INDIAN JOURNAL OF ARID HORTICULTURE
2008, Vol. 3 (2) : 5-10

Characterization of underutilized fruit crops: bael and karonda by RAPD marker

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Abstract

Twenty four accession of Bael, representing four cultivars (Pant Shivani, Pant Urvashi, Pant Sujata and Pant Aparna) and seven accessions of Karonda, representing three cultivars (Pant Manohar, Pant Sudarsan, and Pant Suvarna) have been characterized with RAPD markers. In case of Bael out of the 19 primers screened, 5 were amplified reproducible bands and only three primers were selected for polymorphic study. Out of 10 DNA fragments amplified with three random primers, 8 showed polymorphism. These genotypes were classified into two major groups with each containing three sub-groups. While, in Karonda out of the 19 primers screened, 4 primers amplified reproducible bands and only two primers were selected for the polymorphic study. Out 10 DNA fragments amplified with two random primers, 7 were polymorphic. These genotypes were grouped into two major clusters and one cluster containing two sub- clusters while other consists only one cluster. RAPD data were analyzed by cluster analysis and UPGMA Our analysis revealed that in Bael, some genotypes are identical at DNA level but given different name whereas, Karonda genotypes showed the existence of considerable variation among the test accessions.

Key words: *Bael Germplasm, Karonda, RAPD, DNA, Dendogram*

Introduction

Horticultural crops have also received attention during the last few years related to molecular markers. However, there have been only few attempts in fruit crops. To, date, no information is available on characterization of Bael (*Aegle marmelos* Correa) and Karonda (*Carissa carandus*) germplasm at the molecular level. However, Morphological and phonological traits were used to identified Bael and Karonda genotypes (Misra *et al.* 2003) resulting into the discrimination of different genotypes. But, disease and environmental factors can affect these traits, leading to a wrong identification. Arduous efforts to improve the agronomic traits of wild Bael and Karonda germplasm by traditional selection based on the visible phenotypes started in 1987 and some elite genotypes have been obtained, but genetic improvement has been greatly limited by the limited information on the genetic background of this germplasm. Accurate identification of genotypes is a requisite for efficient conservation, maintenance and utilization of the existing germplasm of Bael and Karonda. In view of there nutritional, medicinal and commercial value, it is desirable to study RAPD markers used to estimate the diversity and relationship among 24 Bael accessions and 7 Karonda accession from different parts of India, maintained at Horticulture Research Centre of Govind Ballabh Pant University and Technology and to identify a 'core collection' from the same.

Materials and methods

Plant materials

Twenty four Bael and seven Karonda accessions (Table1) collected from different states, namely Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and West Bengal and conserved at Horticulture Research Centre, Patharchatta, Pantnagar were utilized in the study. The accessions include diverse types such as four released varieties and twenty genetic stocks of Bael and three released varieties and four genetic stocks of Karonda.

DNA extraction

Total genomic DNA was extracted from recently matured leaves using the CTAB method (Murray and Thompson 1980) with a few modifications. Approximately 2.0 grams of fully expanded fresh leaves were crushed with the help of liquid nitrogen into very fine powdered and was mixed **with 8 ml of extraction buffer (2 % CTAB, 100 mM Tris HCl-pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl), 1 % PVP and 0.2 % β-mercaptoethanol which was pre-heated to 65°C.** The contents were then incubated in a

