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Research Article

GENETIC DIVERSITY OF GUAVA GENOTYPES EVALUATED USING RAPD MOLECULAR MARKER

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Abstract- Genetic diversity among 24 genotypes (22 varieties/collection belonging to *Psidium guajava* and 2 other species) of guava were characterized using Random Amplified Polymorphic DNA (RAPD) markers. Out of 29 RAPD primers used, 10 were found to be monomorphic and 19 showed polymorphism among guava genotypes. Number of alleles detected using polymorphic RAPD primer ranged between 2 (OPA13A) to 11 (OPF02A) with an average of 6 amplicons/primer. High rate of polymorphism was observed reasonably for OPF02A, OPH19A, OPF13A, OPA13A and OPB13A primers. The PIC value ranged from 0.49-0.89 indicates that the markers were quite informative. Based on molecular analysis, Sasri Selection and Sasni Selection were grouped with Allahabad Safeda. Tamil Nadu Selection and Lalit genotypes formed a group at 50% similarity. *Psidum freidrichsthalianum* formed a group with black guava. Molecular analysis showed a high degree of variation among analyzed guava genotypes indicating an important source of genetic diversity that can be used in the guava improvement program.

Keywords- Characterization, Polymorphic Information Content, Jacard's similarity matrix, Genetic Similarity, RAPD

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Introduction

Guava (*Psidium guajava* L.) belongs to the family *Myrtaceae* contains about 150 species [1] of which most widely cultivated is common guava, *P. guajava*. Guava is a native of tropical America [2] and is today widely grown all over the tropical and subtropical areas of the world. Genus *Psidium* It was introduced to India by the Portuguese during the early 17th century [3] currently it has become one of the most common fruit in India. Guavas often included among super fruits, Guava is often marketed as "super fruit", being exceptionally rich in ascorbic acid [2] and is also good source of calcium, phosphorus, and iron [4]. Guava is an open pollinated crop [5] and seedlings from selected progenitors are extensively used to establish commercial orchards. Hence exhibit high level of heterozygosity and genetic variability due to continuous cultivation of heterogeneous seedling trees. Selection from these seedlings can be used to obtain superior strains with respect to fruit yield and quality observed variation in guava seedlings. However, high phenotypic variability prevails among and within trees, which can be detrimental for fresh market but useful to develop new cultivars based on breeding selection. Indeed, a large number of cultivars have been developed in India through selection [6]. This resulted in a great morphological and biochemical variability [7, 8]. Traditionally genetic diversity in the genotypes of guava (wild and cultivated) has been screened and characterized based on morphological [9] and biochemical features [10]. Based on the morphological characters it is not always possible to discriminate between closely related guava genotypes and also genetic variability is very often not expressed by visible characteristics sometimes may change with the cultivation and growth environment. Hence, additional knowledge of genetic diversity as a base for genetic improvement programmes has became extremely important for the development of new varieties with desired traits such as high yields, organoleptic characteristics and resistance to diseases, according to the need of national and international markets. In order to complement the morphology based description, molecular markers were used to assess genetic variability, such as DNA-based markers [11]. Among the different types of molecular markers, Randomly Amplified Polymorphic DNAs (RAPD) are useful for the assessment of genetic diversity [12] owing to their simplicity, speed and relatively low-cost [13]; analyzing genetic relationships, tagging traits for use in marker-assisted selection, and for the rapid construction of a genetic linkage map [14] compared to other types of molecular markers. Molecular markers were considered as powerful tools in the assessment of genetic diversity within and between plant populations [15]. The importance of morphological, anatomical, physiological, biochemical and genetic studies for the conservation, characterization and use of the genetic diversity in several species has been pointed out by several authors [16, 17]. Thus, an attempt was made to assess the genetic variability of *Psidium* genotypes and to estimate the genetic relationship among them. With this background information, the objective of this study aimed to assess the morphological variations through phenotypic, biochemical characteristics among twenty four Psidium guajava genotypes and focus on understanding the genetic relationship among different genotypes by RAPD markers analyses. The preliminary results are may further assist in developing and planning breeding strategies for crop improvement programmes.

Materials and Methods

Plant material

A total of 22 genotypes of *Psidium guajava* and two species of *Psidium* were selected from the experimental orchard of the division of Fruits and Horticultural Technology, IARI, New Delhi, India were used for this study.

