**Molecular Characterization and Genetic Diversity of Guava (Psidium guajava L.) Germplasm**

**ABSTRACT**

**A set of 96 guava germplasm comprised of Kolar Gold Fields 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 Polymorphism Information Content (PIC) value has recorded maximum for the primer mPgCIR09 (0.86).**

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

# 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 genetic diversity present in the guava germplasm.

Guava has gained considerable prominence in India because of its high nutritive value, pleasant aroma, flavour and availability at moderate prices. Besides, it is one of the hardiest fruit crops with high nutritional quality. The fruit contains 165 mg of vitamin C. It is an excellent source of beta carotene, lycopene, potassium and soluble fiber (Radha and Mathew, 2007). Traditionally, different parts of the plant such as leaves, fruits, bark and roots are reported to be helpful in treating of dysentery, gastroenteritis and fungal infections (Jaiswal and Amin, 1992).

Genetic diversity studies are crucial source of information for taxonomic and evolutionary approaches as well as breeding plans. Additionally, the use of molecular methods can contribute for the accurate determination of genetic diversity (Morand *et al*., 2002, Zeid *et al*., 2003., Mehmood, et al., 2014., Singh et al., 2024). The choice of parents for successful hybridization programs would be aided by knowledge of the genetic variation that is available and the provenance of the cultivars. The first step toward effective conservation, maintenance and utilization of existing genetic diversity could be the genetic characterization of genotype coupled with phenotypic traits. Eliminating duplicates from the germplasm, collection through rigorous examination would help to conserve time, space, and resources (Prakash *et al*., 2002). ). Crop improvement greatly benefits from knowledge of the genetic variability of seedling progenies and superiority of hybrids. Keeping in view of the above points, present study was planned.

# MATERIALS AND METHODS

# 2.1 Morphological Characterization

A total of 96 guava genotypes comprised of Kolar Gold Fields 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 evaluated for morphological and molecular traits and the experimental design was augmented block design (91+4 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 SSR markers.

## 2.2 Molecular Characterization

Theyoung leaf samples were collected from individual plants and DNA was extracted by modified CTAB (cetyl trimethyl ammonium bromide) extraction protocol by Doyle and Doyle (1990) with minor modification. The amount of DNA following extractionwas quantified 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 evaluate the genetic diversity of guava’s germplasm (Table 1).

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), 2μl of 10X buffer, 0.8μl of 25 mM MgCl2, 0.7μl 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 Ta°C min and extension at 72°C for 1 min with a final extension at 72°C for 10 min. The annealing temperature (Ta) was kept 2°C above and below Tm 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.

 **Table 1. List of SSR primers used for molecular characterization of guava germplasm**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr.NO.** | **DNA marker locus** | **Forward primer(5'-3')** | **Reverse primer(3'-5')** | **Reference** |
| 1 | mPgCIR01 | TAGTGCTTTGGTTGCTT | GCAGGTGGATATAAGGTC | Zehua Ma et al (2019) |
| 2 | mPgCIR02 | AGTGAACGACTGAAGACC | ATTACACATTCAGCCACTT |
| 3 | mPgCIR05 | GCCTTTGAACCACATC | TCAATACGAGAGGCAATA |
| 4 | mPgCIR08 | ACTTTCGGTCTCAACAAG | AGGCTTCCTACAAAAGTG |
| 5 | mPgCIR07 | ATGGAGGTAGGTTGATG | CGTAGTAATCGAAGAAATG |
| 6 | mPgCIR09 | GCGTGTCGTATTGTTTC | ATTTTCTTCTGCCTTGTC |
| 7 | mPgCIR10 | GTTGGCTCTTATTTTGGT | GCCCCATATCTAGGAAG |
| 8 | mPgCIR11 | TGAAAGACAACAAACGAG | TTACACCCACCTAAATAAGA |
| 9 | mPgCIR015 | TCTAATCCCCTGAGTTTC | CCGATCATCTCTTTCTTT |
| 10 | mPgCIR016 | AATACCAGCAACACCAA | CATCCGTCTCTAAACCTC |

