Original Research Article
Assessment of genetic diversity of seedling origin progenies in guava (*Psidium guajava* L.)

ABSTRACT

A set of 96 guava germplasm comprised of Kolar Gold Field ecotypes and Srirangapatna ecotypes, seedling origin progenies (one year old) and eight released varieties planted at the Department of Biotechnology and Crop Improvement field block, College of Horticulture, Bengaluru were used in this experiment during 2021-2022. The study was conducted to understand the comparative genetic diversity of varieties and seedling origin progenies by using morphological and molecular characterization. Guava seedling origin progenies and released varieties showed significantly higher range of variations for different morphological characters. The maximum leaf blade length was recorded in the genotype BCIG71 (15.10 cm) while the variety Red Guava (6.70 cm) recorded maximum leaf width. The length to width ratio was highest in BCIG53 (2.60). The 96 guava genotypes were also characterized by using a set of 10 SSR primers. A total of 90 alleles were produced by 10 primers; number of alleles per locus varied from 4 (mPgCIR10) to 14 (mPgCIR02) with mean value of 9. The PIC value has recorded maximum for the primer mPgCIR09 (0.86).

***Key words**: Guava, genetic diversity, morphological characterization, molecular characterization*

1. INTRODUCTION

Guava (*Psidium guajava* L.), known as the 'Apple of the tropics' or 'Poor man's fruit' because of its low cost and superior nutritional value (Singh, 2005), is a fruit crop of the family Myrtaceae (Nakasone and Paull, 1998). Guava has gained considerable prominence in India because of its high nutritive value, pleasant aroma, flavour and availability at moderate prices. Since guava is a cross-pollinated crop, every seedling-origin plant possesses a unique gene combination. As a result, morphologically they are diverse from each other and these plants range from poor to exceptional in terms of fruit quality and other growth characters. The morphological characterization is helpful for the initial screening and for tracing genetic variations. Morphological characters are sometimes limited in number, often modified by cultivational practices and environmental factors (Sharma *et al*., 2010). Thus, the combination of morpho-agronomic traits and molecular markers is considered to be a novel approach for evaluating guava germplasm and assessing the level of variation. Hence, the study has carried out to know the extent of diversity present in the guava seedling progenies and some of the released varieties.

2. MATERIALS AND METHODS

2.1 Morphological characterization

A total of 96 guava genotypes comprised of Kolar gold field ecotypes and Srirangapatna ecotypes, seedling origin progenies and eight released varieties were used in this experiment. The genotypes have been planted at a close spacing of 1.8 m x 1.2 m at the Department of Biotechnology and Crop Improvement field block, College of Horticulture, Bengaluru. The genotypes were assessed for morphological and molecular traits and the experimental design was augmented block design (91+4

Commented [AM1]: Please remove/adjust this from there, as it is confusing the title of the Manuscript

Commented [AM2]: Please change the font case of selected words to "Capitalize each word (Without Prepositions)"

Commented [AM3]: Please correct the name as it is "Kolar Gold Fields"

Commented [AM4]: Please replace the sentence (Kolar Gold Field ecotypes and Srirangapatna ecotypes) with "Kolar Gold Fields and Srirangapatna ecotypes"

Commented [AM5]: Please mention the unit of reading

Commented [AM6]: As the abbreviation is used for the very first time here in this manuscript, please write its full form here, then later on use this abbreviated form throughout the manuscript

Commented [AM7]: Somewhere you are mentioning the guava varieties and on the next you are also mentioning it as a germplasm, please use a single word to make it clear either it's germplasm/varieties.

Commented [AM8]: Which type of diversity, as you're doing genetic diversity, please mention here.

Commented [AM9]: Please rewrite the both of sentences to make it clearer and easier to understandable for the reader

Commented [AM10]: Please change the case (Capitalized each word) for the selected heading

Commented [AM11]: Here is again mentioned as genotype, please use a single term to make it clearer, either it's genotype/varieties/germplasm/progenies.

Commented [AM12]: Please write the correct name with proper spelling and as a proper noun. As it's Kolar Gold Fields.

Commented [AM13]: Please remove this word (ecotypes) from here.

Commented [AM14]: The word (assessed) should be replaced with "evaluated".

checks). The vegetative parameters *viz*., leaf length, leaf width, leaf length/width ratio, number of leaves per shoot and number of branches per plant were recorded. The average length of five fully developed leaves excluding petiole were taken using measuring scale and expressed in centimeter (cm). Similarly average width of five fully matured leaves was measured from the middle of the leaf with the help of measuring scale (cm). The number of leaves per shoot was counted for five matured shoot and the average value was recorded in each plant as well as number of branches in each plant was counted and recorded. Further, to assess the genetic diversity, clustering was done using K- mean method.