Molecular analysis using DNA markers

DNA isolation

Total genomic DNA was extracted from young and healthy leaves of all 24 genotypes by a CTAB protocol as per Doyle and Doyle [18] with little modification and 1 gm polyvinyl pyrrolidone (PVP) was added to buffer for removal of phenolics present in the sample. The DNA concentration was estimated using a Nanodrop

(Thermo Fisher, USA). DNA quality was checked by gel electrophoresis on 0.8% agarose gel (TAE buffer, 0.04 M Tris–acetate, 0.001 M EDTA, and pH 8.0, EtBr). The purity and concentration of isolated DNA were determined by gel electrophoresis in 0.8% agarose gel (TAE buffer, 0.04 M Tris–acetate, 0.001 M EDTA, and pH 8.0, EtBr) and compared to 1Kb DNA ladder and diluted to a final concentration of 10 ng/ µl using TE buffer and stored at 4◦C until use.

RAPD Analysis

DNA amplification was based on the method described by Williams's et al. [12]. using 45 decamer primers of arbitrary sequence. The PCR mixture contained 10 ng template DNA, 1 µl of primer, 1 µl dNTPs (MBI, Fermentas, Lithuania, USA), 2.5 µl 10 × PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$) and 0.33U of *Taq* DNA polymerase (G Bioscience, India) in a reaction volume of 25 µl. Template DNA was initially denatured at 94°C for 5 minutes followed by 35 cycles of PCR amplification with the following parameters: 1 minute denaturation at 94°C, 1 minute annealing at 35°C and 1 minute of primer extension at 72°C followed by final extension of 72°C for 5 minutes. To the PCR product, one-tenth volume (2µl) of 6x loading dye containing bromophenol blue and xylene cyanol was added. The amplified products were resolved on a 1.2% agarose gel containing ethidium bromide (10 mg/ml) at a constant voltage of 100V for 2 hours using a horizontal gel electrophoresis system (Biorad, USA). The gel was run in 1X TBE buffer. A 250bp DNA ladder (Genei™, Bangalore in Karnataka, India) was run alongside the amplified products to determine their approximate band size. The amplified fragments were visualized and photographed under UV light using gel documentation (Alphaimager HP and Cell Biosciences, USA). Reproducible DNA bands i.e. bands present in both repetitions of the individual sample were scored manually. Primers were procured from Xcelris Labs Ltd, India. A total of 45 RAPD primers (OPA, OPB, OPC, OPD, OPE, OPF, OPG, OPH, OPJ and OPS series) was used for initial screening using the DNA obtained from Allahabad safeda. All the primers amplified at least one fragment. The 29 best primers, (OPB11, OPB17, OPC5, OPD18, OPG6, OPG19, OPJ-1 and OPJ-4), producing reproducible and clear bands ranging from 100 to 300 bp in size, observed allele size for each primer was almost the same and within the range, as described in the literature [19] were selected and used for further analysis. Band profile was scored based on band size appeared on the gel.

Data analysis

the data was analysed to calculate various parameters such as the total number of bands, the number of polymorphic bands, the percentage of polymorphism, the average number of bands per primer, the effective multiplex ratio (EMR; defined as the number of polymorphic bands/the total number of primers), the polymorphic information content (PIC). PIC was calculated according to Ghislain *et al.* [20] as follows: $PIC = 1 - p2 - q2$ where p was the band frequency and q was band absence frequency. Pair-wise genetic similarity (GS) between genotypes based on RAPD data, growth, and yield related trait's data of parental lines and hybrids were computed by using Jaccard's similarity coefficient [21] as follows: J = a / (n-d). Where 'a' is the number of positive matches (i.e. the number of bands exist in both individuals);'d' is the number of negative matches (i.e. the number of bands absent in both individuals) and 'n' is the total sample size including both the numbers of "matches" and "unmatches". For this purpose, DARwin V.5.0.158 software was used [22]. The resultant similarity matrices based on Jaccard's measure was further analyzed by performing (SHAN) sequential, hierarchical, agglomerative and nested clustering algorithm [23] using the UPGMA method. The results of clustering were plotted in the form of the dendrogram. DARwin V.5.0.158 was used to perform cluster analysis using different data sets *viz.,* morphological data and RAPD data. An analysis of RAPD markers was done after scoring of the bands visually for their presence (1) or absence (0) with each primer. The data transformed as detailed above, and subjected to unweighted hierarchical clustering using the SPSS v.16 software package (SPSS 2007). The results of clustering were plotted in the form of the dendrogram.

