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Genetic diversity among guava genotypes based on seed protein polymorphism

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ABSTRACT

Distinct polymorphism in electrophoretic banding patterns of seed protein following SDS-PAGE was noted in 11 guava genotypes, which led to the detection of 23 polypeptide bands. The highest number of bands (16) was observed in RCG-11 followed by Sangam (14), RCG-3 (13), RCGH-7 (12) and least in Lalit (9). The R_m values ranged from 0.097 to 0.958 in the size range of 97.4 to 14.3 kD. Genetic similarity ranged from 52.6 to 90.90 percent among the genotypes. High similarity index value (90.9%) was recorded in between RCGH-4 and Lucknow-49, Allahabad Safeda and Lucknow-49, Allahabad Safeda and RCG-2 were the genotypes, which relatively close The least similarity value (52.6%) was observed between RCG-11 and RCG-3 followed by RCG-11 and RCG-1 (52.90%). In cluster analysis, total seven clusters were formed and genotypes RCG-1, RCG-3, Sangam, RCGH-7 and RCG-11 were monogenotypic in cluster.

Key words: SDS-PAGE, seed protein, guava genotypes, relative mobility, similarity index.

INTRODUCTION

Guava is an open-pollinated and heterozygous crop with adequate genetic variation for selection of desirable commercial types. The guava clones are varying greatly with respect to their fruit quality and yield potentials. Improvement in any fruit crop needs to be undertaken through breeding and genetic manipulation, which has sufficient genotypes. The chances of success of any crop improvement programme increases to a greater extent due to genetic divergence within the available germplasm.

The fundamental pre-requisite in using genetic variability in plants involves assessment of genetic diversity that exists in the available germplasm (Kahler *et al*., 5). The extent of genetic diversity in germplasm can be assessed through morphological characterization and genetic markers (Ghafoor *et al*., 2).

The seed protein profiles obtained by various extraction procedures is conspicuously species specific and a highly stable character and is affected slightly by environmental conditions or seasonal fluctuations (Gray *et al*., 4). Among biochemical techniques, Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE) is most widely used due to its validity and simplicity for describing genetic structure of crop germplasm. Therefore, electrophoretic procedures have been used for both estimation of genetic diversity and identification of cultivars in several fruit crops like grape (Moreno-Arribas *et al*., 9; Liao *et al*., 5) and citrus (Gogorcena and Ortiz, 3). However, little information is available on varietal differences in seed protein composition among the cultivated guava. Thus, the present study was undertaken.

MATERIALS AND METHODS

The seeds of 11 genotypes were collected from ripen fruit and were grinded into fine powder with the help of pestle and mortar, 20 mg powdered seed material was added to 400 µl of sample buffer and mix thoroughly by gentle tapping. The sample was kept for 1 h at room temperature (28^0-30^0C) and then boiled at 85^0C for 8-10 min. using hot water bath. The samples were kept for overnight at room temperature and centrifuged at 10,000 rpm for 10-12 min. just before loading the supernatant in the gel slots. The supernatant was collected and loaded 20 µl in each slots. The SDS solubilized protein samples were analyzed by one dimensional discontinuous vertical SDS-PAGE with 12.5 percent separating and 4 percent stacking gels using Tris glycine electrode buffer. Electrophoresis was carried out on a vertical slab gel. The samples were electrophoresed at constant voltage of 140-150 V. After electrophoresis, the gels were removed and put in staining tray containing de-ionized water for washing the gel 5 min to remove SDS three times and after that Ezee blue gel stainer was added to cover the gel and staining tray was kept for 2 h. on a gel rocker and then staining solution was drained out. The gel was washed with de-ionized water to remove remaining

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SDS and previous staining solution was added again and gel was kept for overnight at room temperature (28^0-30^0C). Relative mobility (R_m) values of protein bands were calculated from the traveled distances of individual separated polypeptide. The presence and absence of bands were scored in each lane for calculating the similarity index (SI). Based on results of electrophoretic band pattern, similarity index was calculated. The similarity matrix thus generated was used to construct dendrogram by the UPGMA cluster analysis.