Table 1. Similarity matrix for Jaccard's coefficient for twenty four genotyeps of Bael.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20	B21	B22	B23	B24
B1																								
B2	0.667																							
B3	0.713	0.667																						
B4	0.833	0.8	0.833																					
B5	0.667		0.667	0.8																				
B6	0.833	0.5	0.833	0.667	0.5																			
B7	0.667	0.6	0.667	0.8	0.6	0.5																		
B8	0.667	0.333	0.6667	0.5	0.333	0.5	0.6																	
B9	0.667	0.33	0.667	0.5	0.333	0.5	0.6	0.725																
B10	0.667	0.6	0.667	0.8	0.6	0.8	0.6	0.333	0.333															
B11	0.647	0.667		0.833	0.667	0.833	0.667	0.667	0.667	0.667														
B12	0.667	0.333	0.667	0.5	0.333	0.5	0.6	0.80	0.725	0.333	0.667													
B13	0.647	0.667		0.833	0.667	0.833	0.677	0.667	0.667	0.667	0.8	0.667												
B14	0.833	0.8	0.833	0.750	0.8	0.667	0.8	0.5	0.5	0.8	0.833	0.5	0.833											
B15	0.833	0.5	0.833	0.667	0.5	0.667	0.5	0.8	0.8	0.5	0.833	0.8	0.833	0.667										
B16	0.667	0.6	0.667	0.8	0.6	0.8	0.6	0.333	0.333	0.8	0.667	0.333	0.667	0.8	0.5									
B17	0.667	0.333	0.667	0.5	0.333	0.5	0.6	0.785	0.725	0.333	0.667	0.725	0.667	0.5	0.8	0.333								
B18	0.833	0.8	0.833	0.750	0.8	0.667	0.8	0.5	0.5	0.8	0.833	0.5	0.833		0.667	0.8	0.5							
B19	0.833	0.5	0.833	0.667	0.5	0.647	0.5	0.5	0.5	0.8	0.833	0.5	0.833	0.667	0.667	0.8	0.5	0.667						
B20	0.647	0.667	0.647	0.833	0.667	0.833	0.667	0.667	0.667	0.667	0.783	0.667	0.783	0.833	0.833	0.667	0.667	0.833	0.833					
B21	0.833	0.5	0.833	0.667	0.5	0.667	0.5	0.8	0.8	0.5	0.833	0.833	0.833	0.667	0.8	0.5	0.8	0.667	0.667	0.833				
B22	0.674	0.667	0.647	0.833	0.667	0.833	0.667	0.667	0.667	0.667	0783	0.667	0.783	0.833	0.833	0.667	0.667	0.833	0.833	0.833	0.833			
B23	0.647	0.667		0.833	0.667	0.833	0.667	0.667	0.667	0.667	0.8	0.667	0.8	0.833	0.833	0.667	0.667	0.833	0.833	0.783	0.833	0.7 83		
B24	0.0667	0.333	0.667	0.5	0.33	0.5	0.6	0.725	0.725	0.333	0.667	0.725	0.667	0.5	0.8	0.333		0.5	0.5	0.667	0.8	0.667	0.667	

water bath at 65°C for one hour with intermittent shaking. The homogenate was cooled to room temperature and add 10 ml of chloroform : isoamyl alcohol (24 : 1 v/v) was added and shaken well. The tubes were spun in a centrifuge at 8000 rpm for 20 minutes at 27°C and the supernatant transferred to a clean tube. This was repeated three times until a clear solution was obtained and transferred the aqueous phase to a fresh centrifuge tube. To this 0.6 v/v of chilled isopropanol was added, mixed thoroughly and the mixture kept overnight at 4°C to allow DNA to **precipitate. DNA was pelleted the next day by spinning the tubes at 10000 rpm for ten minutes at 4°C and the pellet was washed with 70 % ethanol, dried and dissolved in 500 µl TE buffer (Tris HCl 10 mM and EDTA 1 mM pH 8.0). Five µl of RNase (10 mg/ml) was** added to each sample and incubated overnight at 37°C. The histones were removed by mixing with phenol and centrifuging at 10000 rpm for 5 minutes at 20°C and collecting the supernatant. This step was repeated with phenol, chloroform and isoamyl alcohol (25 **: 24 : 1) and only chloroform : isoamyl alcohol (24 : 1), then the DNA was precipated with 0.6 volume of propanol. The DNA was then dissolved in 500 µl 1x TE and quantified using a 'UV-VIS Spectronic 1210'.**

