DNA Fingerprinting of Guava (*Psidium guajava* L.) Cultivars using RAPD Markers

KK Dahiya[1], Sunil Archak and JL Karihaloo
NRC on DNA Fingerprinting, National Bureau of Plant Genetic Resources, New Delhi-110 012
[1]Department of Entomology, CCS Hisar Agriculture University, Hisar-125 004 (Haryana)

Guava (*Psidium guajava* L.) is one of the important tropical fruit crops of India. Detailed horticultural and systematic study of the species and cultivars is lacking. Molecular markers can complement morphological characterization of cultivars in identification and diversity analysis. RAPD analysis of some guava cultivars of North India is presented. Nine RAPD primers generated 133 amplicons discriminating all the 13 cultivars. Cultivar-specific bands were identified for five cultivars. Statistical analysis revealed moderate genetic diversity among the cultivars investigated. Present analysis demonstrates the feasibility of RAPD technique for DNA fingerprinting of guava cultivars.

Key words: DNA Fingerprinting, Guava, RAPD Analysis

Guava, known as apple of tropics, is one of the important fruit crops of India. Guava is more resistant to drought than any other fruit crop. Originated in tropical America, from Mexico to Peru, guava is in India since early 17th century (Mitra and Bose, 1985). Three species of *Psidium* are reported from India: *P. cattleyanum* Sabine. cultivated in gardens; *P. guineense* Sw. reported as growing wild in Tripura and *P. guajava* L. cultivated in gardens and orchards (Anonymous, 1965). For sustained success in guava improvement programmes, characterization and documentation of the available diversity is of prime importance. Though, medicinal uses of different plant parts of guava are well-documented (Blatter *et al.*, 1995), detailed horticultural and systematic study of the species and cultivars is lacking (Mitra and Bose, 1985). Molecular marker techniques such as RAPD, RFLP, AFLP, ISSR and STMS provide modern tools for plant systematics. They complement morphological characterization. In the era of sovereign rights over national resources and intellectual property protection of life forms, molecular profiles provide robust support to documentation.

Among the various molecular marker techniques developed over the past two decades, RAPD (Welsh and McClelland, 1990; Williams *et al.*, 1990) has been the most widely employed DNA technique because it is easy, quick, simple and economical. Despite questions about its reproducibility, its utility in diversity analysis, mapping and genotype identification has been exploited in many plant species (Harris, 1999; Weising *et al.*, 1995). Neither sequence information nor any prior genetic study is required for these analyses. Data analysis and interpretation can give reliable results if the limitations of RAPD such as low repeatability and non-specificity are internalised. Utility of molecular markers in diversity analysis of guava has been demonstrated (Prakash *et al.*, 2002). The present study attempts to demonstrate the feasibility of RAPD markers in the identification of selected guava cultivars from the northern region of India.

Materials and Methods

Young leaves of 13 cultivars were collected from Research Farm of Department of Horticulture, CCS Haryana Agricultural University, Hisar (Table 1). DNA was extracted based on cTAB method with minor modifications (Archak *et al.*, 2002) to suit guava leaf material. Essentially, the extraction buffer composition was 4% w/v cTAB, 1.4 M NaCl, 100 mM Tris-HCl (pH 8), 20 mM EDTA, 2% PVP w/v, and 0.2% 2-mercapto ethanol v/v. DNA was treated with bovine pancreatic RNase and extracted once with phenol: chloroform (1:1) and twice with chloroform: iso-amyl alcohol (24:1). After precipitation with iso-propanol, a 70% ethanol wash was given. DNA

Table 1. Important morphological features of guava cultivars used in the study

Cultivar	Tree Height (m)	Fruit weight (g)	Flesh colour
Lucknow-49	3.3	107	White
Allahabad Safeda	5.8	87	White
Hisar Safeda	5.6	92	White
Apple Colour	5.4	75	White
Chakaia Rahamnagar	6.1	63	White
Kothrud	6.3	58	Pink
Pear Shaped	7.1	105	White
Red Supreme	5.6	68	Pink
Nagpur Seedling	6.1	102	White
Sindh	6.2	90	White
Tehsidar	6.1	105	White
Nasik	6.1	70	White
Patilo	6.3	–	Pink

