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Morphological and molecular characterization of guava

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Abstract
To characterize 20 genotypes of *Psidium* spp., observations were recorded for leaf blade length and width, petiole length, vein number, leaf surface area, leaf shape, leaf apex and base shape, upper and lower leaf surface colour, leaves colour during winters, leaf texture, pubescence and leaf lamina thickness which revealed significant variations among genotypes. *Psidium chinensis* was most diverse morphologically. Out of 20 decamer RAPD primers used four primers generated strong amplifications and resulted in polymorphic products; these were *viz.*, OPA-03, OPA-05, OPS-04 and OPS-08. Percent polymorphism was 97.43% having a range of 87.5% to 100%. The average number of scoreable bands per primer was 9.75, while average number of polymorphic bands was 9.5. High polymorphism frequency was detected with all the selected primers. The similarity coefficient ranged from 0.42-0.83. The study showed that the genetic base of Indian guava can be rated as moderate to high diversity.

Keywords: Random amplified polymorphic DNA (RAPD), similarity coefficient, polymorphism, Guava, *Psidium* spp., characterization.

1. Introduction
Guava (*Psidium guajava*) is an important tropical fruit crop which belongs to family Myrtaceae. It is the hardiest among tropical fruits and excels most of the other fruit crops in productivity and adaptability. Guava contributes 3.3 per cent share in production of fruits in India [3]. The genus *Psidium* comprises approximately 150 species of small trees and shrubs in which only 20 species produce edible fruits and the rest are wild with inferior quality of fruits. Most commonly cultivated is the common guava, (*P. guajava* L.) and the other cultivated species include the Cattely guava or Strawberry guava (*P. Cattleianum* Sabine), the Brazilian guava (*P. guineense* Sw.) and Costa Rician guava or Chinese guava (*P. friedrichsthalianum* Niedenzu). The other species of *Psidium* are utilized for regulation of vigour, bearing programme, fruit quality improvement and resistance to pest and diseases [7]. The fruits of *P. friedrichsthalianum* are good for jelly making because of their high acidity and this sp. is also reported to be wilt and nematode (*Meloidogyne incognita*) resistant. The interspecific hybrids between *P. guineense* and *P. guajava* have been found resistant to guava wilt and are graft compatible with commercial varieties of *P. guajava* [2]. *P. molle, P. chinensis* and *P. friedrichsthalianum* are recognized as donor parents for imparting dwarfness. For continued improvement of guava cultivars through breeding to overcome threats from diseases, insect-pests or biotic stresses and to evolve varieties suited to consumer preferences, a diverse gene pool is essential. Cultivar identification and estimation of diversity using phenotypic markers, though generally used since time immemorial, have several limitations, especially in perennial crops.

With technological advancements, molecular markers have come to the focus which is not only quick but also highly accurate when compared to the earlier techniques. Now it is possible to single out differences present at the molecular level, which is more authentic and less affected by environmental factors. Among the different types of molecular markers, Random Amplified Polymorphic DNAs (RAPD) are useful for the assessment of genetic diversity owing to their simplicity, speed and relatively low-cost. RAPD markers are efficient in the studies of genetic diversity [11]. They have been used in phylogeny and systematics [17], genetic linkage mapping [5] and gene tagging [15]. RAPD markers were used to estimate molecular diversity of guava genotypes earlier [9, 4, 1].

Predominantly guava is produced in north India and many famous varieties of guava are commercially grown in this area. Lot of confusion exists within *Psidium* species, as the different literatures arrange them in different order and the synonyms used in the nomenclature makes it more confusing, therefore, molecular marker assessed identification of duplicates in the germplasm is essential for the maintenance, commercialization and conservation of this genus. This study focuses on assessing the genetic variability among the *Psidium* species and establishment of the genetic relationships between them.

Material and Methods

Experimental material

The experimental materials consisted of 20 different germplasm lines of guava maintained at Horticultural Research Centre, Pattharchatta, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. The university farm is situated at 29°N latitude, 79.30°E longitude and at an altitude of 243.8 meter above the mean sea level. The guava germplasm comprised of 16 cultivated varieties and four accessions of other species of genus *Psidium* (Table 1).