PCR amplification, gel electrophoresis and statistical analysis

Amplification was achieved by the protocol described by William *et al.* (1990) with some modification. The DNA was amplified with four random 10 mer primers which produced the maximum number of amplified product after screening 19 primers. Ingredients for each reaction include template DNA 35-40 ng, 100 **µM dNTPs each, Taq DNA polymerase 0.5 unit, 1 X magnesium chloride buffer and 5 pmoles primers (Genie, Bangalore, Pvt. Ltd.). The reaction mixtures were** overlaid with mineral oil and amplification was performed in a thermal cycler (Eppendorf). Total reaction consisted of 40 cycles, each cycle consisting three steps, denaturation at 94°C for 30 sec, annealing at 36°C for 1 min and primer extension at 72°C for 5 min. Amplification fragment were separated on 1.6 % agarose (HIMEDIA) gels containing ethidium

Table 1. List of accessions under study and their place of collection

List of Bael Genotypes					
S. N.	Genotypes	Place of collection	S. N.	Genotypes	Place of collection
1.	PB 1	Deoria, U.P.	13.	PB 13	Pantnagar,Uttarakhand
2.	PB 2	Deoria, U.P.	14.	PB 14	Pantnagar,Uttarakhand
3.	PB 3	Deoria, U.P.	15.	PB 15	Sultanpur, U.P.
4.	PB 4	Deoria, U.P.	16.	PB 16	Sultanpur, U.P.
5.	Pant Shivani	Jaunpur, U.P.	17.	PB 17	Pantnagar,Uttarakhand
6.	Pant Urvashi	Samastipur, Bihar	18.	PB 18	Pantnagar,Uttarakhand
7.	PB 7	Gonda, U.P.	19.	PB 19	24-Parganas, W.B.
8.	PB 8 (NB I)	Faizabad	20.	PB 20	24-Parganas, W.B.
9.	Pant Aparna	Faridpur	21.	PB 21	24-Parganas, W.B.
10.	PB 10	Pantnagar,Uttarakhand	22.	PB 22	24-Parganas, W.B.
11.	PB 11	Pantnagar,Uttarakhand	23.	PB 23	24-Parganas, W.B.
12.	Pant Sujata	Pantnagar,Uttarakhand	24.	PB24	24-Parganas, W.B.
List of Karonda Genotypes					
1.	PK 1	U.P.	5.	Pant Suvarna	Pantnagar,Uttarakhand
2.	Pant Manohar	Pantnagar,Uttarakhand	6.	PK 6	U.P.
3.	PK 3	U.P.	7.	PK 7	U.P.
4.	Pant Sudharsan	Pantnagar,Uttarakhand			

Table 2. The amplification profile of three RAPD primers

Primer No.	Sequence 5'? 3'	Amplified Range	RAPD loci	Polymorphic %
23SS10AT7	AGCCAGCGAA	600 - 1000 bp	3	100
26SS10C10	TTCCGAACC	500 – 1000 bp	3	66.67
28SS10C12	GGACCCTTAC	550 – 890 bp	4	75

Table 3. The amplification profile of three RAPD primers

Primer No.	Sequence 5'? 3'	Amplified Range	RAPD loci	Polymorphic %
25SS10T9	GTCCCGTGGT	170 - 790 bp	4	50
OPBF 06	TCCACGGGCA	230 – 650 bp	6	83.33

Table 5. Similarity matrix for Jacard's coefficient of seven genotypes of Karonda