was dissolved to appropriate dilution in TE buffer and quantified in a fluorometer. Subsequent to screening of primers, nine random primers (Operon) were selected based on amplification pattern. Amplification reactions contained 2.5 mM $MgCl_2$; 50 µM KCl; 10 mM Tris-HCl (pH 9); 0.1% Triton X-100; 200 µM of each of dNTPs; 0.5 µM primer; 30 ng template DNA; and 1 unit Taq DNA polymerase (Bangalore Genei, Bangalore) in a reaction volume of 25 µl. After a pre-denaturation step of 4 min at 94°C, DNA amplification reactions were cycled 40 times at 94°C for 1 min, 35°C for 1 min and 72°C for 2 min in a Perkin Elmer 9600 thermocycler. A final extension was allowed for 5 min at 72°C. Upon completion of the amplification, reaction mixture was mixed with 6x loading dye containing 0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol. Electrophoresis was carried out in a 1.2% agarose/1x Tris-Borate EDTA gel at 4 V/cm. Sizes of the identified bands were determined relative to a GeneRuler 100 bp DNA ladder (MBI Fermentas). Following ethidium bromide staining, amplified products were visualised on a UV trans-illuminator. Bands were scored as '1' for presence and '0' for absence to prepare the binary data matrix. Nei and Li (1979) coefficient was used to derive pair-wise relationships, and a dendrogram was developed based on neighbour-joining algorithm (Saitou and Nei, 1987). Once the profiles were generated, the probability of identical match by chance (Ramakrishna *et al.,* 1994) was calculated as: Pi = (Average similarity index)^n, where, n = average number of amplified products/ cultivar.

Results and Discussion

Nine primers produced 133 bands ranging from 300 bp to 3000 bp in size, of which 74.7% were polymorphic (Table 2). A typical amplification pattern is given in Fig. 1. All the bands generated by primers OPA-13 and OPE-13 were polymorphic. On the other hand, OPB-09 produced six bands of which only one was polymorphic.

Table 2. Primer-wise details of the amplification in guava cultivars

Primer	Number of Amplicons	Polymorphic amplicons
OPA-05	12	9
OPA-13	10	10
OPB-05	18	13
OPB-09	6	1
OPC-13	17	16
OPD-06	21	18
OPE-09	11	5
OPE-13	21	21
OPE-19	17	12

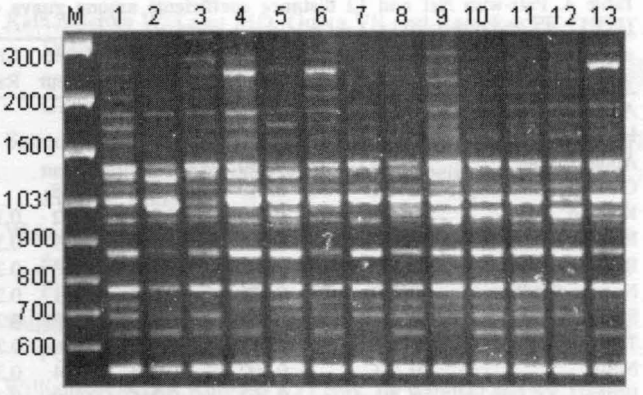

Fig. 1. RAPD profiles of 13 guava cultivars obtained using primer OPE-9. Lanes from 1-13 represent the cultivars Lucknow-49, Allahabad Safeda, Hisar Safeda, Apple Colour, Chakaia Rahamnagar, Kothrud, Pear Shaped, Red Supreme, Nagpur Seedling, Sindh, Tehsidar, Nasik and Patilo. M represents molecular weight marker; sizes of the bands are in base pairs.

Based on the total band profile all the cultivars could be discriminated from each other. However, cultivar specific markers could be identified for only five cultivars (Table 3). Inter-relationships between cultivars were

Table 3. Cultivar-specific amplicons generated by random primers in guava cultivars

Cultivar	Primer	Size of amplicon (base pairs)
Chakaia Rahamnagar	OPC-13	550
Kothrud	OPD-06	1100
Lucknow-49	OPA-05	600
	OPE-19	850 and 800
Pear Shaped	OPA-05	750
Red Supreme	OPD-06	950

revealed by computing pair-wise distance coefficient. Cultivars exhibited an average pair-wise distance of 0.242 (Table 4). Hisar Safeda and Allahabad Safeda were the closest pair of cultivars with a distance of 0.051 on a scale of zero to one. This was predictable since Allahabad Safeda is the female parent of Hisar Safeda, a hybrid cultivar. Cultivars Pear Shaped and Red Supreme were most distantly placed in relation to each other with a distance of 0.423. Average similarity index among 13 cultivars was 0.604 and on an average 81.85 bands were amplified/cultivar. Therefore, employing all the 9 primers, the probability of identical match by chance was 1.2×10^{-18} indicating a high degree of confidence in identification.

