	5.03 ± 0.18	2.32 ± 0.05	0.67 ± 0.07	52.13 ± 0.35	0.17 ± 0.041	0.05 ± 0.007	1.229 ± 0.098	4.33 ± 0.29	0.03 ± 0.01	0.58 ± 0.05
Allahabad Safeda	Ovate	Cordate	Apiculate	Present	Light Green	Light Green	Twisted	Thick	11.80 ± 0.31	5.80 ± 0.10	2.03 ± 0.04	0.90 ± 0.06	50.80 ± 0.58	0.21 ± 0.047	0.06 ± 0.008	1.804 ± 0.114	4.23 ± 0.03	0.06 ± 0.02	0.24 ± 0.05
Hisar surkha	Obovate	Obtuse	Apiculate	Present	Green	Light Green	Twisted	Thick	9.57 ± 0.43	5.00 ± 0.15	1.91 ± 0.06	0.57 ± 0.09	44.47 ± 0.68	0.22 ± 0.055	0.04 ± 0.009	1.210 ± 0.133	5.53 ± 0.18	0.10 ± 0.02	0.61 ± 0.05
Lalit	Obovate	Obtuse	Acute	Present	Green	Green	Twisted	Thick	14.87 ± 1.43	6.00 ± 0.21	2.47 ± 0.16	0.83 ± 0.09	40.27 ± 0.53	0.17 ± 0.060	0.37 ± 0.010	1.082 ± 0.145	4.73 ± 0.09	0.03 ± 0.01	0.51 ± 0.05
Lucknow 49	Oblance-olate	Obtuse	Obtuse	Present	Dark Green	Green	Twisted	Thick	14.63 ± 0.61	6.27 ± 0.12	2.33 ± 0.07	1.07 ± 0.03	50.73 ± 0.58	0.33 ± 0.037	0.21 ± 0.006	1.895 ± 0.091	7.30 ± 0.27	0.15 ± 0.02	0.72 ± 0.05

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Table 1 (*Concluded*)