RESULTS AND DISCUSSION

The protein bands were stacked according to their molecular weight, *i.e.*, high molecular weight proteins were in the upper region and the low molecular weight proteins in the middle and lower regions of the gel. The electrophoregram (banding patterns) is presented in Fig. 1. The electrophoretic pattern of seed proteins of 11 genotypes in terms of presence and absence of different bands is shown in Table 1. A total of 23 bands could be resolved in seed protein profile of all the guava genotypes under study. The highest number of bands (16) was observed in RCG-11 followed by Sangam (14), RCG-3 (13), RCGH-7 (12), while, Lalit had the least number of bands (9).

The R_m value ranged from 0.097 to 0.9 58 in the size range of 97.4 to 14.3 kD. The protein banding pattern was characterized by four distinct zones, *viz*., A, B, C and D in the increasing order of electrophoretic mobility (Table 2). Zone A represented by seven bands, *viz*., A_1, A_2, A_3, A_4, A_5, A_6 and A_7, which varied in intensity and thickness in different genotypes. The R_m value of these zones ranged from 0.097 to 0.222. The zone B comprised of thick dark, medium dark and light intensity bands and was subdivided into B_1,

Table 1. Banding pattern of seed proteins in guava genotypes.

Bands No.	RCG-1	RCG-2	RCG-3	RCGH-1	Lalit	Sangam	Lucknow-49	Allahabad Safeda	RCGH-4	RCGH-7	RCG-11
1	0	0	0	0	0	0	0	0	0	0	1
2	0	0	0	0	0	0	0	0	0	0	1
3	0	1	0	0	0	0	0	0	0	1	1
4	0	0	1	0	0	0	0	0	0	0	0
5	0	0	0	0	0	1	0	0	0	1	1
6	0	0	1	1	0	0	0	0	0	0	0
7	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1
10	0	0	1	0	0	0	0	0	0	0	0
11	0	0	0	0	0	1	0	0	0	0	0
12	0	0	0	0	0	1	1	0	0	0	1
13	0	0	0	0	0	0	0	0	1	0	0
14	1	0	0	0	0	1	0	0	0	0	0
15	0	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1
21	0	0	0	0	0	0	0	0	0	1	0
22	0	0	0	0	0	0	0	0	0	0	1
23	1	1	1	0	0	1	1	1	1	0	1
Total	10	11	13	10	9	14	11	10	11	12	16

1, 0 = Presence and absence of band, respectively

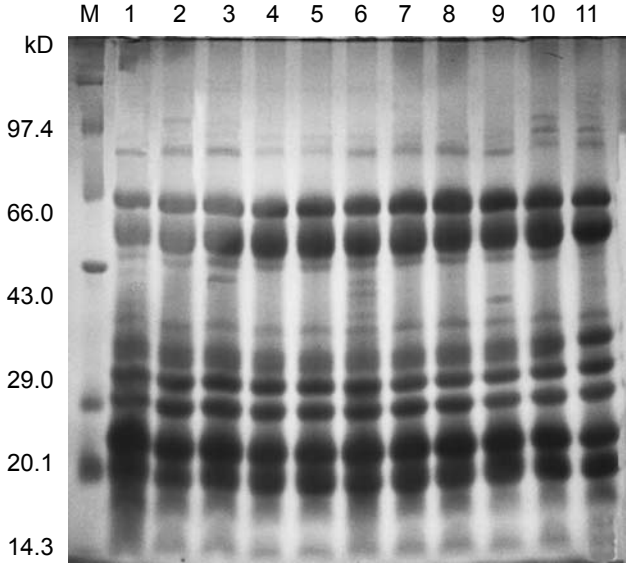

Fig. 1. SDS-PAGE of guava genotypes. M-Marker; Lane 1-RCG-1, Lane 2- RCG-2; Lane 3- RCG-3; Lane 4- RCGH-1; Lane 5- Lalit; Lane 6- Sangam; Lane 7- Lucknow-49; Lane 8- Allahabad Safeda; Lane 9 - RCGH-4; Lane 10 - RCGH-7; Lane 11 - RCG-11.