Dendrogram provided a clear picture of how the cultivars are placed in relation to each other (Fig. 2). Cultivars Kothrud and Pear Shaped formed a distinct cluster. Nasik, Patilo and Tehsildar were clustered together

Table 4. Pair-wise Nei and Li distance coefficients among guava cultivars

	Lucknow-49	Allahabad Safeda	Hisar Safeda	Apple Colour	Chakaia Rahamnagar	Kothrud	Pear Shaped	Red Suprme	Nagpur Seedling	Sindh	Tehsidar	Nasik
Allahabad Safeda	0.250											
Hisar Safeda	0.257	0.051										
Apple Colour	0.230	0.146	0.094									
Chakaia Rahamnagar	0.256	0.149	0.156	0.138								
Kothrud	0.225	0.200	0.207	0.202	0.157							
Pear Shaped	0.378	0.366	0.347	0.319	0.348	0.285						
Red Supreme	0.302	0.242	0.238	0.246	0.247	0.253	0.423					
Nagpur Seedling	0.259	0.178	0.162	0.191	0.227	0.221	0.388	0.152				
Sindh	0.293	0.161	0.156	0.198	0.200	0.229	0.376	0.202	0.091			
Tehsidar	0.244	0.218	0.202	0.246	0.247	0.241	0.376	0.236	0.182	0.176		
Nasik	0.293	0.300	0.283	0.294	0.346	0.289	0.386	0.268	0.222	0.256	0.154	
Patilo	0.291	0.262	0.246	0.280	0.305	0.275	0.422	0.233	0.200	0.232	0.171	0.107

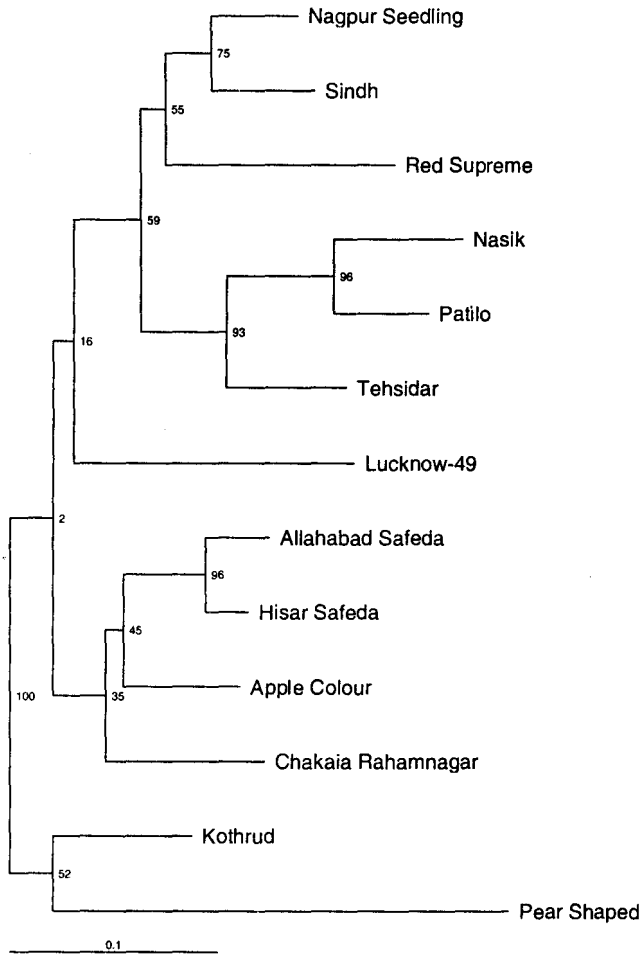

Fig. 2. Neighbour-joining tree of 13 guava cultivars. The bar represents Nei and Li coefficient

at a bootstrap probability of more than 93%. Hisar Safeda and Allahabad Safeda were grouped together with a bootstrap value of 96%. Rest of the groups did not have bootstrap probabilities high enough to be distinguished as clusters. The clustering pattern neither corresponded to the known attributes of the cultivars such as flesh colour or place of origin as reflected in their names. Guava is an allogamous tree crop, which is highly heterozygous. Correspondence between molecular markers and morphological traits may not be apparent. In the present experiment, the highest distance index was only 0.423, indicating high similarity among the cultivars analysed. This observation can be indicative only and cannot be conclusive. An accurate measure of molecular diversity in Indian guava cultivars may be obtained if a large number of them are screened using a higher number of markers. Nevertheless, the present report demonstrated that RAPD could be effectively used to identify cultivars. The utility of the specific RAPD markers can be increased by sequencing their termini and designing longer primers (SCARS) for specific amplification.

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