Recording of morphological characters

Leaf characters were recorded in the month of January 2013. A random sample of ten fully developed leaves from current growth of each plant was selected from the outside branches at the middle of canopy for the purpose of data recording. Rating of various characters was done in accordance with 'Guava Descriptor' published by All India Coordinated Research Project on Subtropical Fruits, Central Institute for Subtropical Horticulture, Lucknow, under Indian Council of Agricultural Research in the year 2011. Observations were recorded for different leaf characters *viz.*, length of leaf blade, width of leaf blade, petiole length, number of veins, surface area of leaf (Li- COR portable leaf area meter LI-3000), shape of leaf, shape of leaf apex and base, colour of upper and lower leaf surface, colour of leaves during winters, texture of leaf, pubescence and leaf lamina thickness.

Collection of experimental Tissues

The young leaf samples were collected in the early morning hours from individual tree of all the 20 genotypes and wrapped in aluminium foil. Samples were placed in an ice box and brought to laboratory. Five gram of fresh sample was weighed with electronic balance and placed in -20 °C refrigerator.

Genomic DNA isolation

Genomic DNA was isolated using the modified protocol of Porebski *et al*. [8]. The isolated DNA was purified using the standard protocol [10] and stored in TE buffer at -20 °C for further experimentation.

PCR amplification

Twenty oligonucleotide primers were got custom synthesized by Banglore Genei Pvt. Ltd., India. The genomic DNA isolated form 20 samples were suitably diluted and its 100 ng per PCR tube was used as a template in PCR amplification. After repeated PCR reactions a reaction mixture was standardized for 20 guava genotypes which gave strong amplifications. Final reactions were performed with the volume of 25 µl PCR reaction mixture (PCR buffer 1x; dNTPs, 215 µM each; $MgCl_2$, 2 mM; Primer, 10 pmoles; *Taq* DNA polymerase, 1 U; DNA template, 100 ng). Gradient PCR was used for standardizing the PCR reactions for 20 primers with all the 20 genotypes. The amplification conditions starts with initial denaturation at 94 °C for 5 min followed by 42 cycles of denaturation at 94 °C for 1 min, annealing at 42 °C for 1 min and extension at 72 °C for 1 min, and end with a final extension at 72 °C for 7 min. PCR products were subjected to electrophoresis in 2% (w/v) agarose (Bangalore Genei Pvt. Ltd.) gel with 1x TAE buffer. Electrophoresis was done by running the gel at 75 volts for 45 min. The gel was removed and stained with ethidium bromide (EtBr) solution for 10 min followed by distaining in distilled water for 5 min. The gel image was photographed using Gel Documentation system. All the amplified bands were scored as present or absent for each DNA sample.

Data analysis

Analysis of quantitative morphological data was done with 1 way ANOVA applied using SPSS software version 19 whereas analysis of qualitative morphological data was done using DARwin5 software, version 5.0.158 developed by CIRAD, research unit: Genetic Improvement of Vegetatively Propagated Crops. In order to analyze the genetic relatedness among the species, a dendrogram based on unweighted pair group method with arithmetic average (UPGMA) was obtained. Data were analyzed to obtain Jaccard's similarity coefficients among the genotypes by using NTSYS-pc version 2.02e. The SIMQUAL program was used to calculate the Jaccard's similarity coefficients. The genetic distance tree was also constructed using the band scoring through the DARwin5 software.