Commented [AM15]: Please mention the name of experimental design chosen for this study with reference. And also reconfirm the (91+4). If there is any check of the experiment, it should be mentioned in detail.

Commented [AM16]: As you mentioned earlier the genetic diversity's study is executed using SSR markers, here you have to mention the same thing to make it less confusing for the reader.

2.2 Molecular characterization

Commented [AM17]: Please change the case (Capitalized each word) for the selected heading

The young leaf samples were collected from individual plants and DNA was extracted by modified CTAB (cetyl trimethyl ammonium bromide) extraction protocol by Doyl and Doyl (1990) with minor modification. The amount of DNA following extraction was quantified and purification and quality was checked by using Nanodrop Spectrophotometer (NanoDrop Technologies, Thermoscientific) and also by gel electrophoresis (0.8 % agarose gel used to check the quality of DNA). DNA samples were diluted with appropriate amount of TE buffer to yield a working concentration of 100 ng / µl and stored at -20° C temperature. A total of ten SSR primers were used to assess the genetic diversity of seedling progenies as well as eight released varieties of guava genotypes (Table 1).

Commented [AM18]: Please cite the reference appropriately, and rewrite it with correct spellings as it's, "Doyle and Doyle "

Commented [AM19]: Please delete the selected words from the sentence to make it clearer.

Commented [AM20]: Please mention the source of this line and also make sure that is this solely used for the quality assessment of DNA?

Commented [AM21]: Please remove space from here

Commented [AM22]: Please use a single term for the SSR, either they are primers or markers.

Commented [AM23]: Replace the word with evaluate

Commented [AM24]: Please change the sentence as, "genetic diversity guava's germplasm."

Gradient PCR was set for each primer with selected samples to standardize the temperature of amplification. Genomic DNA was diluted to prepare working stocks of 100 ng/µl. The PCR reaction was set in a total volume of 10µl containing 1µl genomic DNA (100ng/µl), 3µl of 10X buffer, 0.8µl of 25 mM $MgCl_2$, 0.4µl of 10mM dNTPs, 0.5µl of each primer (10nmol), 1U of Taq DNA polymerase (Fermentas, Life Sciences, USA) and 5µl distilled water. Amplification was performed in a thermocycler using following programme. Initial denaturation at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 30 s, annealing at T_a˚C min and extension at 72˚C for 1 min with a final extension at 72˚C for 10 min. The annealing temperature (T_a) was kept 2˚C above and below T_m of that particular primer sequence. The amplified products were analyzed on 2.5 % metaphor agarose gel containing ethidium bromide (10 mg/ml) at a constant voltage of 80V for 2 hours using a horizontal gel electrophoresis system (Biorad, USA). Gel pictures were recorded under UV light gel documentation System (Alpha Imager®, USA). Reproducible DNA bands of individual sample were scored manually and analyzed for genetic diversity, population genetic parameters. Allele frequency, heterozygosity were computed using GenAlex V6.0. Further, cluster analysis has done using DARwin software.

Commented [AM25]: Please rewrite the correct recipe of 10ul PCR.

Commented [AM26]: Please rephrase the sentence to make it easier and clearer for the reader.

Commented [AM27]: Please Rewrite the sentence, as there is major mistake about the annealing temperature of PCR. Also cite the reference for the described conditions of PCR.

Table 1. List of SSR markers used for molecular characterization of guava genotypes

Commented [AM28]: Clarify it either its Marker/Primer?

Commented [AM29]: Replace the word with germplasm

DNA marker locus	Forward primer(5'-3')	Reverse primer(3'-5')
mPgCIR01	TAGTGCTTTGGTTGCTT	GCAGGTGGATATAAGGTC
mPgCIR02	AGTGAACGACTGAAGACC	ATTACACATTCAGCCACTT
mPgCIR05	GCCTTTGAACCACATC	TCAATACGAGAGGCAATA
mPgCIR08	ACTTTCGGTCTCAACAAG	AGGCTTCCTACAAAAGTG
mPgCIR07	ATGGAGGTAGGTTGATG	CGTAGTAATCGAAGAAATG
mPgCIR09	GCGTGTCGTATTGTTTC	ATTTTCTTCTGCCTTGTC
mPgCIR10	GTTGGCTCTTATTTTGGT	GCCCCATATCTAGGAAG
mPgCIR11	TGAAAGACAACAAACGAG	TTACACCCACCTAAATAAGA

mPgCIR015	TCTAATCCCCTGAGTTTC	CCGATCATCTCTTTCTTT
mPgCIR016	AATACCAGCAACACCAA	CATCCGTCTCTAAACCTC

RESULTS AND DISCUSSION

3.1 Morphological characterization

The guava seedling progenies and released varieties revealed significant variation for morphological characters. The seedling progeny BCIG71 (15.10 cm) noticed the maximum leaf blade length and the minimum leaf blade length was recorded in the genotype BCIG03 (7.50 cm). Similar results were reported by Singh *et al*. (2016). With respect to the leaf width, the highest value recorded in the genotype Red Guava (6.70 cm) whereas the minimum leaf width was recorded in the genotype BCIG38 (3.03 cm). Additionally, the leaf length and width ratio was found to be maximum in BCIG53 (2.60) whereas the minimum ratio was observed in BCIG02 (1.41) (Table 2). Earlier studies by Sharma *et al*. (2010) and Kumari *et al*. (2018) reported similar results and they attributed these traits to be genotypic and thus varying among individual genotype, could also due to the prevailing agro-climatic conditions.