Genotype	Leaf shape	Leaf base shape	Leaf apex shape	Pubescence	Colour of leaf lamina (adaxial)	Colour of lamina (abaxial)	Petiole orientation	Lamina thickness	Leaf length (cm)	Leaf width (cm)	Leaf length/ width (ratio)	Petiole length (cm)	Leaf area (cm^2)	Catalase activity (µmol/ mg protein/ min)	Peroxidase activity (µmol/mg protein/ min)	SOD activity (unit/mg protein/ min)	A (µmol/ m^2/s)	gs (mmol/ m^2/ sec)	E (mmol/ m^2/s)
Punjab Pink	Oblanceolate	Cordate	Acute	Absent	Green	Light Green	Twisted	Intermediate	12.83 ± 1.15	6.17 ± 0.26	2.10 ± 0.27	0.90 ± 0.10	52.73 ± 0.58	0.18 ± 0.049	0.08 ± 0.008	1.839 ± 0.120	7.70 ± 0.31	0.22 ± 0.02	0.16 ± 0.05
Pant Prabhat	Obovate	Cordate	Acute	Present	Green	Green	Twisted	Intermediate	14.67 ± 1.01	7.60 ± 0.31	1.93 ± 0.09	0.80 ± 0.06	60.33 ± 0.47	0.28 ± 0.038	0.04 ± 0.007	1.655 ± 0.092	3.87 ± 0.12	0.11 ± 0.02	0.34 ± 0.05
Purple Guava	Obovate	Cordate	Apiculate	Absent	Purple	Pinkish red	Straight	Thin	8.13 ± 0.20	3.87 ± 0.09	2.10 ± 0.01	0.23 ± 0.03	32.87 ± 0.75	0.72 ± 0.042	0.05 ± 0.008	1.139 ± 0.103	5.20 ± 0.23	0.09 ± 0.02	0.44 ± 0.05
Shweta	Oblong	Round	Attenute	Absent	Green	Light Green	Twisted	Thick	13.33 ± 0.43	5.50 ± 0.35	2.44 ± 0.10	0.67 ± 0.03	50.40 ± 0.23	0.24 ± 0.040	0.09 ± 0.007	1.047 ± 0.097	9.63 ± 0.09	0.25 ± 0.02	0.23 ± 0.05
Thai Guava	Oblong	Round	Attenute	Absent	Dark green	Light Green	Twisted	Thick	10.77 ± 0.34	5.60 ± 0.31	1.93 ± 0.09	1.10 ± 0.06	41.47 ± 0.44	0.15 ± 0.043	0.14 ± 0.008	1.960 ± 0.106	5.27 ± 0.22	0.07 ± 0.02	0.44 ± 0.05
GH 2018-1	Lanceolate	Cordate	Obtuse	Absent	Green	Light Green	Straight	Intermediate	7.57 ± 0.62	4.10 ± 0.31	1.85 ± 0.05	0.17 ± 0.03	49.60 ± 0.20	0.43 ± 0.036	0.03 ± 0.006	1.711 ± 0.087	6.33 ± 0.29	0.09 ± 0.01	0.72 ± 0.09
GH 2018-2	Ovate	Round	Round	Absent	Dark green	Green	Straight	Intermediate	8.50 ± 0.52	5.07 ± 0.17	1.68 ± 0.06	0.23 ± 0.03	73.40 ± 0.50	0.19 ± 0.038	0.19 ± 0.007	1.863 ± 0.091	6.20 ± 0.27	0.05 ± 0.01	0.54 ± 0.09
GH 2018-3	Lanceolate	Cordate	Obtuse	Absent	Dark green	Green	Staraight	Thick	10.17 ± 0.09	4.83 ± 0.18	2.11 ± 0.08	0.20 ± 0.00	63.53 ± 0.41	0.24 ± 0.043	0.06 ± 0.005	1.794 ± 0.105	5.30 ± 0.27	0.05 ± 0.01	0.34 ± 0.09
GH 2018-4	Obovate	Cordate	Apiculate	Sparse	Dark green	Green	Twisted	Thick	9.50 ± 0.30	4.43 ± 0.15	2.14 ± 0.03	0.17 ± 0.03	54.93 ± 0.44	0.20 ± 0.034	0.02 ± 0.006	1.676 ± 0.081	6.40 ± 0.25	0.08 ± 0.01	0.44 ± 0.09
GH 2018-5	Oblong	Cordate	Obtuse	Sparse	Dark green	Green	Staright	Intermediate	9.97 ± 0.80	4.97 ± 0.09	2.00 ± 0.13	0.30 ± 0.00	43.47 ± 0.85	0.26 ± 0.043	0.03 ± 0.008	1.724 ± 0.106	3.77 ± 0.20	0.12 ± 0.01	0.36 ± 0.09
GH 2018-6	Ovate	Cordate	Rounded	Absent	Green	Green	Straight	Thin	10.83 ± 0.62	4.67 ± 0.24	2.33 ± 0.11	0.40 ± 0.00	45.13 ± 0.93	0.18 ± 0.032	0.03 ± 0.005	1.369 ± 0.077	4.67 ± 0.26	0.16 ± 0.01	0.33 ± 0.09
GH 2018-7	Lanceolate	Cordate	Obtuse	Absent	Dark green	Green	Straight	Intermediate	10.13 ± 0.29	4.33 ± 0.22	2.35 ± 0.07	0.43 ± 0.03	41.07 ± 0.47	0.22 ± 0.036	0.22 ± 0.006	1.775 ± 0.212	5.40 ± 0.15	0.17 ± 0.01	0.78 ± 0.09
GH 2018-8	Lanceolate	Cordate	Obtuse	Absent	Dark green	Green	Straight	Intermediate	9.23 ± 0.95	4.30 ± 0.25	2.17 ± 0.29	0.33 ± 0.07	38.67 ± 0.48	0.21 ± 0.037	0.13 ± 0.006	1.089 ± 0.089	8.73 ± 0.09	0.26 ± 0.01	0.45 ± 0.09
GH 2018-9	Ovate	Round	Obtuse	Absent	Green	Light green	Straight	Thick	9.27 ± 0.53	5.13 ± 0.12	1.80 ± 0.07	0.23 ± 0.03	39.07 ± 0.64	0.29 ± 0.042	0.06 ± 0.008	1.426 ± 0.103	4.60 ± 0.17	0.03 ± 0.01	0.29 ± 0.06
GH 2018-10	Oblong	Cordate	Rounded	Absent	Dark green	Green	Twisted	Thick	13.93 ± 0.47	6.17 ± 0.09	0.05	0.53 ± 0.03	67.40 ± 0.53	0.23 ± 0.034	0.03 ± 0.006	1.408 ± 0.137	8.44 ± 0.28	0.03 ± 0.01	0.40 ± 0.04
CD (P=0.05)									1.99	0.59	0.34	0.15	1.65	0.020	0.004	0.101	0.63	0.02	0.06
Mean									11.07	5.24	2.00	0.54	49.62	0.25	0.10	1.53	5.88	0.11	0.45

