

Variability among selective guava (*Psidium guajava* L.) varieties revealed by morphology and RAPD marker

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Abstract

The research was conducted for the assessment of genetic diversity using both morphological and random amplified polymorphic DNA (RAPD) analysis of twelve guava (*Psidium guajava* L.) varieties growing in Bangladesh. Morphological characterization of guava varieties showed a wide range of variation. The highest variability was observed between Poly and Jelly varieties. Polymerase chain reaction with 5 arbitrary 10-mer and 3 arbitrary 12-mer RAPD primers produced a total of 50 bands of which 75.23 percent were polymorphic. The highest percentage of polymorphic loci (100%) was observed for primer A and the lowest (50%) for A03 primer. The UPGMA dendrogram revealed the segregation pattern and the difference of evolutionary changes. Guava varieties were separated into two main groups, one of them was made up of Chinese, Jelly, Kazi, Apple, L-49, Local-2 and Local-3. The other one was made up of Local-1, Poly, Kashi, Thai and Bombay. The highest genetic distance between Apple and Kazi peyara indicate that these varieties might be interesting in breeding programme for improving trait of interest. This scientific information could be used for further improvement of guava.

Key words: Polymorphic, genetic diversity, RAPD, *Psidium guajava* L.

INTRODUCTION

Guava (*Psidium guajava* L.) belongs to the family Myrtaceae, is almost universally known (Morton, 1987). This is native to tropical South America, but it is cultivated in many tropical and subtropical parts of the world (Mishra *et al.*, 2007). In Bangladesh, common cultivars of guava are kazi, deshi, swarupkhathi, lal peyara, kushum peyara etc. Kazi peyara is the most popular variety with commercial cultivation taking all over Bangladesh. Popular cultivars of India are Apple Colour, Behat Coconut, Lucknow 42, Lucknow 49, Banaras, Chittidar, Allahabad safeda, Baruipur, Pear shaped, Seedless etc. were introduced in Bangladesh. *Psidium guajava* had the chromosome count of $n = 22$ and $2n = 44$. The karyotype of the species was slightly asymmetric with a little variation in chromosome length. The species revealed 4 pairs of metacentric, 12 pairs of nearly metacentric and 6 pairs of sub-metacentric chromosome.

Guava is an allogamous fruit crop, which is highly heterozygous. Several guava cultivars have emerged as a result of seedling selection and seedling of these cultivars are being commercially exploited through seed propagation which has indirectly given rise to

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several types which are not true to the commercial type and vary in several characters from the parent population. Morphological characters may not be reliable to discriminate between closely related guava genotypes although several morphological traits such as fruit color, leaf shape and size may be useful but they lose their usefulness for assessment of genetic diversity between closely related guava genotype. Molecular markers can be gainfully employed to discriminate between species and cultivars of guava (Prakash, *et al.*, 2002). Among the different types of molecular markers, randomly amplified polymorphic DNA (RAPD) are useful for the assessment of genetic diversity (Williams *et al.*, 1990) owing to their simplicity, speed and relatively low-cost (Rafalski & Tingey, 1993) compared to other types of molecular markers. RAPD is considered to be one of the major techniques commonly used in germplasm characterization and similar studies have been carried out in horticultural crops including cherimoya (Rahman *et al.*, 1997), mango (Farzana *et al.*, 2009), banana (Das *et al.*, 2009) and guava (Tseng *et al.*, 2007). RAPD markers have been used extensively to classify accessions, identify cultivars and hybrids (Meng *et al.*, 1996), and analyze genetic diversity (Lee *et al.*, 1996; Levi *et al.*, 2001; Garcia *et al.*, 1998; Silberstein *et al.*, 1999; Gwanama *et al.*, 2000; Sureja *et al.*, 2006).