Results and Discussion

Morphological characterization

Analysis of variance for the quantitative traits (Table 2) revealed that varieties were significantly different from each other taking each of these characters individually. Leaf blade length was maximum in cv. Lalit (14.89 cm), whereas it was minimum in the case of *P. chinensis* (5.31cm). Variation in length of leaf blade in different guava genotypes was also reported by Sehgal and Singh [12]. Leaf blade breadth was maximum in *P. guineense* (6.79 cm), closely followed by Sardar (6.69 cm) and minimum in *P. chinensis* (1.62 cm). Petiole length was found to be maximum in cv. Sardar (1.08 cm) closely followed by Pant Prabhat (1.02 cm) and minimum in *P. chinensis* (0.395 cm). Maximum number of veins was in cv. Riverside (23.4) and minimum in *P. friedrichsthalianum* (10). Surface area of leaf was maximum in the case of *P. guineense* (106.12 cm^2) and minimum in genotype *P. chinensis* (15.04 cm^2). Significant differences were observed among genotypes considering leaf shape, shape of leaf apex and base and leaf colour during winters (Table 4). These leaf morphological characters were quite informative and useful in characterizing these genotypes, as some genotypes could easily be identified using a combination of these characters. For example, if leaf shape is oblanceolate, shape of leaf apex is obtuse and texture of leaf is coriaceous then the genotype is *P. cujavillis* and if leaf shape is lanceolate and shape of leaf apex is attenuate then this information is enough to conclude that this genotype is *P. friedrichsthalianum.*

By drawing Similarity tree considering both of these qualitative and quantitative morphological characters using DARwin5 software Figure 1 was obtained. The tree was constructed based on UPGMA. It was clear from the tree that genotype number 20 *i.e., P. chinensis* was the most diverse morphologically forming a completely different node from the other 2 clusters. There were two major clusters apart from

P. chinensis. The bigger cluster (supposedly 'Cluster-A') comprised of 12 genotypes. In Cluster-A three main sub-clusters were formed. In the first sub-cluster 4 genotypes Red Fleshed, Allahabad Safeda, *P. cujavillis* and Chittidar are placed. In the second sub-cluster Lalit and Pant Prabhat appeared to be quite similar morphologically forming a separate node at a very later stage, whereas, Sardar and *P. guineense* formed a cluster together within this sub-cluster. The third sub-cluster consisted of KG-1 Guava and Kayamganj. Apart from these two Hisar Safeda and Shweta also were parts of the same sub-cluster. The smaller cluster (supposedly Cluster-B) consisted of 7 genotypes including Seedrolf, Baraf Khana, Karela, Riverside, Sangareddy and *P. friedrichsthalianum*.

Molecular characterization

In the present study, a polymerase chain reaction (PCR) based random amplified polymorphic DNA (RAPD) method was able to detect the heterogeneity of amplified DNA from the germplasm of *Psidium* species. Four RAPD markers were found to be polymorphic and informative for this set of 20 genotypes of *Psidium* species out of the 20 markers initially screened. A total of 376 RAPD bands were amplified in the range of 0.24 to 2kb. Percent polymorphism was 97.43% having a range of 87.5% to 100% (Table 5 and 6). The average number of scoreable bands per primer was 9.75, while average number of polymorphic bands was 9.5 (having a range from 8-11 bands per primer). High frequency of polymorphism was detected with all the selected primers. The present investigation shows more or less similarity with the works of Dahiya *et al*. [6] who scored 133 loci ranging from 300 bp to 3000bp in size with 74.7% polymorphic bands using 9 RAPD markers. Prakash *et al*. [9] observed 93 loci varied in size from 100 to 3000 bp with an average of 11.2 loci per primer while analysisng guava using 8 informative RAPD primers. Chen *et al.* [4] analyzed *P. guajava* L. from indigenous tribes of Taiwan using 4 RAPD primers and amplified 82 polymorphic RAPD patterns. Sharma *et al*. [13] scored 347 loci with 92.29% polymorphism while analysing *Psidium* species using RAPD markers. The high level of polymorphism in the present investigation and also supported by earlier reports may be because of outbreeding nature of guava. The present study reveals 97.43% polymorphism with the RAPD primers *viz.*, OPA-03, OPA-05, OPS-04 and OPS-08. Dahiya *et al*. [6] reported 100% polymorphism in OPA-13 while Ahmed *et al.* [1] reported highest polymorphic loci 37.5% in OPA-03 in molecular characterization of 33 *Psidium* germplasm of South-western Bangladesh. Thus, the four RAPD markers selected out of the 20 initially screened seems to be useful and informative in terms of characterization and identification of guava genotypes.