B_2, B_3, B_4 and B_5 in the R_m value ranged from 0.306 to 0.472. However, Zone C comprised of thick and medium dark intensity band with R_m value in range of 0.500 to 0.681. Zone C was also subdivided into six bands, *viz*., C_1, C_2, C_3, C_4, C_5 and C_6. The fourth and lower most zone was characterized by five bands, *viz*., D_1, D_2, D_3, D_4 and D_5 with the R_m value range of 0.764 to 0.958. Out of total 23 band, A_7 (R_m = 0.222), B_1 (R_m = 0.306), B_2 (R_m = 0.361), C_4 (R_m = 0.583), C_5 (R_m = 0.639), C_6 (R_m = 0.681), D_1 (R_m = 0.764), D_2 (R_m = 0.819) were common in all the cultivars. Several bands were present or absent in specific genotypes like A_1 (R_m = 0.097) and A_2 (R_m = 0.125) were present only in genotype RCG-11, while A_4 (R_m = 0.194) and B_3 (R_m = 0.444) were specific to RCG-3. Band B_4 (R_m = 0.469) was specific to genotype Sangam. Similarly, band C_1 (R_m = 0.500), D_3 (R_m = 0.875) and D_4 (R_m = 0.889) were unique to RCGH-4, RCGH-7 and RCG-11, respectively. Band A_3 (R_m = 0.153) was common for RCG-2, RCGH-7 and RCG-11 and it was absent in other genotypes. Likewise, A_5 (R_m = 0.208) was present only in genotype Sangam, RCGH-7 and RCG-11. Band B_5 (R_m = 0.472) was specific to Sangam, Lucknow-49 and RCG-11.

The common band may serve as a reference for enter-gel comparison and presence and absence of band can be used for cultivar identification from the gene pool. Variation in protein band profiling among guava genotypes was also reported by Raghava and Tiwari (11). Variation in band intensity observed within each electrophoregrams may be attributed to large variations in the amount of the various polypeptides present in the extract (Misra *et al*., 8).

The genetic similarity among the guava genotypes was analysed based on presence and absence of electrophoretic seed protein bands to evaluate the degree of closeness among different genotypes and to study the evolutionary relationship. A perusal of results presented in Table 3 indicated that the genetic similarity ranged from 52.6 to 90.90 percent among the genotypes. The high similarity index value (90.9%) was recorded in between RCGH-4 and Lucknow-49, Allahabad Safeda and Lucknow-49, Allahabad Safeda and RCG-2 followed by SI value of 90.0% between Lalit and RCGH-1, suggesting these genotypes to be close to each other. The high SI values indicated a close relationship among the genotypes. The closeness may be due to common parentage or confluence of similar gene from different parents in the development of varieties. The least similarity value (52.6%) was observed between RCG-11 and RCG-3 followed by RCG-11 and RCG-1 (52.90%). Thus, SI values may be used to establish relationship among the genotypes. The variation in polypeptide and isozyme pattern may reflect true genetic diversity rather than the variation in diverse horticultural traits, which are generally influenced by the surrounding environment (Mandal *et al*., 7). Dendrogram was made on the basis of seed protein banding patterns (Fig. 2) using UPGMA method. The clustering pattern revealed that all the genotypes were grouped together. However, genotypes RCG-1, RCG-3, Sangam, RCGH-7 and RCG-11 were found as monogenotypic in cluster. On the basis of 0.91 (Nei and Li's) similarity coefficient, total seven clusters were formed. The cluster analysis resolved the eleven guava genotypes into two major groups. First cluster comprises of two genotypes Lalit and RCGH-1. The maximum 4 genotypes (RCG-2, Allahabad Safeda, Lucknow-49 and RCGH-4) were present in cluster II. Lalit and RCGH-1 showed the maximum similarity in cluster I followed by Allahabad Safeda and RCG-2 of cluster II. Among the guava genotypes, RCG-11 was most diverse in cluster analysis and can be used as a parental line in breeding programme for the desirable traits like, less seed content (Babu *et al*., 1). Clustering of the genotypes signifies close genetic proximity between/among the varieties. Band on distance between varieties of different clusters, contrasting parents may be identified and used in the breeding programme for generating wider variability for selection and crop improvement. However, parental lines of narrow genetic base may also necessitate the

Table 2. Relative mobility (R_m) values among guava genotypes for seed proteins.