Results and Discussion

A total of 29 RAPD primers used to study the diversity analysis among different guava genotypes [Table-1]. Out of 29 primers, 10 were found monomorphic and 19 revealed polymorphism among different guava genotypes. A total of 109 alleles were observed, a number of alleles per locus varied from 2 to 11 with an average of 6 alleles per locus. The values for the polymorphism information content ranged from 0.49 to 0.89 with a mean of 0.77. There was a reasonably high rate of polymorphism for OPF02A, OPH19A, OPF13A, OPA13A, and OPB13A primers, point towards the scope for further utilization of these markers for characterization of guava variants. The occurrence of unique alleles or rare alleles provides an immense opportunity for the generation of comprehensive fingerprint database [Fig-1]. The PIC value ranging from 0.49 to 0.89 shows that the markers had been quite informative. The PIC value is influenced through the occurrence of variants per locus as well as the relative distribution of the alleles. The range of alleles per locus found was one to three with most of them amplifying more than one band. In the present study OPF02A, OPH19A, OPF13A and OPA13A primers consistently amplified a number of bands ranged from 8-10 [Table-1]. The Jacard's similarity matrix dendrogram constructed using the DARWIN method showed that 24 guava genotypes were grouped into 4 major clusters. However, the Arka Mridula, Sour type guava, Red Peel guava and *Psidium pumilum* remained as a separate group [Fig-2].

Table-1 *RAPD primers sequence with number of bands amplified, number of polymorphic bands, PIC value.*

S. No.	Name of the primer	Sequence (5' ➔ 3')	Number of polymorphic amplicons	GC%	PIC
1	OPA2A	TGCCAAGCTG	6	70	0.82
2	OPA5A	AGAGGTCTTG	7	60	0.84
3	OPA13A	CAGAACCCAC	7	70	0.84
4	OPA19A	CAAGCGTCGG	6	60	0.83
5	OPA20A	GTTACGATCC	6	60	0.81
6	OPB5A	TGAGCCCTTC	4	70	0.74
7	OPB11A	GTAGACACGT	10	60	0.84
8	OPC5A	GACGACCGCC	9	70	0.87
9	OPD 18A	GAAAGCCAAC	11	60	0.89
10	OPE 13A	CCAGATTCGG	5	70	0.79
11	OPF02A	GAGGATCACT	11	60	0.89
12	OPF 13A	GGCTGCAGAC	8	60	0.87
13	OPF20A	GGTCTAGAGC	6	60	0.75
14	OPG 3A	GAGCACTCCA	5	70	0.64
15	OPG6A	GTGCCTATCC	9	60	0.83
16	OPG19A	GTCAGAGCAA	6	60	0.82
17	OPH19A	CTGACCAGAC	10	70	0.88
18	OPJ14A	CACACGGATG	6	70	0.80
19	OPS 5	TTTGATGCCTA	8	70	0.87
Total			140		
Range			3-11		0.64-0.89

In the first main cluster, Sasri Selection and Sasni Selection were grouped with Allahabad safeda and Hisar Safeda. This may be due to the fact that, Sasri Selection and Sasni Selection are the open pollinated seedling population of Allahabad Safeda. The second cluster is comprised of Pant Prabhat, Yellow type, Lucknow-49 and Hisar Surkha. Tamil Nadu Selection and Lalit genotypes formed a group at 50% similarity. These two genotypes have similarity in terms of red blush on the peel. A critical examination of these clusters clearly indicated that *Psidium freidrichstahlianum* formed a group with Black guava.

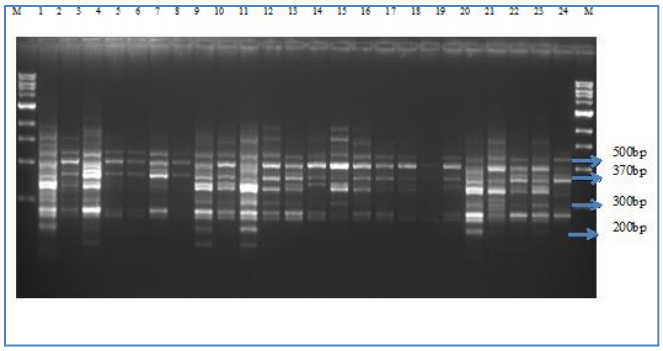

Fig-1 RAPD amplification pattern of different Guava germplasm

M-molecular marker (2% Agrose gel), 1. Allahabad Safeda, 2. Arka Amulya, 3. Behat Coconut, 4. Hafsi Red, 5. Hisar Safeda, 6. Hisar Surka, 7. Lalit 8. Lucknow-49, 9. Pant Prabhat, 10. Punjab Pink, 11. Red Type, 12. Red Peel, 13. Black Guava, 14. Sasni Selection, 15. Sasri Selection, 16. Snow White, 17. Shweta, 18. Tamil Nadu Selection, 19. Thai, 20. Yellow Type 21. *P. friedrichsthalianum,* 22.*P. pumilum,* 23.Sour Type and 24.Arka Mridula.