DNA polymorphism can be used as molecular markers and is considered to have significant impact on applied plant breeding. The polymerase chain reaction (Mulis & Faloon, 1987) provides a rapid, safe and efficient method for screening large population. Unlike Southern blot analysis, PCR can directly distinguish polymorphism including insertion or deletion and point mutation events. Besides, RAPD analysis provided DNA polymorphism even among related species rapidly and feasibly (Williams *et al.*, 1990). RAPD fingerprinting can be used to trace genetic or epigenetic changes at the genome level. The goals of this study were to assess the morphological variations through phenotypic study, to investigate the genetic variability at the genomic level and to select parents for better and more production.

MATERIALS AND METHODS

Plant material: Twelve varieties (Table 1) of Guava were collected from Germplasm Center of Khulna University and Khulna University campus, Bangladesh.

Table 1. Guava varieties used in RAPD analysis

Sl no.	Variety	Sl no.	Variety
1	Chinese	7	Thai
2	Apple	8	Bombay
3	Kashi	9	L-49
4	Kazi	10	Local-1
5	Poly	11	Local-2
6	Jelly	12	Local-3

Genomic DNA extraction: The modified CTAB method (Doyel & Doyel, 1987) was adopted to extract genomic DNA from the plant leaves at Plant Biotechnology Lab of Khulna University, Khulna. In this experiment, 0.1g leaves were used instead of 0.5-1.0g leaves. Liquid nitrogen and sterile sand were avoided during grinding. For genomic DNA extraction isolation buffer, lysis buffer and CTAB buffer were used separately instead of CTAB isolation buffer.

PCR reaction and RAPD analysis: Genomic DNA polymorphism was determined by the random amplified polymorphic DNA (RAPD) method (Williams *et al.*, 1990). Five 10 mer primers and three 12 mer primers were used for DNA amplification (Table 2). Amplification was performed in sterile 0.2 ml Eppendorf tubes in 10 μ l reaction volume. One microliter (2ng) of genomic DNA was mixed with 9 μ l of PCR master mixture containing PCR buffer with additional 1.5 mM MgCl₂; 200 μ M each of dATP, dCTP, dGTP, dTTP; 0.004 μ M primer; 2 units of *Taq* DNA polymerase (Bioneer corporation, Korea). Amplification was conducted in a thermocycler (Eppendorp master cycler) for 5 min at 94°C (preliminary denaturation), the thermal cycle was 1 min at 94°C (denaturation), 1 min at 35°C (annealing) and 2 min at 72°C (elongation). This cycle was repeated for 30 times and an elongation period was carried out for 5 min at 72°C. Finally the reactions were held at 4°C.

Table 2. List of random primers used in genetic variability analysis of guava

Sl no.	Primer code	Sequence (5' – 3')	GC content(%)
1	A	GAACGGACTC	60%
2	B	AATSSGCTGG	60%
3	C	AACGGTGACC	60%
4	D	TCTGCCATCC	60%
5	E	TTCCCCCAG	70%
6	A01	AGCAGCGCTCA	66%
7	A03	TGCCTCGACCA	66%
8	A05	CCGAGTTAGAT	50%

After PCR reaction, amplified products were analyzed by electrophoresis in 2% agarose gel. A Lambda/Hind III marker was run for each agarose gel, for estimation of fragment size. The gel was stained and visualized under UV Transilluminator. Gels were photographed using a gel documenter fitted with camera.

Each accession was scored for the presence (1) or absence (0) of a particular amplification product. Data was analyzed by the software component 2.0 (CPW3) for phylogenetic analysis. The unweighted pair group method with arithmetic averages (UPGMA) based dendrogram was constructed from distance matrix. The possible pair-wise genetic distance values were calculated according Nei & Li, (1979)

$$d_{xy} = 1 - [2n_{xy} / (n_x + n_y)],$$

where, n_x and n_y are the numbers of bands amplified in individuals x and y, respectively, and n_{xy} is the number of bands shared by those individuals. The distances between each pair of accessions were used to construct a dendrogram using the unweighted pair group method with arithmetic averages (UPGMA).