	PK 1	Pant Manohar	PK 3	Pant Sudarshan	Pant Suvarna	PK 6	PK 7
PK 1	1						
Pant Manohar	0.747	1					
PK 3	0.572	0.572	1				
Pant Sudarshan	0.680	0.733	0.572	1			
Pant Suvarna	0.647	0.572	0.940	0.572	1		
PK 6	0.940	0.747	0.572	0.680	0.572	1	
PK 7	0.747	0.813	0.572	0.680	0.647	0.747	1

bromide (0.5 µg per 10 µl) solution for 20 min and photographed under Gel Documentation System (Alpha Innotech Corporation, USA). Each PCR was conducted as an experiment with controls to test purity and viability of reagents. Two controls namely 'no template' (distilled water instead of template DNA) and 'positive control' (with template DNA) were included in all the PCR reactions. Bands were manually scored 1 for presence and 0 for absence and the binary data were used for statistical analysis. The matrix of different RAPD phenotypes of all the primers was assembled. The sizes of the fragments (molecular weight in base pairs) were estimated by using 100-bp ladder marker, which was run along with the amplified products. Genetic similarities (GS) for the RAPD data were calculated using the Jaccard algorithm, and phenograms were constructed using the clustering method of the unweighted pair group method of arithmetic averages (UPGMA). All the above analysis was performed using the NTSYS-PC version.2.11. (Rohlf, 2000).

Results and Discussion

Diversity of the Bael accessions

The 24 accessions of Bael were characterized and a total of ten RAPD loci were detected. Out of nineteen tested primer, purchased from Genie, Bangalore, Pvt. Ltd. only five gave results and from five, only three primers (Table 2) produced polymorphic products. The average number of bands per primer was 3.33 and these amplified products ranging from 550 to 1000 bp by three primers. About 50 % of the samples were repeated to test for reproducibility and only the reproducible and unambiguous bands were for the analysis. Out of 10 loci, 8 were polymorphic and only two bands were found to monomorphic. The average of polymorphic bands per primer was 2.67. The scored from 24 accessions of Bael with three RAPD (10 mer) primers were used to generate similarity coefficient. Jaccard's similarity matrix presented in Table 4, showed the pair wise similarities among Bael genotypes with similarity coefficient ranged from 0.333 to 1.00.

UPGMA cluster analysis categorizes twenty four germplasm of Bael into two main clusters. In cluster I, there are 16 accessions of Bael which again sub-divided into two Subgroups. Subgroup I^1 contains 8 accessions viz., PB 16, PB 10, PB 4, Pant Shivani, PB 2, PB 18, PB 14 and PB 1 with coefficient of 0.71. The PB 1 shows 83.3 % similarity with PB 14 and PB 18 while PB 2 and PB 5 are related to PB 1, PB 14 and PB 18 with 78 % similarity. The other subgroup I^2 comprised PB 3, PB 11, PB 13, PB 23, PB 19, PB 20, PB 22 and Pant Urvashi. The genotypes in this group showed highest similarity of 1. In cluster II consists of eight Bael genotypes which again sub-divided into Subgroup II^1 and Subgroup II^2. In Subgroup II^1 only one genotype i.e., PB 7 exist with coefficient of 0.667. Subgroup