Table 2. Characterization of seedling origin guava progenies and varieties based on morpho-physical traits (quantitative characters)

Sl. No.	Genotypes	Leaf blade length (cm)	Leaf blade width (cm)	Length /width ratio	Number of leaves per shoot	Number of branches per plant
1	BCIG01	7.93	4.26	1.86	15.09	12.00
2	BCIG02	8.73	6.20	1.41	14.10	3.00
3	BCIG03	7.50	4.23	1.77	15.25	11.00
4	BCIG04	10.33	6.30	1.64	13.40	12.00
5	BCIG05	11.40	6.00	1.90	15.19	14.00
6	BCIG06	10.06	5.83	1.73	14.20	15.00
7	BCIG07	11.06	5.70	1.94	21.84	16.00
8	BCIG08	9.83	5.23	1.88	23.44	14.00
9	BCIG09	9.20	5.33	1.73	21.71	12.00
10	BCIG10	10.16	5.50	1.85	18.00	8.00
11	BCIG11	9.00	5.43	1.66	19.88	8.00
12	BCIG12	10.50	5.75	1.83	27.00	15.00
13	BCIG13	8.70	4.53	1.92	12.93	10.00
14	BCIG14	8.30	4.66	1.78	16.00	5.00
15	BCIG15	9.46	5.30	1.78	27.20	9.00
16	BCIG16	10.76	5.60	1.92	16.11	13.00
17	BCIG17	8.43	4.76	1.77	15.59	14.00
18	BCIG18	8.70	5.40	1.61	21.55	9.00
19	BCIG19	9.96	5.40	1.84	24.00	5.00
20	BCIG20	8.36	4.80	1.74	21.33	10.00
21	BCIG21	8.63	4.80	1.80	21.53	5.00
22	BCIG22	8.83	4.80	1.84	16.14	6.00
23	BCIG23	8.96	4.80	1.87	19.87	5.00

Commented [AM30]: Please add a column on the right side to cite the reference of each primer set use. And also make column on the left side to add the Sr. No. to make it clearer that how many primers set are used.

Commented [AM31]: Move the heading to extreme left of the page by deleting single tab space before the heading. And also give it number 3 to continue the series from top.

Commented [AM32]: Replace the selected words with germplasm.

Commented [AM33]: Ite the reference properly according the citation style of the journal.

Commented [AM34]: Correct it as, "leaf blade width"

Commented [AM35]: Replace the word with "germplasm"

Commented [AM36]: Correct it as, "leaf blade width"

Commented [AM37]: Replace the word with germplasm

Commented [AM38]: Cite the reference according to the citation style of the journal

Commented [AM39]: Remove the extra space between word (Table) and Word (2).

Commented [AM41]: Replace it with germplasm

Commented [AM42]: Replace w with W

Commented [AM40]: Please replace the word Sl. No with Sr. No.