**3. RESULTS AND DISCUSSION**

# 3.1 Morphological characterization

The guava germplasm 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 germplasm BCIG03 (7.50 cm). Similar results were reported by Singh et al. (2016). With respect to the leaf blade width, the highest value recorded in the germplasm Red Guava (6.70 cm) whereas the minimum leaf width was recorded in the germplasm 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)**

| **Sr. No.** | **Germplasm** | **Leaf blade length (cm)** | **Leaf blade Width (cm)** | **Length/Widthratio** | **Number of leaves per shoot** | **Number of branchesper 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 |
| 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 |
| 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** |

The K-mean clustering grouped 96 germplasm 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). Similar grouping of clusters are in agreement with the previous findings of Gangappa *et al*. (2022), Sharma *et al*. (2010) and Kumari *et al*. (2018).

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

|  |  |  |
| --- | --- | --- |
| **Cluster number** | **Number of germplasm** | **Germplasm 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** |

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

**3.2 Molecular characterization**

The 96 guava germplasm 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) which was higher than 4.5 alleles per locus reported by Risterucci et al. (2005) for guava germplasm from six different origins (Camaeroon, Colombia, Cuba, Florida, Hawaii, and Martinique) and Rodriguez *et al*. (2010) for Cuban cultivars and accessions. The genetic diversity values ranged from 0.87 (mPgCIR09) to0.63 (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 5), 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.

The pattern of allelic diversity obtained in the present study was higher in comparison to the gene diversity observed by Chaithanya et al. (2014). 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).

 

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

 **Table 5. Properties of SSR primers and the degree description of the polymorphism obtained in seedling origin guava progenies and varieties**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sr. 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 |

**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.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that **NO generative AI technologies** such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**REFERENCES**

29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56

 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28

Chaithanya, M. V. N., Dinesh, M. R., Vasugi, C., Reddy, D. C. L., Sailaja, D. & Aswath, C. (2014). Assessment of genetic diversity in guava (*Psidium guajava*) germplasm using microsatellites. *Journal of Horticultural Sciences*, *9*(2):117-125.

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

Gangappa, N., Singh, C., Verma, M., Thakre, M., Sevanthi, A., Singh, R., Srivastav, M., Raghunandan, K., Anusha, C., Yadav, V. & Nagaraja, A. (2022). Assessing the genetic diversity of guava germplasm characterized by morpho-biochemical traits. *Frontiers in Nutrition*, *9*.

Jaiswal, V. S., & Amin, M. N. (1992). Guava and Jackfruit,. In: F. A. Hammerschlag and R. E. Litz (Eds.). Biotechnology of perennial fruit crops, biotechnology in agriculture, Vol. 8. CAB International, Wallingford, UK, 421-431.

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.

Morand, S., Simkova, A., Matejusova, I., Plaisance, L., Verneau, O. & Desdevises, Y. (2002). Investigating patterns may reveal processes: evolutionary 68 ecology of ectoparasitic monogeneans. *International Journal Parasitol*., *32*: 111-119.

Mehmood, A., Jaskani, M. J., Khan, I. A., Ahmad, S., Ahmad, R., Luo, S., & Ahmad, N. M. (2014). Genetic diversity of Pakistani guava (Psidium guajava L.) germplasm and its implications for conservation and breeding. Scientia Horticulturae, 172, 221-232.

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

Prakash, D. P., Narayanaswamy, P., & Sondur, S. N. (2002). Analysis of molecular diversity in guava using RAPD markers. *Journal of Horicultural Sciences and Biotechnology*, *77*: 287-93.

Radha, T., & Mathew, L. (2007). Fruit crops. New India Publ. Agency, New Delhi, India.

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 Deepak., Chandra Shekhar Pandey., Pandey, S. K., Umesh Kumar Chanderia., Sanjay Kumar Singh., & Shailendra Sagar Prajapati, (2024). “Estimating the Morphological Variability of Guava (Psidium Guajava L.) Genotypes in Jabalpur District, India”. Journal of Experimental Agriculture International 46 (5):716-23

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.

Zehua Ma., Shunzhi Liu., Ziewei, Shejin Xu., & Weirong Hu, (2019). Analysis of Genetic Diversity of 45 Guava Germplasm Evaluated Using SSR Markers.*International Journal of Fruit Science,20* (3):385-393