The marker OPA-03 detected 8 loci among the 20 genotypes. Size of loci ranged from 240 bp to 2000 bp and just one amplicon was monomorphic (Figure 2). This primer produced 87.5% polymorphic loci. A unique locus at around 1500 bp size was detected only in *P. guineense* which seems to be a landmark for identification and differentiation of *P. guineense* from the rest of the genotypes. A total number of 11 loci varied in size from 280 bp to 2020 bp were amplified by OPA-05. All the loci were polymorphic thus producing 100% polymorphism. The marker OPA-05 was able to detect two unique loci at around 410 bp size in Hisar Safeda and at 1400 bp size in *P. cujavillis*. Thus, the marker OPA-05 can be used to identify and differentiate these two genotypes considering these unique bands as reference. The OPS-04 amplified 10 polymorphic loci varied in size from 450 bp to 2000 bp (Figure 3). No unique band was observed in the amplification produced by this primer. A total number of 10 polymorphic loci were amplified by OPS-08 primer. Sizes of the loci were found to vary from 290 bp to 1500 bp. No unique band was observed in the amplification produced by this primer.

The dendrogram generated using molecular data on 20 guava genotypes revealed its grouping in 2 clusters (Figure 4). The first group (Group A) consisted of only *P. chinensis* whereas the remaining 19 genotypes were grouped together in a second group (Group B). These 2 major groups had a genetic linkage distance of 48%. The group B was further divided into two main clusters. Cluster I consisted of 6 genotypes all of which were seedling selections or close relatives of the mother of these cultivars- Allahabad Safeda, so they naturally associated with it. Allahabad Safeda and Kayamganj were found to be most genetically similar (83%). Sardar, which is a seedling selection of Allahabad Safeda, was 78% to the previously mentioned two varieties. Other three varieties which were present in this group were Chittidar, Hisar Safeda and Pant Prabhat all of which had Allahabad Safeda as a common parent. The second main cluster of Group-B consisted of 13 genotypes. In this cluster Lalit and Shweta, both selections made at CISH, Lucknow had 77.5% genetic similarity to each other. This might be due to the fact that, both of them have Apple color as their common parent. Apart from these two genotypes, this cluster also consisted of two introductions, Seedrolf and Riverside which might have grouped together probably because of their non-Indian origin. The other species of genus *Psidium* that were a part of this study were dispersed randomly in the dendogram suggesting that all these species could be subspecies of *P. guajava.* KG-1 guava and *P. cujavillis* also exhibited similarity in their genetic make-up apart from being morphologically similar in some respects like shape of leaf apex and leaf base, length of leaf blade and colour of leaf lamina. Genotypes which showed similar morphological and genetic trends were grouped more or less together. Allahabad Safeda and Chittidar showed close morphological similarity and were also found to be genetically close. All the selections of Allahabad Safeda formed a part of the same cluster both in morphological and molecular studies of the present investigation confirming Allahabad Safeda as the mother of all these varieties. *P. chinensis* was found to be most diverse both genetically and morphologically from.

Table 1: Genotypes of guava and wild relatives undertaken for molecular characterization study

Code	Name	Source	Code	Name	Source
1	Red Fleshed	Allahabad	11	Pant Prabhat	GBPUAT,Pantnagar
2	Chittidar	Allahabad	12	Seedrolf	CISH, Lucknow
3	Karela	Rewa	13	Shweta	CISH, Lucknow
4	Riverside	CISH, Lucknow	14	Lalit	CISH, Lucknow
5	Sardar	CISH, Lucknow	15	KG-1 Guava	Local selection
6	Sangareddy	FRS, Sangareddy	16	Black Guava	Local selection
7	Kayamganj	Govt. nursery, Bareily	17	*Psidium guineense*	CISH, Lucknow
8	Baraf Khana	Local selection	18	*Psidium friedrichsthalianum*	CISH, Lucknow