Commented [AM43]: Bold this lowest value to make it prominent value

Commented [AM44]: Bold this lowest value to make it prominent value

Sl. No.	Genotypes	Leaf blade length (cm)	Leaf blade width (cm)	Length /width ratio	Number of leaves per shoot	Number of branches per plant
24	BCIG24	7.80	4.30	1.81	46.00	4.00
25	BCIG25	9.66	5.03	1.92	20.13	12.00
26	BCIG26	10.16	5.80	1.75	29.64	6.00
27	BCIG27	10.43	5.66	1.84	31.22	4.00
28	BCIG28	10.86	5.83	1.86	15.98	13.00
29	BCIG29	10.40	5.00	2.08	24.57	8.00
30	BCIG30	11.50	6.60	1.74	22.00	6.00
31	BCIG31	9.50	5.63	1.69	24.10	5.00
32	BCIG32	11.26	5.43	2.07	31.63	8.00
33	BCIG33	9.63	5.06	1.90	16.60	13.00
34	BCIG34	10.20	5.66	1.80	13.97	8.00
35	BCIG35	9.46	5.16	1.83	14.14	16.00
36	BCIG36	9.96	5.53	1.80	12.41	14.00
37	BCIG37	14.60	6.40	2.28	17.00	6.00
38	BCIG38	7.75	3.03	2.56	12.29	8.00
39	BCIG39	11.76	6.06	1.94	14.55	12.00
40	BCIG40	13.83	5.96	2.32	18.14	17.00
41	BCIG41	10.46	5.86	1.78	11.06	9.00
42	BCIG42	10.26	5.56	1.85	11.67	14.00
43	BCIG43	9.50	4.93	1.93	17.88	5.00
44	BCIG44	9.80	5.40	1.81	13.98	11.00
45	BCIG45	8.20	3.70	2.22	39.85	4.00
46	BCIG46	9.70	5.53	1.75	23.87	16.00
47	BCIG47	13.63	5.83	2.34	31.79	13.00
48	BCIG48	14.03	6.20	2.26	24.75	11.00
49	BCIG49	12.73	6.43	1.98	25.78	14.00
50	BCIG50	12.73	5.80	2.19	23.45	16.00
51	BCIG51	14.93	6.20	2.41	14.50	15.00
52	BCIG52	8.83	5.13	1.72	21.08	15.00
53	BCIG53	14.36	5.53	2.60	25.93	11.00
54	BCIG54	10.76	6.00	1.79	24.00	12.00
55	BCIG55	9.00	5.26	1.71	20.96	13.00
56	BCIG56	10.03	5.53	1.81	18.12	10.00
57	BCIG57	14.30	6.30	2.27	34.09	11.00
58	BCIG58	12.26	6.10	2.01	27.53	16.00
59	BCIG59	10.13	5.76	1.76	20.03	17.00
60	BCIG60	11.16	6.03	1.85	24.46	13.00
61	BCIG61	10.23	5.90	1.73	25.08	18.00
62	BCIG62	10.66	5.80	1.84	22.69	12.00
63	BCIG63	11.66	5.86	1.99	19.25	11.00

Commented [AM41]: Replace it with germplasm

Commented [AM42]: Replace w with W

Commented [AM40]: Please replace the word Sl. No with Sr. No.

Commented [AM45]: Bold this lowest value to make it prominent value

Commented [AM46]: Bold this highest value to make it prominent value

Sl. No.	Genotypes	Leaf blade length (cm)	Leaf blade width (cm)	Length /width ratio	Number of leaves per shoot	Number of branches per plant
64	BCIG64	10.56	6.13	1.72	28.43	7.00
65	BCIG65	11.26	4.80	2.35	25.13	4.00
66	BCIG66	9.56	5.36	1.78	21.92	26.00
67	BCIG67	14.03	5.93	2.37	26.22	11.00
68	BCIG68	13.36	5.33	2.51	15.64	17.00
69	BCIG69	13.00	5.40	2.41	15.96	15.00
70	BCIG70	12.70	5.60	2.27	30.75	10.00
71	BCIG71	15.10	6.63	2.28	23.65	17.00
72	BCIG72	14.40	6.56	2.20	16.91	13.00
73	BCIG73	8.13	4.16	1.95	20.70	8.00
74	BCIG74	10.36	4.96	2.09	20.86	9.00
75	BCIG75	14.43	5.83	2.48	18.00	10.00
76	BCIG76	13.06	5.50	2.37	25.14	8.00
77	BCIG77	13.33	5.70	2.34	27.00	9.00
78	BCIG78	12.76	5.66	2.25	15.99	15.00
79	BCIG79	9.23	5.43	1.70	17.10	15.00
80	BCIG80	11.93	5.16	2.31	14.91	5.00
81	BCIG81	12.63	5.30	2.38	17.33	4.00
82	BCIG82	15.06	6.36	2.37	22.92	10.00
83	BCIG83	12.23	5.76	2.12	26.93	6.00
84	BCIG84	11.60	4.76	2.44	14.66	5.00
85	BCIG85	8.90	4.46	2.00	7.66	8.00
86	BCIG86	10.83	5.63	1.92	19.53	8.00
87	BCIG87	13.56	5.76	2.35	22.71	4.00
88	BCIG88	14.30	6.10	2.34	12.32	13.00
89	Arka Rashmi	9.10	5.00	1.82	35.00	12.00
90	L-49	11.60	5.30	2.19	43.75	34.00
91	Red Guava	12.13	6.70	1.81	42.04	26.00
92	Seedless Guava	10.66	4.93	2.16	30.85	9.00
93	Arka Poorna	11.96	5.73	2.09	32.30	14.00
94	Allahabad Safeda	13.73	6.35	2.16	25.04	10.00
95	Arka Kiran	11.66	5.60	2.08	41.54	36.00
96	White local	11.10	5.43	2.04	41.89	34.00
	Mean	**10.95**	**5.49**	**1.99**	**21.81**	**12.08**
	S.E m±	**0.203**	**0.07**	**0.027**	**0.80**	**0.68**
	C.D (5%)	**1.97**	**0.74**	**0.14**	**9.28**	**2.12**

Commented [AM41]: Replace it with germplasm

Commented [AM42]: Replace w with W

Commented [AM40]: Please replace the word Sl. No with Sr. No.