leaf base shape (13) was recorded for cordate leaf base, and lowest (03) was for obtuse shape. Most of the guava genotypes were devoid of the pubescence, except six genotypes. The adaxial leaf lamina colour varied among the tested guava genotypes. The light green and purple lamina colour showed the lowest frequency (01), while dark green and green colour proved most common (09). Further, green coloured abaxial leaf lamina was most common (11) among tested guava genotypes, while pinkish-red colour was noticed in 01 genotype. The studied guava genotypes were grouped into two petiole orientation groups, i.e. straight and twisted petiole with equal frequency (10 in each). The guava genotypes were grouped into three different groups based on their leaf lamina thickness. Most of the guava genotypes (10) have the thick lamina, while it was thin in only 01 genotype. Earlier, Pandey *et al*. (2017) and Sharma *et al.* (2010) also observed the foliage characters to be the valid criteria to characterize the guava genotypes. Furthermore, Methela *et al*. (2019) included the qualitative leaf morphological parameters for the characterization of the guava germplasm and deciphered a large variability for the studied traits as also observed in the present study.

The guava genotypes including the newly bred hybrids had large variability for quantitative leaf parameters (Table 1). Lalit tended to show the highest leaf length (14.87±1.43 cm) followed by Pant Prabhat and L 49 without any significant difference. The lowest leaf length was recorded in GH 2018-8 (9.23±0.95 cm), however it was found statistically similar with Hisar Surkha, Purple Guava and all the hybrids except GH 2018-10. Pant Prabhat also showed the broadest leaf (7.60±0.31 cm) while Purple Guava had the lowest leaf width (3.87±0.09 cm). The highest and lowest leaf length to width ratio was recorded in GH 2018-7 (2.35±0.07) and GH 2018-2 (1.68±0.06), respectively. The longest petiole length was recorded in Thai Guava (1.10±0.06cm) statistically *at par* with L 49, while it was lowest in GH 2018-1 (0.17±0.03cm). The highest leaf area was recorded in GH 2018-10 (67.40±0.53 cm^2), while lowest in GH 2018-8 (38.67±0.48 cm^2). Pandey *et al*. (2017) characterized the guava germplasm using the leaf morphological parameters including the leaf area and recorded high variability. Likewise, Kareem *et al*. (2018) also included the quantitative leaf parameters for diversity assessment of guava genotypes. In the present study, the guava genotypes including newly bred hybrids showed high degree of variations for the qualitative leaf parameters.