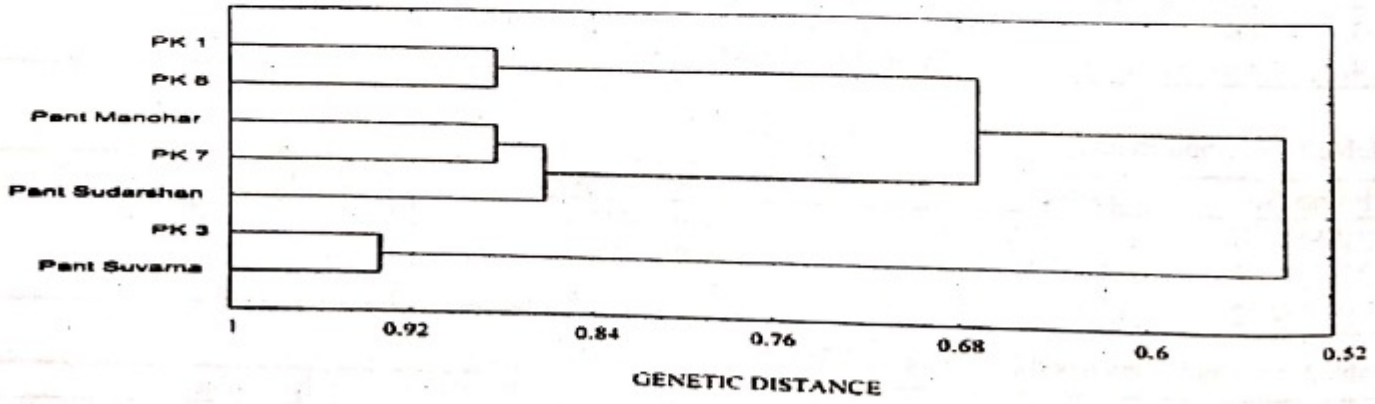

Fig. 1. Dendogram of 24 varieties of Bael genotypes constructed by using UPGMA based on Jaccards similarity Coefficient

II[2] contains seven genotypes i.e., NB 1, Pant Sujata, Pant Aparna, PB 17, PB 24, PB 15 and PB 21 with coefficient 0.8. PB 17 is related to PB 24 and shows coefficient of 1 and are similar to each other. However, PB 15 and PB 21 are related to other genotypes of cluster II with 0.74 genetic similarity. It was clear that some accessions from the same geographical regions clustered together along with other accessions from other places. Our findings agree with those of Prakash *et al.* (2002) who also observed that gropus of cultivars were clustered according to their origin in Indian guava. Similarly Cortes *et al.* (2001) reported for olive cultivars that provinces are not geographically isolated, because of the movement and exchange of germplasm among countries is very improbable that unique cultivars evolved in a specific country. Similar findings were reported by Misra *et al.* (2003) in Bael genotypes using seed protein electrophoresis, Deng *et al.* (1995) in lemons, Hancock *et al.* (1994) in strawberry and Matsumota *et al.* (1996) in rose cultivars. From above study it is concluded that there was a great variability between different accessions of same origin as well as similarity was also observed in between accessions of same origins which clearly indicating a low to moderately high genetic diversity among the accessions of Bael. Pant Shivani, Pant Urvashi, NB 1, and PB 7 were found great variability from other genotypes of Bael. So these genotypes could be used as 'core collection' for the improvement and new release of cultivars in Bael.

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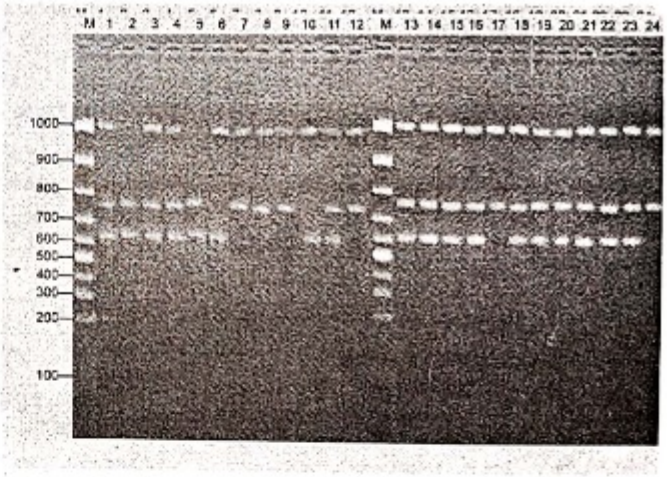

Fig. 2. The RAPD profile of 24 Bael genotypes generated by the primer 23SS10AT7 on 1.6 per cent agarose gel