9	Allahabad Safeda	Allahabad	19	*Psidium cujavillis*	CISH, Lucknow
10	Hisar Safeda	CCSHAU, Haryana	20	*Psidium chinensis*	CISH, Lucknow

Table 2: Results of Analysis of Variance (ANOVA) for quantitative morphological characters

S. No.	Mean sum of Squares	Leaf blade length	Leaf blade width	Petiole length	Number of veins	Surface area
1.	Between genotypes	3807.931**	973.183**	17.956**	76.385**	2983.127**
2.	Error	109.612	26.370	1.404	3.400	27.686
** indicates highly significant values						

Table 3: Characterization of guava genotypes on the basis of morpho-physical traits (quantitative characters)

S. N.	Varieties	Leaf blade Length (cm)	Leaf blade width (cm)	Petiole length (cm)	Number of veins	Surface area (cm^2)
1.	Red Fleshed	12.42	6.06	0.925	19.8	86.46
2	Chittidar	11.49	5.77	0.893	20.2	78.86
3	Karela	10.90	4.64	0.604	21.2	49.75
4	Riverside	10.36	4.54	0.755	23.4	72.43
5	Sardar	13.54	6.69	1.08	21	101.06
6	Sangareddy	10.18	4.75	0.59	14.8	58.74
7	Kayamganj	11.99	6.14	0.840	17.8	78.77
8	Baraf Khana	10.52	4.08	0.417	19.4	45.19
9	Allahabad Safeda	12.98	6.57	0.90	17.2	81.50
10	Hisar Safeda	13.69	6.23	0.797	19.6	53.90
11	Pant Prabhat	14.81	6.60	1.02	19.6	85.09
12	Seedrolf	12.89	5.73	0.849	22	64.14
13	Shweta	14.80	6.06	0.921	19.6	65.72
14	Lalit	14.89	6.09	0.811	20	87.07
15	KG-1 Guava	13.47	5.95	0.809	19.4	67.48
16	Black Guava	8.88	4.86	0.663	15.2	38.02
17	*P. guineense*	14.70	6.79	0.899	10.6	106.12
18	*P. friedrichsthalianum*	7.35	3.30	0.610	10	34.03
19	*P.cujavillis*	13.20	6.21	0.790	10.6	90.60
20	*P.chinensis*	5.31	1.62	0.395	21.2	15.04

Table 4: Characterizations of guava genotypes on the basis of morpho-physical traits (qualitative characters)

Varieties	Leaf shape	Shape of leaf apex	Shape of leaf base	Texture of leaf	Colour(upper leaf surface	Colour(lower leaf surface)	Lamina thickness	Pubescence	Colour of leaf during winters	Petiole Orientation
Red Fleshed	Oblanceolate	Obtuse	Rounded	Glabrous	Green	Light green	Intermediate	Absent	Coppery	Straight
Chittidar	Oblanceolate	Rounded	Obtuse	Glabrous	Green	Green	Intermediate	Absent	Brick red	Straight
Karela	Oblong	Obtuse	Rounded	Glabrous	Green	Green	Thin	Absent	Pink	Straight
Riverside	Oblong	Obtuse	Obtuse	Glabrous	Green	Green	Intermediate	Absent	Pink	Straight
Sardar	Oblong	Rounded	Rounded	Glabrous	Green	Green	Intermediate	Absent	Red	Straight
Sangareddy	Oblanceolate	Obtuse	Obtuse	Glabrous	Light green	Light green	Intermediate	Absent	Brick red	Straight
Kayamganj	Oblong	Obtuse	Obtuse	Glabrous	Light green	Light green	Thin	Absent	Pink	Straight
Baraf Khana	Oblong	Obtuse	Rounded	Glabrous	Green	Green	Intermediate	Absent	Brick red	Straight
Allahabad Safeda	Oblong	Obtuse	Rounded	Glabrous	Green	Green	Intermediate	Absent	Brick red	Straight
Hisar Safeda	Oblong	Rounded	Rounded	Glabrous	Green	Green	Intermediate	Absent	Brick red	Straight
Pant Prabhat	Oblong	Obtuse	Rounded	Glabrous	Green	Light green	Intermediate	Absent	Brick red	Straight
Seedrolf	Oblong	Acute	Rounded	Glabrous	Light green	Light green	Intermediate	Absent	Pink	Straight
Shweta	Oblanceolate	Rounded	Rounded	Glabrous	Green	Green	Intermediate	Absent	Red	Straight
Lalit	Lanceolate	Obtuse	Rounded	Glabrous	Light green	Light green	Intermediate	Absent	Brick red	Straight
KG-1 Guava	Oblong	Obtuse	Cordate	Glabrous	Green	Green	Intermediate	Absent	Pink	Straight
Black Guava	Oblanceolate	Obtuse	Rounded	Glabrous	Dark red	Red	Intermediate	Absent	Brown	Straight
P. guineense	Oblong	Acute	Rounded	Coriaceous	Dark green	Light green	Thick	Sparse	Red	Straight
P. friedrichsthalianum	Lanceolate	Attenuate	Obtuse	Glabrous	Light green	Light green	Thin	Absent	Pink	Straight
P. cujavillis	Oblanceolate	Obtuse	Rounded	Coriaceous	Dark green	Green	Thick	Sparse	Red	Straight
P. chinensis	Oblong	Obtuse	Obtuse	Glabrous	Green	Light green	Thin	Absent	Pink	Straight