Morphological features among the varieties were obtained by measuring the tree, leaves and fruits from randomly selected samples with assistance of the horticulture expert of Agro technology discipline, Khulna University. Fruit height was measured in meter. Ten mature fruits were collected for each variety. Fruit length and diameter were measured and the final value was obtained from average of those values. Fruits shapes were measured by adjusting the fruit shape chart.

RESULTS AND DISCUSSION

Morphological characterization: Morphological characteristics of twelve varieties of guava have been presented in (Table 3) such as length, width, fruit color, shape, seed producing rate, taste etc. Height of the tree of Kazi and L-49 vary from 3.9 m to 2.3-3.4 m, Kashi is tallest among twelve varieties (5.2m). All the varieties except Poly are green to light green in color, L-49 has primrose yellow and variety Poly is reddish in color. Fruits of four varieties (Chinese, Thai, Poly and Local-2) are round and other five varieties (Jelly, Bombay, L-49, Local-1, local-3) are rounded ovoid in fruit shape. Apple, Kashi and Kazi are spherical, ovoid and globose in shape, respectively. Fruits of thai variety contains less numbers of seed comparing with other varieties. Apple, kasha and bombay contain medium number of seeds. High amount of seeds is observed in L-49. Longitudinal section of fruits were presented in Figure 1. Only Poly is red fleshed guava. Jelly guava has pinkish colored flesh and sour taste. Kazi variety is considered as the most popular commercial variety in Bangladesh.

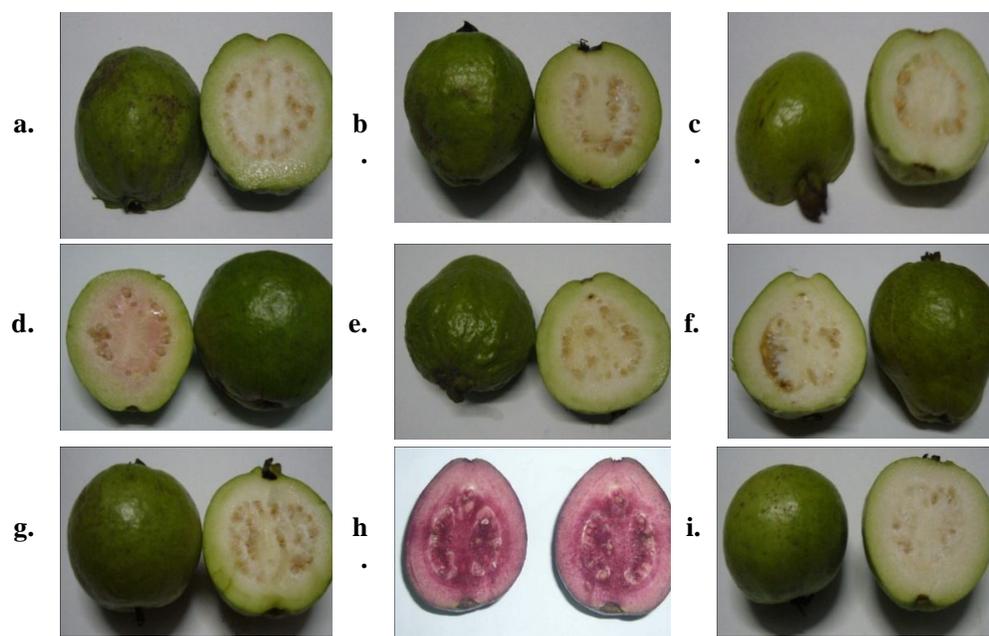


Fig. 1. Longitudinal section of guava varieties (a) Apple (b) Bombay (c) Chinese (d) Jelly (e) Kashi (f) Kazi (g) L-49 (h) Poly and (i) Thai