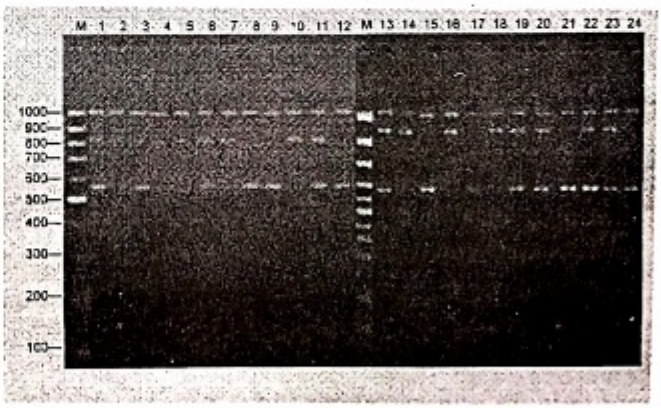

Fig. 3. The RAPD profile of 24 Bael genotypes generated by the primer 26SS10C10 on 1.6 per cent agarose gel

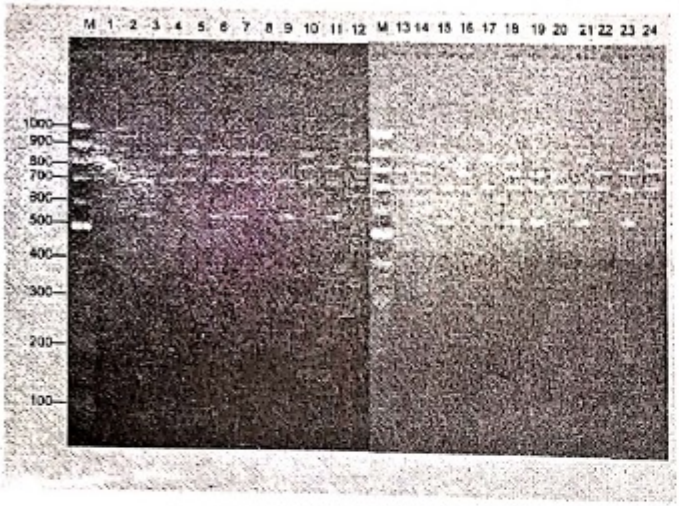

Fig. 4. The RAPD profile of 24 Bael genotypes generated by the primer 28SS10C12 on 1.6 per cent agarose gel