Table 5: Polymorphism expressed by different RAPD primers among the guava genotypes

Primer	Sequence	Number of amplicons scored	Number of polymorphic amplicons	Percent Polymorphism
OPA-03	AGTCAGCCAC	8	7	87.5%
OPA-05	AGGGGTCTTG	11	11	100%
OPS-04	CACCCCCTTG	10	10	100%
OPS-05	TTCAGGGTGG	10	10	100%
Total		39	38	97.43%
Average		9.75	9.5	97.43%
Range		8-11	8-11	87.5-100%

Table 6: RAPD profile based on four polymorphic primers in 20 genotypes of guava

Primer	Range of Bands	Total no. of Bands	Number of Average Bands	Total Number of Polymorphic Bands	Average number of Polymorphic Bands
OPA- 03	3-7	100	5.00	80	4.00
OPA- 05	3-7	96	4.80	96	4.80
OPS- 04	2-6	71	3.55	71	3.50
OPS- 08	3-8	109	5.45	109	5.45

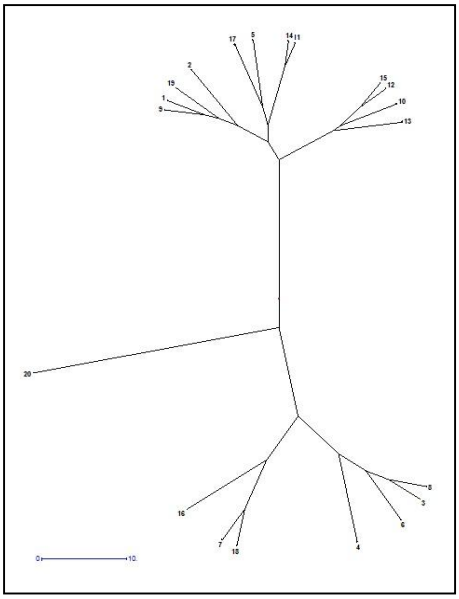

Fig 1: Tree construction based on morhological characters of *Psidium* genotypes constructed using DARwin5 software