Table 3. Morphological features of selective guava varieties

No. of variety	Name of variety	Average plant height (m)	Fruit length (cm)	Fruit diameter (cm)	Fruit color	Fruit shape	Taste of fruit	Seed quality
V1	Chinese	3.8	3.7	4.3	Yellowish green	Round	High sweet	Low
V2	Apple	4-5.2	5.3	5.7	Light green	Spherical	Sweet	Medium
V3	Kashi	5.25	6.5	5.8	Light yellow	Ovoid	Sweet	Medium
V4	Kazi	3.9	8.0	7.0	Yellowish green	Globose	Medium sweet	Low
V5	Poly	4-4.5	5.2	4.8	Blackish red	Round	Sweet	Low
V6	Jelly	4.2-4.6	7.3	6.6	Yellowish green	Rounded ovoid	Sour	Low
V7	Thai	3.8-4.4	5.8	5.9	Light green	Round	Sweet	Very low
V8	Bombay	4.5-5	5	4.8	Yellowish green	Rounded ovoid	Sweet	Medium
V9	L 49	2.3-3.4	5.5	6	Primrose yellow	Rounded ovoid	sweet	High
V10	Local-1	4.5	6.8	6.5	Yellowish green	Rounded ovoid	Sweet	Low
V11	Local-2	5.0	5.2	5.9	Light green	Round	Medium sweet	Low
V12	Local-3	5.2	5.3	5.5	Light green	Rounded ovoid	Sweet	Medium

Molecular characterization: Randomly selected eight primers were used in RAPD analysis. These primers could amplify different genomic regions of 12 guava varieties and produced different banding patterns, ranging from 2 to 10. The average bands per primer was 6.25. Of the 50 bands scored, 40 (75.23%) were found to be polymorphic and 10 bands were found to be shared (Table 4), whereas 77% polymorphism has been seen among other 22 varieties of guava (Shiva *et al.*, 2017). The polymorphism is low comparing to mango (85.71%) (Farzana *et al.*, 2009). Fukuoka *et al.* (1992) got average 0.8 bands per primer with 40% and 6.1 bands per primer with 50% GC content for rice varieties. The correlation between GC content of the primer and the number of bands could be explained as the greater hydrogen bonding; because of G is pairing with C by three hydrogen bonds shows the high stability of base complementation than that of complementation of A with T by two hydrogen bonds (Fukuoka *et al.*, 1992). Dahiya *et al.* (2002) got 74.7% polymorphic loci by RAPD analysis using 9 primers but Sharma *et al.* (2007) obtained 92.29% polymorphism among 22 guava varieties. The primers were selected according to their performance with mango (Farzana *et al.*, 2009). In this case the average level of polymorphic bands was (5.0) per primer where as Priyamvada *et al.* (2017) obtained average 9.5 polymorphic bands while analyzing 20 guava varieties by 20 primer in RAPD analysis.

Table 4. RAPD primers with corresponding bands scored and their size range together with polymorphic bands observed in 12 guava varieties

Sl No.	Primercode	Number of total band	Number of common band	Number of polymorphic band	% of polymorphic loci
1	A	10	0	10	100
2	B	5	1	4	80
3	C	7	2	5	71.43
4	D	5	2	3	60
5	E	5	1	4	80
6	A01	9	1	8	88.89
7	A03	2	1	1	50
8	A05	7	2	5	71.43
Total		50	10	40	601.75
Average for 8primers		6.25	1.25	5.0	75.23

The frequencies of polymorphic bands varied from primer to primer. Though no accession-specific marker was identified, the high level of polymorphism revealed by the proportion of polymorphic loci (75.23%) indicated that RAPD markers could be considered as effective tools for estimating genetic diversity in different accessions of guava. Maximum number of shared bands has been observed for primer A03 and primer A gives maximum polymorphic bands.

Figure 2 presents the pictures of agarose gel showing polymorphisms observed with primers A and A01.