Band No.	RCG-1	RCG-2	RCG-3	RCGH-1	Lalit	Sangam	Lucknow-49	Allahabad Safeda	RCGH-4	RCGH-7	RCG-11
A_1	-	-	-	-	-	-	-	-	-	-	0.097
A_2	-	-	-	-	-	-	-	-	-	-	0.125
A_3	-	0.153	-	-	-	-	-	-	-	0.153	0.153
A_4	-	-	0.194	-	-	-	-	-	-	-	-
A_5	-	-	-	-	-	0.208	-	-	-	0.208	0.208
A_6	-	-	0.215	0.215	-	-	-	-	-	-	-
A_7	0.222	0.222	0.222	0.222	0.222	0.222	0.222	0.222	0.222	0.222	0.222
B_1	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306
B_2	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361
B_3	-	-	0.444	-	-	-	-	-	-	-	-
B_4	-	-	-	-	-	0.469	-	-	-	-	-
B_5	-	-	-	-	-	0.472	0.472	-	-	-	0.472
C_1	-	-	-	-	-	-	-	-	0.500	-	-
C_2	0.514	-	-	-	-	0.514	-	-	-	-	-
C_3	-	0.542	0.542	0.542	0.542	0.542	0.542	0.542	0.542	0.542	0.542
C_4	0.583	0.583	0.583	0.583	0.583	0.583	0.583	0.583	0.583	0.583	0.583
C_5	0.639	0.639	0.639	0.639	0.639	0.639	0.639	0.639	0.639	0.639	0.639
C_6	0.681	0.681	0.681	0.681	0.681	0.681	0.681	0.681	0.681	0.681	0.681
D_1	0.764	0.764	0.764	0.764	0.764	0.764	0.764	0.764	0.764	0.764	0.764
D_2	0.819	0.819	0.819	0.819	0.819	0.819	0.819	0.819	0.819	0.819	0.819
D_3	-	-	-	-	-	-	-	-	-	0.875	-
D_4	-	-	-	-	-	-	-	-	-	-	0.889
D_5	0.958	0.958	0.958	-	-	0.958	0.958	0.958	0.958	-	0.958

Table 3. Similarity index (SI) among guava genotypes for seed proteins.

Genotype	RCG-1	RCG-2	RCG-3	RCGH-1	Lalit	Sangam	Lucknow-49	Allahabad Safeda	RCGH-4	RCGH-7	RCG-11
RCG-1	-	75.0	64.3	66.7	72.7	71.4	75.0	81.8	75.0	57.1	52.9
RCG-2		-	71.4	75.0	81.8	66.7	83.3	90.9	83.3	76.9	68.8
RCG-3			-	76.9	69.2	58.8	71.4	76.9	71.4	56.3	52.6
RCGH-1				-	90.0	60.0	75.0	81.8	75.0	69.2	52.9
Lalit					-	64.3	81.8	90.0	81.8	75.0	56.3
Sangam						-	78.6	71.4	66.7	62.5	66.7
Lucknow-49							-	90.9	83.3	64.3	68.8
Allahabad Safeda								-	90.9	69.2	62.5
RCGH-4									-	64.3	58.8
RCGH-7										-	64.7
RCG-11											-

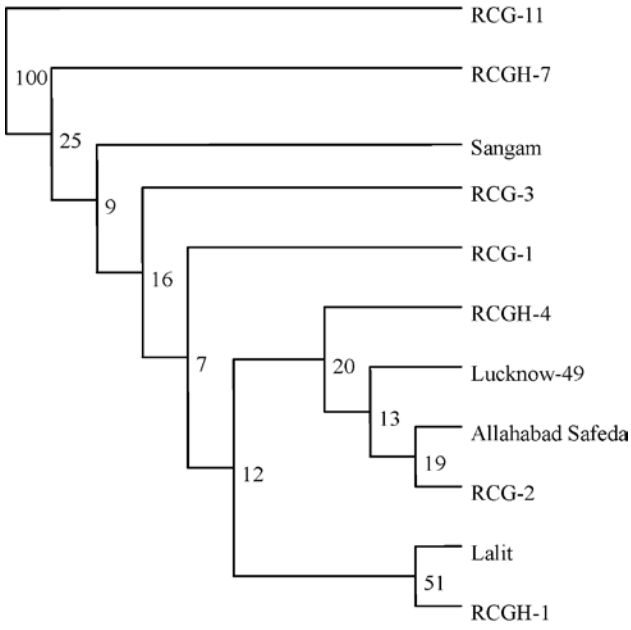

Fig. 2. UPGMA cluster analysis of 11 guava genotypes and the dendrogram generated based on seed protein electrophoresis.

use of techniques like wide hybridization or mutation for the creation of genetic variability. Prakash *et al.* (10) also observed low to moderate genetic diversity in guava using RAPD markers.

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