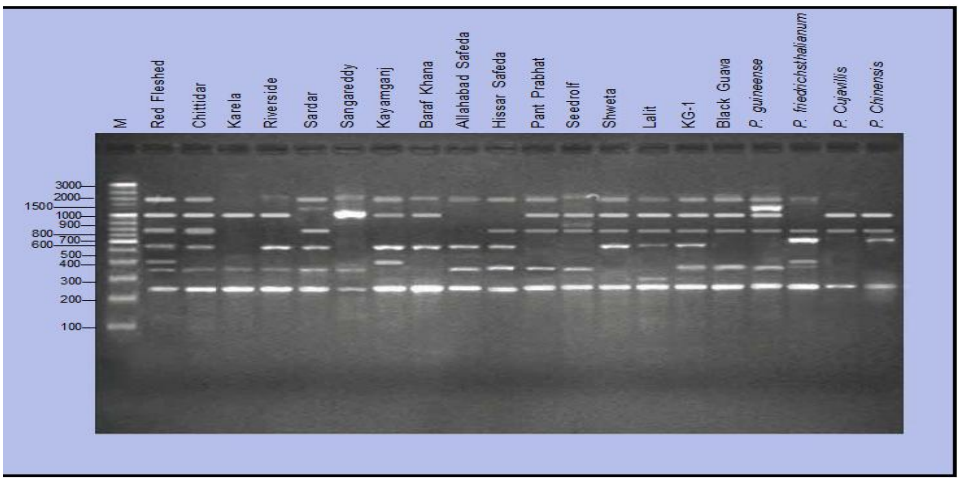

Fig 2: RAPD pattern of 20 guava genotypes with Primer OPA-03

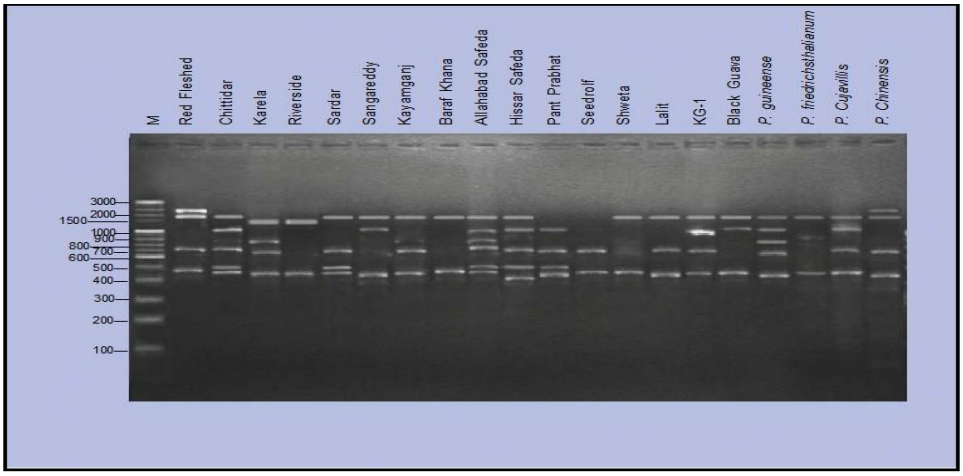

Fig 3: RAPD pattern of 20 guava genotypes with Primer OPS-04

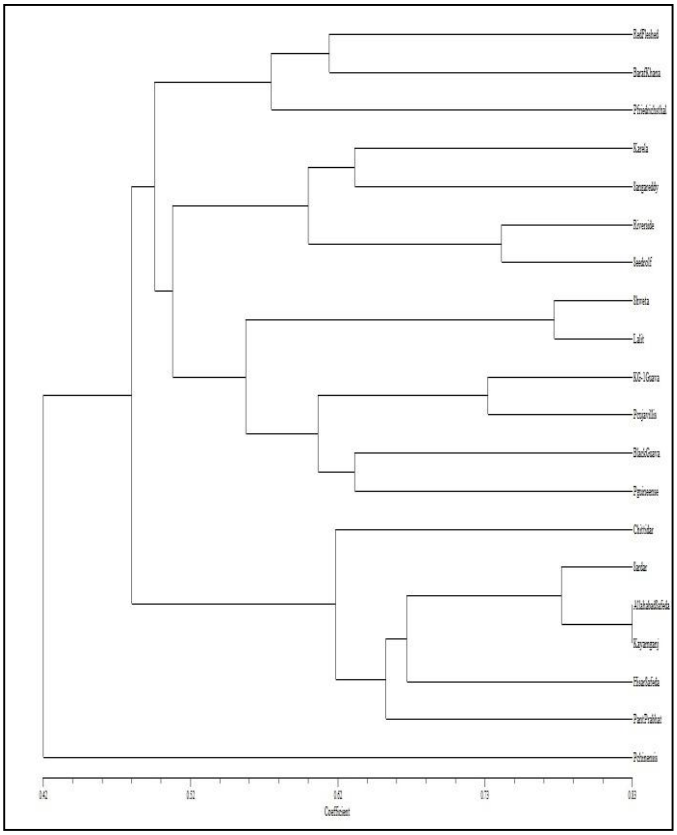

Fig 4: Jaccard's Coefficient based UPGMA dendogram, showing clustering of 20 genotypes of *Psidium* based on RAPD primers. The numbers plotted represent individual genotypes.

Conclusion

Study of genetic diversity among cultivars and populations will benefit guava breeding programs, by facilitating decisions on parental genotypes for crosses, and for germplasm management to maximize the conserved diversity. The use of neutral, codominant, and highly informative markers, such as microsatellites, is essential to fulfil those objectives. The present study has generated valuable information for the horticulturists, breeders, nurserymen and the guava growers. It would be helpful for the documentation, management and conservation of guava genetic resources of the region. The study showed that the genetic base of Indian guava can be rated as moderate to high diversity. The results of this study indicate that RAPD markers can be used to study the genetic diversity among genotypes. The results of this work will also provide a good foundation for future research on this crop.

References

1. Ahmed B, Mannan MA, Hossain SA. Molecular characterization of guava (*Psidium guajava* L.) germplasm by RAPD analysis. Int. J. Nat. Sci. 2011; 1(3):62-67.
2. Anonymous. Annual Report, Central Institute for Subtropical Horticulture, Lucknow, 2003-04, 10-11.
3. Anonymus. Indian Horticulture Database. National Horticulture Board, Gurgaon, Haryana, 2011.
4. Chen T, Nog C, Wang C, Shyu Y. Molecular Identification and Analysis of *Psidium guajava* L. from Indigenous Tribes of Taiwan. J. Food Drug Anal. 2007; 15(1):82-88.