Pair wise Nei's genetic distance values between twelve guava varieties are shown in Table 5. The value ranged from 0.2054 to 0.7837 with the minimum between Bombay and Local-3 and the maximum between kazi and apple. In UPGMA dendrogram the eight selected varieties are grouped into two clusters (Fig. 3). Cluster 1 consists with chinese, jelly, kazi, apple, L-49, local-2 and Local-3. The other one was made up poly, local-1, kashi, Thai and Bombay plotted separately in the dendrogram.

Genetic distance was ranged from 0.2054 to 0.7837. The highest dissimilarity coefficient (0.7837) was observed between genotypes local-3 vs Bombay. The lowest (0.2054) similarity coefficient was revealed between the Kazi and Apple. Using the Nei's 1979 genetic distance a dendrogram was constructed to obtain the clustering of the accessions. Kanupriya *et al.* (2011) showed the heterozygosity among 9 guava varieties which was from 0.392 to 0.961 with a mean of 0.824 with 23 microsatellite markers. The dendrogram shows that all the accessions were found to be grouped in two major clusters designated as I and II (Fig. 3). Cluster I is broad which includes 7 accessions. Cluster II includes the highest genetic distance.

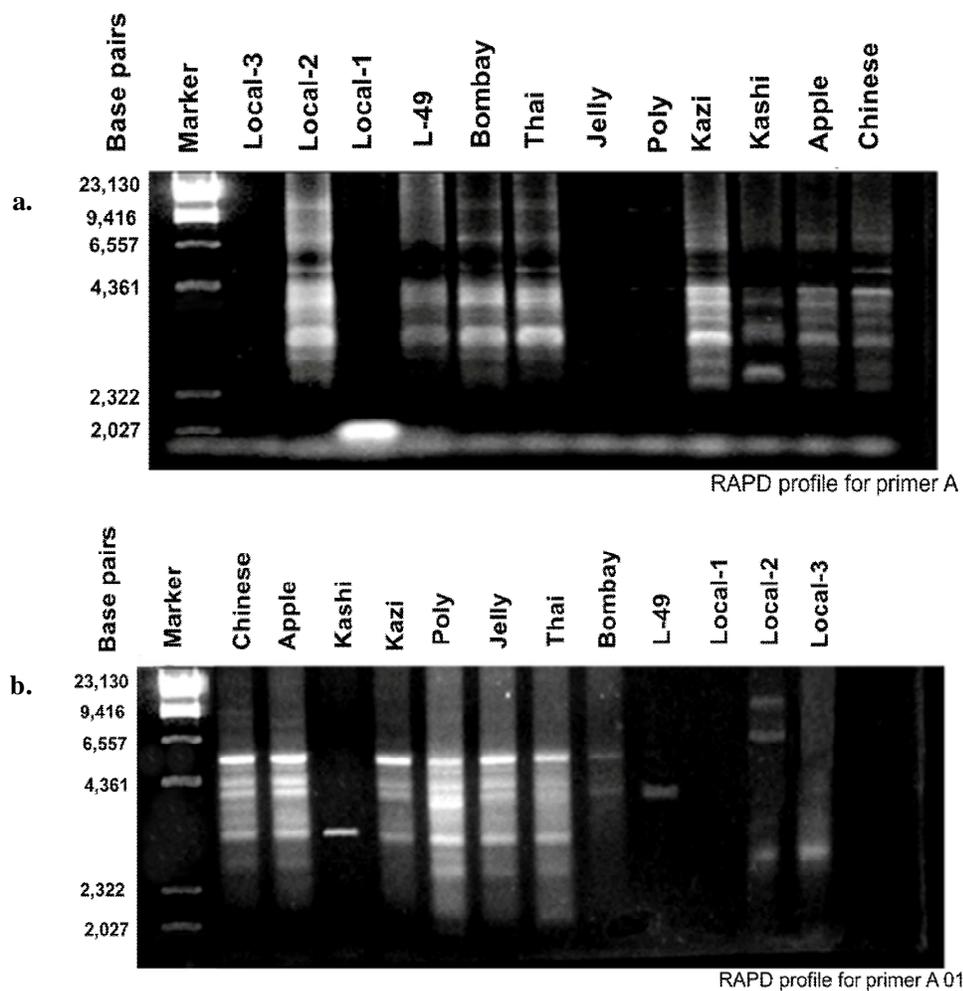


Fig. 2. RAPD profile for primer A (a) and primer A01 (b) of twelve guava varieties

Maximum number of shared bands was amplified with primer A, C, D, A05 but primer A and A01 amplified maximum number of polymorphic bands 100% and 88.89% respectively.