In physio-biochemical parameters, viz. stomatal conductance (g_s), transpiration rate (E), net photosynthetic rate (A), CAT, POD and SOD activities were found to varying significantly among the studied guava genotypes (Table 1). The highest activity of CAT was observed in Purple Guava (0.72 ± 0.42 μmol/mg protein/min) statistically, while it was low in Arka Kiran and Lalit. The activity of POX was noticed to be the highest in Lalit (037 ± 0.010 μmol/mg protein/min), while GH 2018-1, GH 2018-4, GH 2018-5 and GH 2018-6 were found to have very low activity of POX (0.02 ± 0.006 to 0.03 ±0.008 μmol/mg protein/min). The highest and lowest activity of SOD was recorded in the leaves of Thai Guava (1.96 ± 0.106 unit/mg protein/min) and GH 2018-8 (1.089 ± 0.089 unit/mg protein/min), respectively. Shweta and GH 2018-8 proved their superiority for high A (9.63 ± 0.09 μmol/m^2/s in Shweta and 8.73 ± 0.09 μmol/m^2/s) in GH 2018-8 and gs (0.25 ± 0.2 mmol/m^2/s in Shweta and 0.26 ± 0.01 mmol/m^2/s in GH 2018-8. The lowest A and gs was noticed in Pant Prabhat (3.78 ± 0.12 μmol/m^2/s), Arka Kiran and GH 2018-9 (0.03 ± 0.01 mmol/m^2/s in each), respectively. GH 2018-7 had highest E (0.78 ±0.09 mmol/m^2/s), while it was lowest in Allahabad Safeda (0.24 ± 0.05 mmol/m^2/s). A total of 21 SSR loci screened for their allelic polymorphism among the guava genotypes including the newly bred hybrids. Out of the 21 SSR loci, only eight were found polymorphic and informative among the guava genotypes (Table 2). The allelic size ranged from 170 to 300 bp among the eight SSR loci. The number of alleles amplified by the SSR loci varied from 2 (mPgCIR13 and mPgCIR24) to 6 (mPgCIR2) with an average of 3.75 alleles per locus. The major allele frequency among the SSR loci varied from 0.250 (mPgCIR22) to 0.900 (mPgCIR13) with an average of 0.497. The gene diversity or expected heterozygosity among the SSR loci ranged from 0.180 (mPgCIR13) to 0.794 (mPgCIR22) with an average value of 0.599. The observed heterozygosity varied from 0 (mPgCIR13, mPgCIR24, mPgCIR19, mPgCIR20 and mPgCIR11) to 0.350 (mPgCIR22) with an average of 0.071. The highest PIC value (0.761) recorded for the SSR locus, mPgCIR22 and lowest (0.164) for locus, mPgCIR13 with an average of 0.542. The SSR loci, viz. mPgCIR2, mPgCIR11, mPgCIR12, mPgCIR19, mPgCIR20 and mPgCIR22 had PIC value > 0.500 among the studied SSR loci thus, had high discrimination power. Ma *et al*. (2020) studied diversity indices of 15 SSR markers among the 45 guava genotypes and recorded average PIC value of 0.60, which is much in congruent to the present investigation. Recently, Kumar *et al*. (2020) estimated the genetic diversity statistics of 26 polymorphic SSR loci among the 40 guava genotypes and estimated average PIC value of 0.46, similar to the present findings. Thus, the selected SSR markers set had substantial

Table 2 The details of genetic diversity statistics of microsatellite loci among the guava genotypes

Marker	Major allele frequency	Allele No.	Gene diversity	Heterozygosity	PIC
mPgCIR22	0.250	5.000	0.794	0.350	0.761
mPgCIR12	0.528	3.000	0.600	0.167	0.528
mPgCIR13	0.900	2.000	0.180	0.000	0.164
mPgCIR24	0.579	2.000	0.488	0.000	0.369
mPgCIR19	0.400	4.000	0.685	0.000	0.623
mPgCIR20	0.353	5.000	0.740	0.000	0.697
mPgCIR2	0.400	6.000	0.719	0.050	0.674
mPgCIR11	0.563	3.000	0.586	0.000	0.520
Mean	0.497	3.750	0.599	0.071	0.542