Commented [AM47]: Bold this highest value to make it prominent value

Commented [AM48]: Bold this highest value to make it prominent value

The K-mean clustering grouped 88 seedling progenies and eight released guava varieties into six clusters (Table 3; Fig.1). Among the six clusters, cluster-III was the largest with 24 seedling origin progenies followed by cluster-I with 23 entries. The highest inter cluster distance was noticed in between cluster-VI and cluster-V (6.06) followed by the inter cluster distance between cluster-V and cluster-IV (5.05). This interpreted that the genotypes in cluster-VI and cluster-V were having high variability in turn contributing to diversity (Table 4).

Commented [AM49]: Replace the selected words with 96 germplasm

Table 3. Classification of seedling origin guava progenies and varieties into different clusters

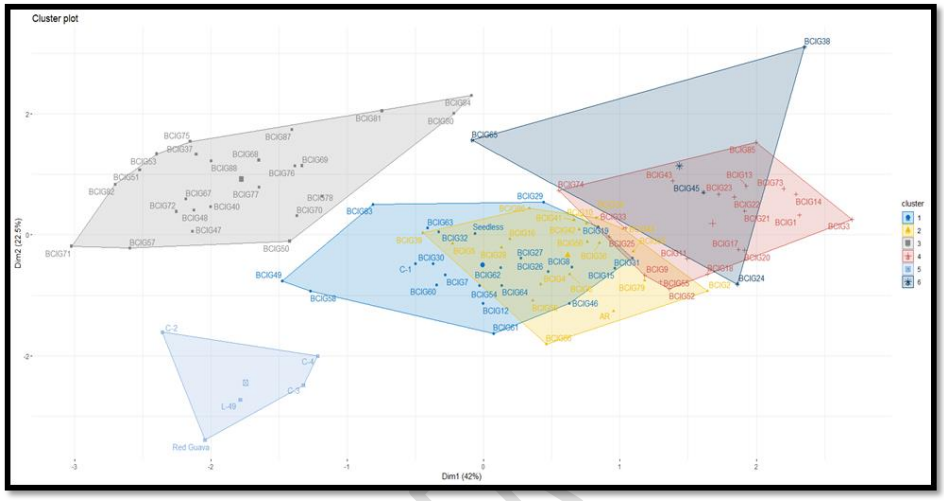
Cluster number	Number of genotypes	Genotypes and checks in cluster
Cluster I	23	Arka Poorna, BCIG07, BCIG08, BCIG12, BCIG15, BCIG19, BCIG26, BCIG27, BCIG29, BCIG30, BCIG31, BCIG32, BCIG46, BCIG4, BCIG54, BCIG58, BCIG60, BCIG61, BCIG62, BCIG63, BCIG6, BCIG83 and Seedless Guava

Cluster II	20	BCIG2, BCIG4, BCIG5, BCIG6, BCIG10, BCIG16, BCIG28, BG34, BCIG35, BCIG36, BCIG39, BCIG41, BCIG42, BCIG44, BCIG56, BCIG59, BCIG66, BCIG79, BCIG86 and Arka Rashmi
Cluster III	24	BCIG37, BCIG40, BCIG47, BCIG48, BCIG50, BCIG51, BCIG53, BCIG57, BCIG67, BCIG68, BCIG69, BCIG70, BCIG71, BCIG72, BCIG75, BCIG76, BCIG77, BCIG78, BCIG80, BCIG81, BCIG82, BCIG84, BCIG87 and BCIG88
Cluster IV	20	BCIG01, BCIG03, BCIG09, BCIG11, BCIG13, BCIG14, BCIG17, BCIG18, BCIG20, BCIG21, BCIG22, BCIG23, BCIG25, BCIG33, BCIG43, BCIG52, BCIG55, BCIG73, BCIG74 and BCIG85
Cluster V	5	Allahabad Safeda, Arka Kiran, White local, L-49 and Red Guava
Cluster VI	4	BCIG24, BCIG38, BCIG45 and BCIG65

Table 4. Average intra and inter cluster distances among the six clusters of seedling origin guava progenies and varieties

Clusters	I	II	III	IV	V	VI
I	**1.72**	2.16	2.94	2.45	4.08	4.22
II		**1.47**	3.33	2.11	4.47	4.78
III			**1.92**	3.87	4.37	4.83
IV				**1.44**	5.05	3.82
V					**2.64**	6.06
VI						**3.94**

Fig. 1. Dendrogram depicting the clustering pattern of seedling progenies and varieties of guava genotypes