5. Cheung WY, Champagne G, Hubert N, Landry BS. Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea.* Theor. Appl. Genet. 1997; 94:569-582.
6. Dahiya KK, Sunil A, Karihaloo JK. DNA fingerprinting of Guava cultivars using RAPD markers. Indian J. Plant Genet Res. 2002; 15(2):112-115.
7. Morton JF. Myrtaceae. In: Fruits of warm climates. Curits F, Domling JA. ed. Farmer Regional Administration, Bureau of Plant Inspection, Division of Plant Industry, Florida Department of Agriculture and Consumer Source. 1987, 356-392.
8. Porebski SL, Bailey G, Baum RB. Modification of CTAB DNA extraction protocol for plant containing high polysaccharides and polyphenol components. Plant Mol. Bio. Rep. 1997; 5:8-15**.**
9. Prakash DP, Narayanaswamy P, Sondur SN. Analysis of molecular diversity in guava using RAPD markers. J. Hort. Sci. Biotech. 2002; 77(3):287-293.
10. Sambrook J, Fritsch EF, Maniatis T. In: Molecular Coloning - A Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989.
11. Sarkhosh A, Zamani Z, Fatahi R, Ebadi A. RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.) genotypes. Sci. Hortic. 2006; 111:24-29.
12. Sehgal OP, Singh R. The classification and description of some important varieties of guava. Indian J Hort. 1965; 22:25-32.
13. Sharma AS, Sherawat SK, Singhrot RS, Boora KS. Assessment of genetic diversity and diversity relationships among *Psidium* spp. through RAPD analysis. Acta Hortic. 2007; 735:71-77.
14. Sun Q, Ni Z, Liu Z, Gao J, Huang T. Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. Euphytica. 1998; 99(3):205-211.
15. Tiwari KR, Penner GA, Warkentin TD. Identification of coupling and repulsion phase RAPD markers for powdery mildew resistance gene er-1 in pea. Genome. 1998; 41(3):440-444.