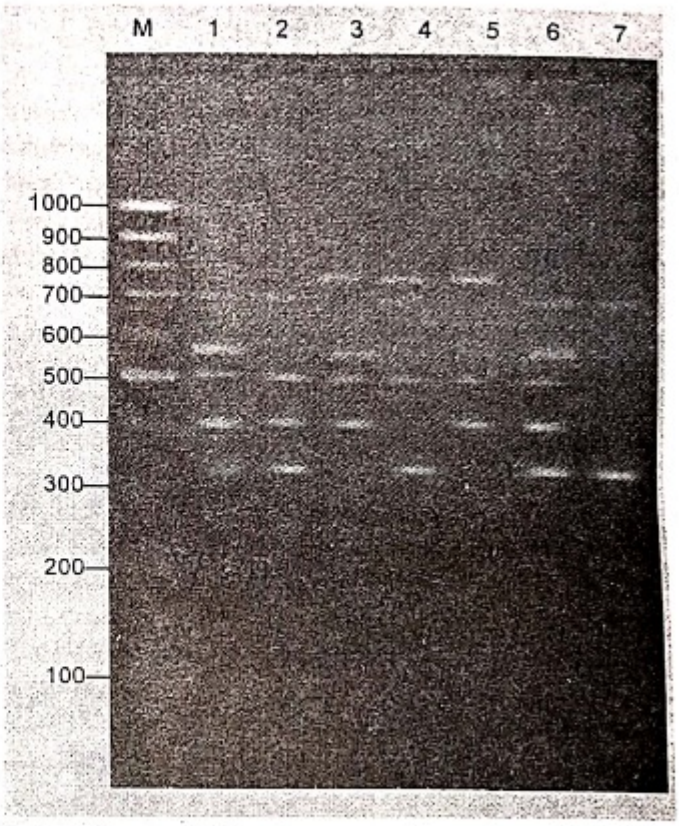

Fig 6. Polymorphic bands generated by primer OPBF06

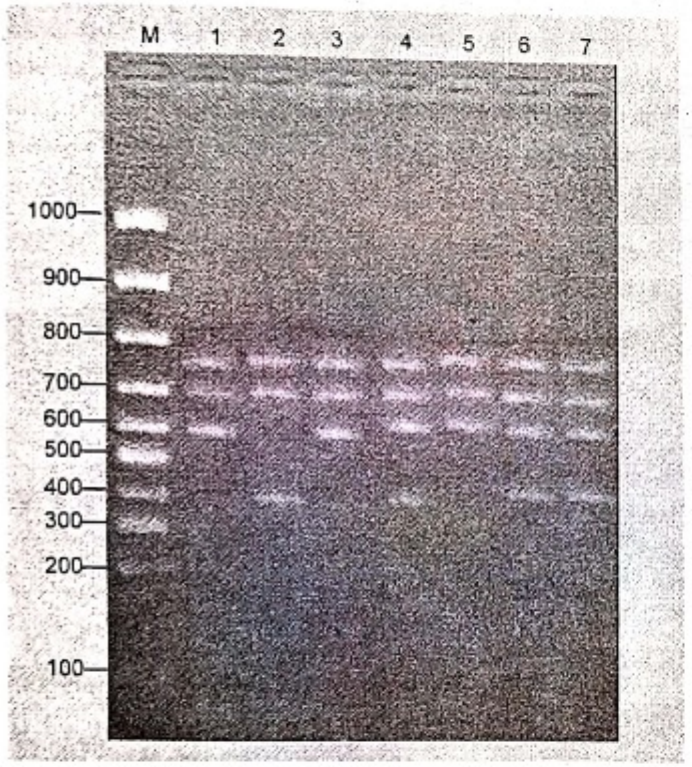

Fig 5. Polymorphic bands generated by primer

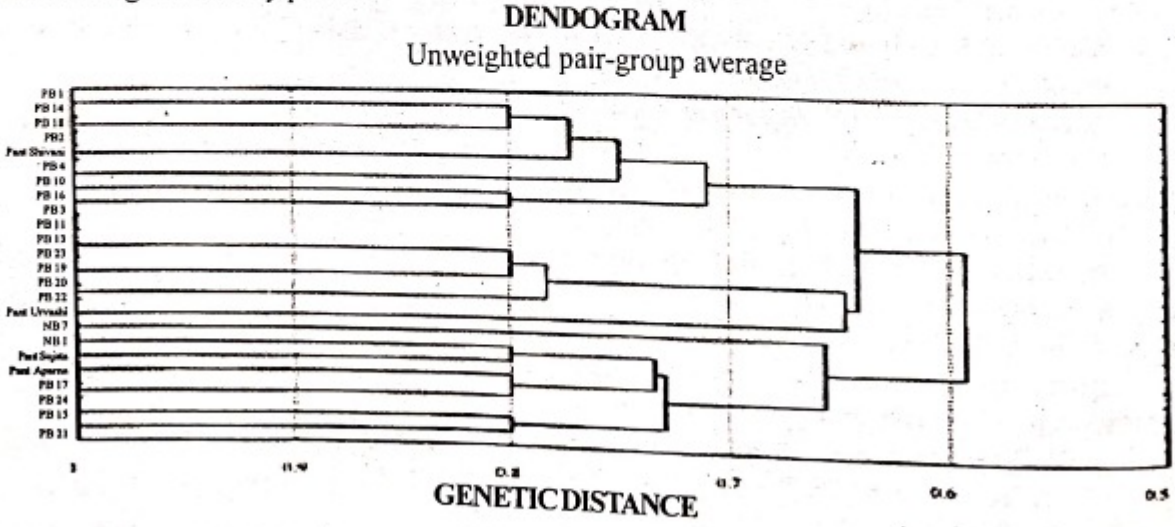

Fig 7. Dendogram of 7 varieties of Karonda genotypes constructed by using UPGMA based on Jaccard's similarity