3.2 Molecular characterization

The 96 guava genotypes were characterized using a set of 10 SSR primers. A total of 90 alleles were produced by 10 primers, the number of alleles per locus varied from 4 (mPgCIR10) to 14 (mPgCIR02) (Table 5) which was higher than 4.5 alleles per locus reported by Risterucci *et al.* (2005) for guava samples from six different origins (Camaeroon, Colombia, Cuba, Florida, Hawaii, and Martinique) and Rodriguez *et al*. (2007) for Cuban cultivars and accessions. The genetic diversity values ranged from 0.87 (mPgCIR09) to 0.63 (mPgCIR16). Furthermore, PIC values were highest for the primer mPgCIR09 (0.86) and lowest was in the primer mPgCIR16 (0.60) with a mean value of 0.70 (Table 6), as previously reported by Kanupriya *et al.* (2011) who did cultivar identification and genetic fingerprinting of guava using microsatellite markers, with PIC values ranging from 0.34 to 0.90 with a mean of 0.75.

Commented [AM50]: Rewrite the full form as "Figure"

Commented [AM51]: There is no table with this number, please mention it.

Commented [AM52]: Cite the reference properly according to the citation style of the journal.

Commented [AM53]: Word (Samples) should be replaced with germplasm.

Commented [AM54]: 2007 should be replaced with 2010

Commented [AM55]: Cite the reference properly according to the citation style of the journal.

Commented [AM56]: Cite the reference properly according to the citation style of the journal.

The pattern of allelic diversity obtained in the present study was higher in comparison to the gene diversity observed by Kumari *et al*. (2018), who reported a mean value of 2.34 alleles per locus and Valdes-Infante *et al*. (2007) reported 4.57 allele 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 genotypes studied by them. The SSR marker generated across the seedling progenies as well as released varieties were assessed for genetic distance and the dissimilarity matrix which was used for cluster development using the neighbour joining (NJ) method and showed three major clusters on the basis of genetic similarity (Fig. 2).

Commented [AM57]: Cite the reference properly according to the citation style of the journal.

Commented [AM58]: Cite the reference properly according to the citation style of the journal.

Table 6.Properties of SSR markers and the degree description of the polymorphism obtained in seedling origin guava progenies and varieties

Sl. No.	SSR marker	Sample size	Allele number	Major allelic frequency	Gene diversity	Heterozygosity	PIC
1	mPgCIR01	96	11	0.52	0.69	0.00	0.66
2	mPgCIR02	96	14	0.38	0.72	1.00	0.68
3	mPgCIR05	96	7	0.40	0.76	0.00	0.73
4	mPgCIR07	96	13	0.34	0.83	0.06	0.81
5	mPgCIR08	96	6	0.52	0.67	0.00	0.64
6	mPgCIR09	96	11	0.19	0.87	0.04	0.86
7	mPgCIR10	96	4	0.42	0.69	0.00	0.63
8	mPgCIR11	96	5	0.29	0.74	0.00	0.69
9	mPgCIR015	96	8	0.30	0.77	0.00	0.74
10	mPgCIR016	96	11	0.57	0.63	0.00	0.60
	Mean	9	9.00	0.39	0.74	0.11	0.70

Commented [AM59]: Replace the word, “Sl. No.” with “Sr. No.”

3. CONCLUSION

Commented [AM60]: Conclusion should not be given, no 3. As results and discussion are already given this number, It should be just with the heading of Conclusion

Based on the results obtained in the present study, it is concluded that the guava seedling origin progenies and released varieties showed significantly higher range of variations for different morphological characters. Amongst various seedling guava genotypes evaluated, BCIG53, BCIG66, BCIG68 and BCIG71 were found to be diverse in terms of different morphological parameters. Out of 10 SSR markers studied, the primers mpgCIR09 and mpgCIR07 were highly informative.

Commented [AM61]: Please recheck these both of germplasm as they are not directly associated with any significant results.