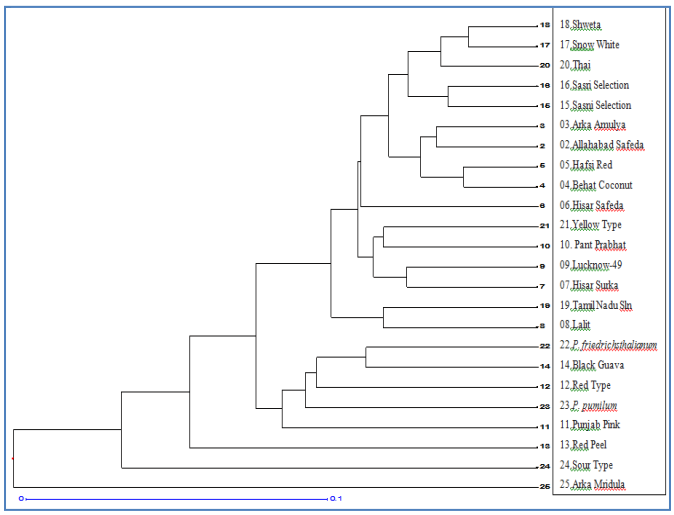

Fig-2 Dendrogram of 24 diverse guava genotypes based on 19 RAPD marker using DARwin method

The morphological data pertaining to Black guava also revealed significant variation from the varieties belonging to *Psidium guajava* species. It is evident from the present study that Black guava have more similarity to *P. freidrichstahalianum* than *P. guajava* [Fig-2]. The Jacard's similarity matrix dendrogram constructed using the UPGMA method showed that Sasri Selection and Sasni Selection were grouped with Allahabad safeda and Hisar Safeda. This may be due to the fact that Sasri Selection and Sasni Selection are the open pollinated seedling population of Allahabad Safeda. Tamil Nadu Selection and Lalit genotypes make a group at 50% similarity. These two genotypes have commonality in terms of red blush on the peel. *Psidium freidrichstahlianum* formed a group with Black guava. The morphological data pertaining to Black guava also revealed significant variation from the varieties belonging to *Psidium guajava* species. It is evident from the present study that Black guava have more similarity to *P. freidrichstahalianum* than *P. guajava*. From the present study, it can be said that a high degree of molecular polymorphism was exhibited by RAPD markers used in the present study. Among these, PCR-based techniques of random multilocus analysis (RAPD, AFLP and ISSR) were effectually utilized in genotyping, genome mapping and phylogenetic studies in horticultural crops. A foremost advantage of RAPD markers over some other DNA based markers is that they necessitate no prior sequence information and no prior knowledge regarding any particular gene in a target taxon. Molecular diversity of guava germplasm have been studied by several workers like Prakash *et al* [3], Chen *et al*., [24], Ahmed *et al* [25] and Mani *et al*., [26]. No clear cut pattern, especially for different clustering (i.e., genetic variance) and source population diversity can be found in the present study. This might be attributed to the less number of markers used and greater molecular changes in the genotypes under study. From this present investigation, it can be concluded that the guava germplasm used for the present study seems to be divergent.

Conclusion

Based on the results obtained in the present study, it can be concluded that wide variation in terms of molecular level. Recently molecular markers have been used as a tool to investigate the plant genoypic diversity. Banding patterns can be converted into informative data for diversity analyses. The shortcoming of RAPD method is the reproducibility in amplification. In this study, the PCR reactions were performed in optimal conditions and informative RAPD fragments were obtained with high reproducibility. RAPD analysis is efficient and accurate for the investigation of distribution of commercial guava or local guavas. The RAPD analysis is useful in the fingerprinting of each guava sample. The geographical locations, growth altitude, and climates may contribute the polymorphic RAPD of guava trees in India. This result confirmed a high degree of variant amongst analyzed guava genotypes indicating an important source of genetic diversity that can be used within the guava improvement programme.

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Author Contribution: All author equally contributed

Abbreviations: RAPD-Random Amplified Polymorphic DNA, CTAB- Cetyl trimethylammonium bromide,
EDTA- Ethylenediaminetetraacetic acid, EtBr- Ethidium Bromide, dNTPs-deoxynucleotide triphosphates,
PCR- Polymerase Chain Reaction, TBE-Tris/Borate/EDTA buffer, EMR -Effective Multiplex Ratio
PIC -Polymorphic Information Content, GS-Genetic Similarity
SHAN- Sequential, hierarchical, agglomerative and nested clustering algorithm
UPGMA- Unweighted Pair Group Method with Arithmetic Mean.

Conflict of Interest: None declared

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