Table 5. Pair wise genetic distance values in of twelve guava varieties

	Chinese	Apple	Kashi	Kazi	Poly	Jelly	Thai	Bom bay	L-49	Local -1	Local -2	Local -3
Chinese	-											
Apple	0.2727	-										
Kashi	0.6551	0.375	-									
Kazi	0.4328	0.2054	0.323	-								
Poly	0.4909	0.4426	0.5094	0.4516	-							
Jelly	0.4901	0.4385	0.5918	0.4482	0.4347	-						
Thai	0.3333	0.3333	0.5737	0.4	0.5172	0.4074	-					
Bombay	0.5714	0.5483	0.7407	0.4603	0.6078	0.7446	0.3559	-				
L-49	0.5471	0.6610	0.5686	0.5333	0.7083	0.7272	0.4035	0.2653	-			
Local-1	0.56	0.5714	0.5616	0.6140	0.6888	0.5609	0.6296	0.4782	0.4883	-		
Local-2	0.4642	0.4193	0.444	0.3650	0.4901	0.6595	0.5	0.5384	0.7142	0.7826	-	
Local-3	0.7073	0.5744	0.6923	0.625	0.7222	0.75	0.7777	0.7837	0.7647	0.7064	0.7297	-

*Bold numbers are presenting minimum and maximum genetic distance values

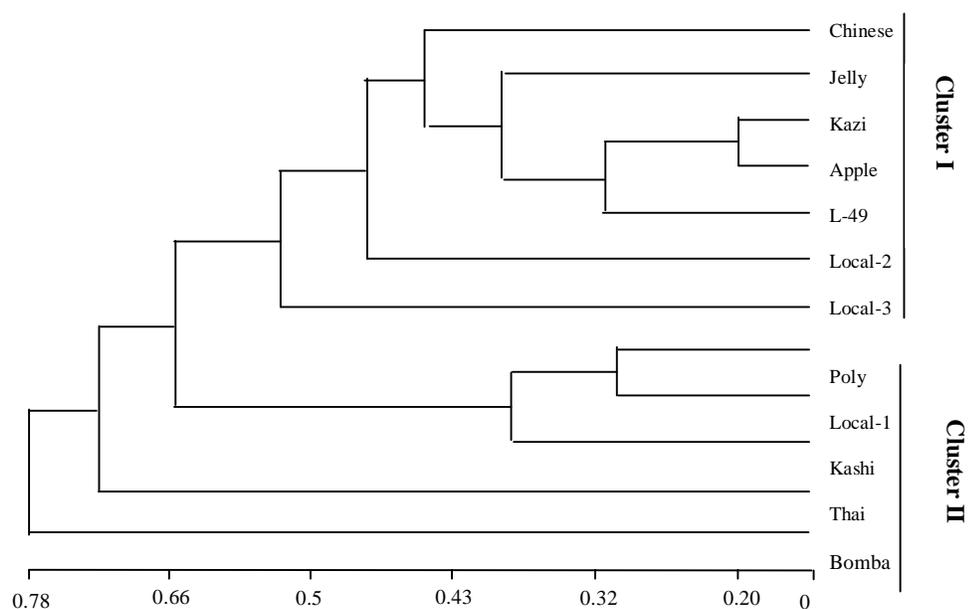


Fig. 3. The UPGMA dendrogram based on Nei's genetic distance (1978) summarizing the data on differentiation between twelve guava samples

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