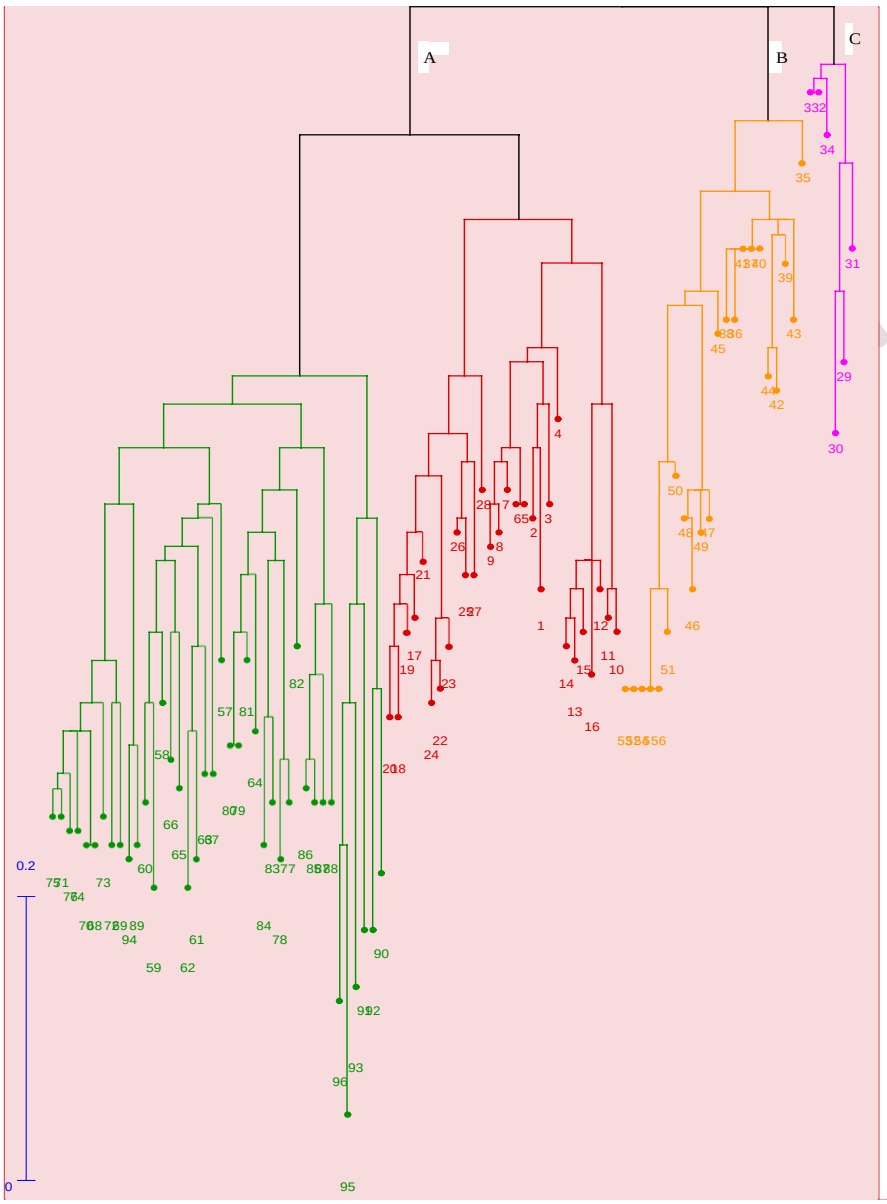


Fig. 2. Dendrogram generated using Neighbor Joining (NJ) method of DARWin programme from the computed genetic distance of simple matching coefficient.

Commented [AM62]: This picture should be moved to the results and discussion section of the manuscript.