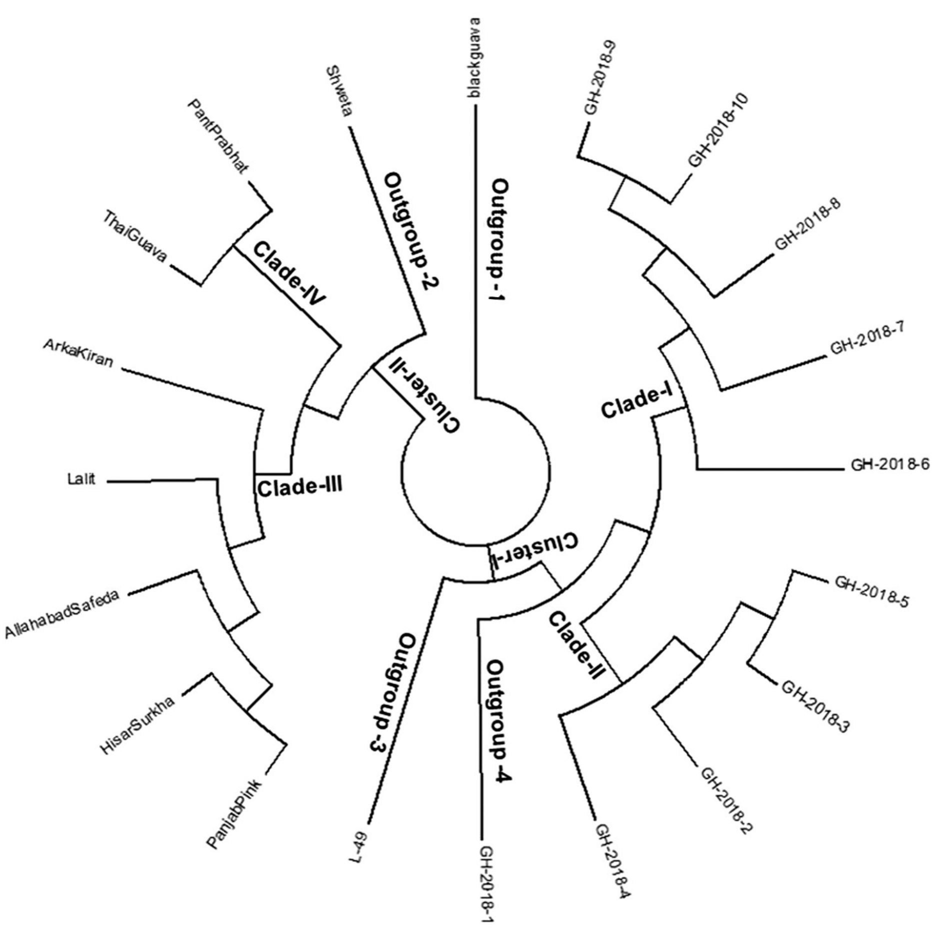

Fig 1 The N-J tree of guava genotypes based on the microsatellite marker data.

genetic diversity indices among the guava genotypes, including the newly bred hybrids.

The N-J tree grouped the guava genotypes, including the newly bred hybrids into two clusters and one out-group (Fig 1). Earlier, Sitther *et al*. (2014) found the genetic relationship among the guava genotypes using the N-J tree based on 20 microsatellite markers. Furthermore, Kumar *et al*. (2020) also constructed a phylogenetic tree using N-J method based on 26 SSRs and classified 40 guava genotypes into different genetic groups. In the present study, the guava genotypes, including the newly bred hybrids, form other clusters, clades and out-groups in the N-J tree. Thus, microsatellite based genetic fingerprinting deciphered a substantial level of genetic diversity among guava genotypes and hybrids. The findings of the present investigation suggested that the guava genotypes including newly bred hybrids are quite diverse for their morphological, physio-biochemical parameters and also at genetic levels. The genetic differentiation of recently bred guava hybrids elucidated that the crossing between the heterozygous guava parents may yield novel recombinants for evolving superior genotypes.

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