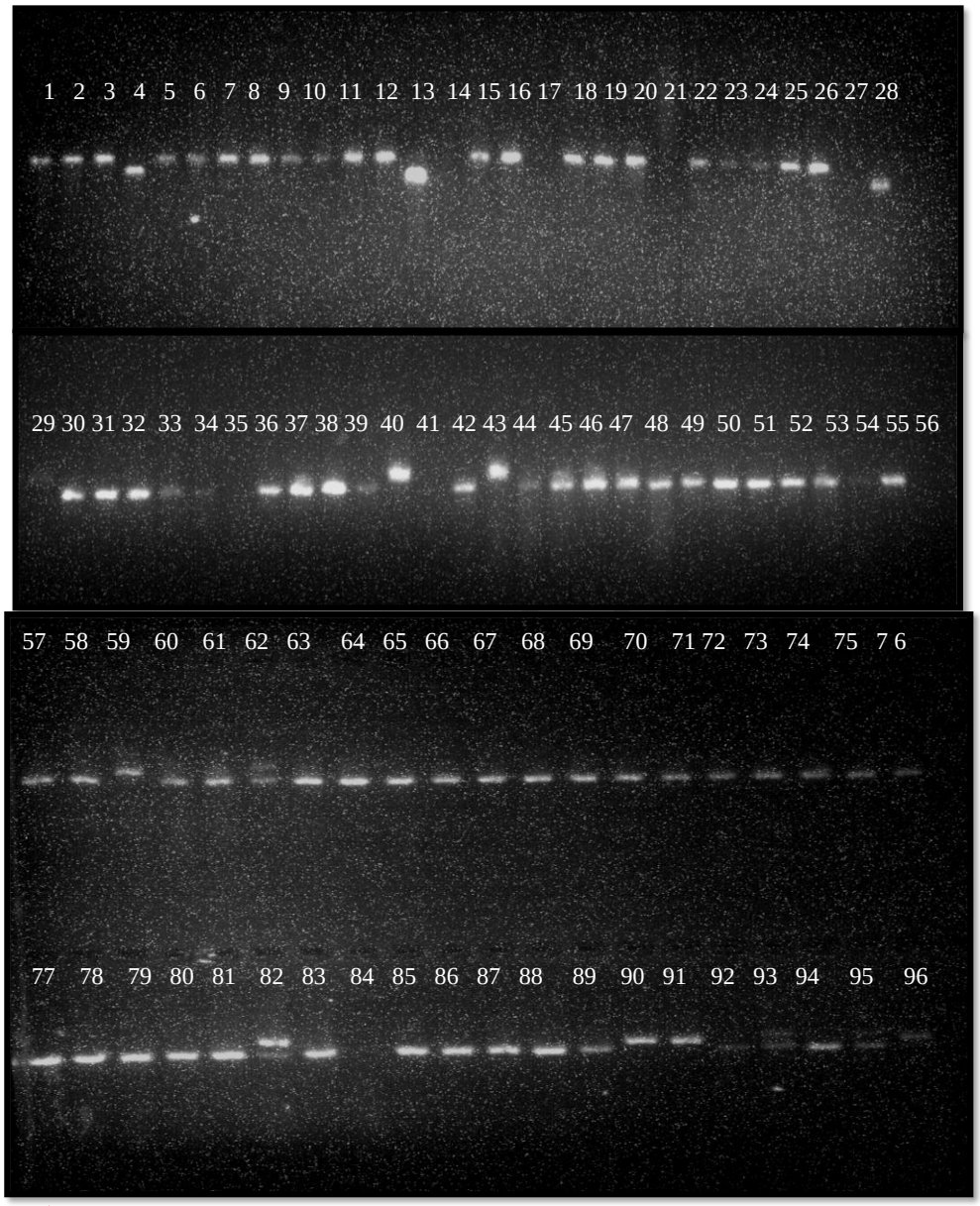


Fig. 3. Agarose gel image showing amplification for the guava seedling progenies and released varieties for the primer mpgCIR09

Commented [AM63]: All the gel images should be labelled properly by indicating the amplified product length in bp (base pairs). Also group the images properly to make it more attractive for the reader. If possible, high-resolution images should be inserted. And the Picture should be added in the results section after primer's table. There should be no picture after conclusion and before references.

REFERENCES

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *12*:13-15.

Kanupriya., Latha., P. M., Aswath, C., Reddy, L., Padmakar, B., Vasugi, C., & Dinesh, M. R. (2011). Cultivar identification and genetic fingerprinting of guava (*Psidium guajava*) using microsatellite markers. *International Journal of Fruit Science*., *11*(2):184-196.

Kumari, S., Arumugam, N., Singh, R., Srivastav, M., Banoth, S., Mithra, A. C., Arun, M. B., Goswami, A. K., & Khan, A. (2018). Diversity analysis of guava *(Psidium guajava)* germplasm collection. *Indian Journal of Agricultural Sciences*, *88*(3):489-497.

Nakasone, H. Y., & Paull, R. E. (1998). Tropical fruits.Crop production in science in horticulture., CAB International, Wallingford, UK. pp. 468.

Risterucci, A. M., Duval, M. F., Rohde, W., & Billotte, N. (2005). Isolation and characterization of microsatellite loci from *Psidium guajava* L. *Mol. Ecol. Notes 5*:745–748.

Rodriguez, M. N. N., Valdes, I. J., Velasques, B., Rivero, D., Martinez, F., Risteruci, A.M., Billotte, N., Becker, D., Ritter, E., & Rohde, W. (2010). Indivisual versus combined data set for moeular characterization of Cuban guava (*Psidium gujava* L.) germplasm. *Acta. Horticulturae*, *849*:163-172.

Sharma, A., Sehrawat, S. K., Singhrot, R. S., & Tele, A. (2010). Morphological and chemical characterization of *Psidium* species. *Not. Bot. Horti. Agrobot. Cluj-Napoca*, *38*(1): 28-32.

Singh, D., Gill, M. I. S., Boora, R. S., & Arora, N. K. (2016). Genetic diversity analysis in guava (*Psidium guajava*) on the basis of morphological and physic-chemical traits. *Indian Journal of Agricultural Scences, 85* (5):678-683.

Singh, G. (2005). Strategies for improved production in guava (eds.). Proc. 1st Intl. Guava Symp. CISH, Lucknow, India, 26–39.

Valdes-Infante, J., Rodriguez, N. N., Becker, D., Velázquez, B., Sourd, D., Espinosa, G., & Rohde, W. (2007). Microsatellite characterization of guava (*Psidium guajava* L.) germplasm collection in Cuba. *Cultivos Tropicales, 28*: 61– 67.