International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 11(1): 93-96, February 2018
DOI: 10.30954/0974-1712.2018.00178.12

GENETICS AND PLANT BREEDING

Genetic Divergence Analysis in Papaya (*Carica papaya* L.) Genotypes using Molecular Markers

P.N. Kore*, M.J. Patel, A.S. Desai, A.A. Sakure, D.A. Patel and R.R. Acharya

Department of Horticulture, B.A.C.A. Anand Agricultural University, Anand-388 110, Gujarat, India

*Corresponding author: koreprabhu111@gmail.com (ORCID ID: 0000-0002-0430-3688)

Paper No. 655 Received: 08-09-2017 Accepted: 10-01-2018

ABSTRACT

The present investigation consisting of genetic divergence of papaya genotypes was conducted during the year 2014-15 and 2015-16 at Horticultural Research farm of B. A. College of Agriculture, Anand Agricultural University, Anand. Molecular characterization of twelve papaya genotypes was carried out by 20 RAPD primers. The highest PIC value (0.97) was obtained with primer OPE-7, while the lowest (0.28) with primer OPA-18. Highest genetic similarity (0.714) was found between Pusa Dwarf and Madhu Bindu whereas the lowest (0.113) between CO 8 and Pune Selection 3. Dendogram analysis revealed that Pune selection 3 was genetically diverse from other papaya genotypes. Over all (63.40%) polymorphism was observed among all the 12 papaya genotypes.

Highlights

- Highest genetic similarity (0.714) was found between Pusa Dwarf and Madhu Bindu.
- Pune selection 3 was found to be very diverse among the other papaya genotypes.

Keywords: Papaya diversity, RAPD marker, dendogram

Papaya (*Carica papaya* L.), belonging to the family Caricaceae is one of the most important fruits cultivated throughout the tropical and subtropical regions of the world. Recently another taxonomic revision was proposed and supported by molecular evidence that genetic distances were found between papaya and other related species (Kim *et al.* 2002). Some species that were formerly assigned to *Carica* family were classified in the genus *Vasconcella* (Badillo 2001).

Accordingly, the classification of *Caricaceae* has been revised to comprise *Cylicomorpha*, *Carica*, *Jacaratia*, *Jarilla*, *Horovitzia* and *Vasconcella*, with *Carica papaya* being the only species within the genus *Carica* (Badillo, 2001). Genetic diversity is a key component of any agricultural production system. The material from diverse origin of the crop species can help to ensure conservation of co-adapted gene complexes (Frankel *et al.* 1995). The application of genetic variation can also be utilized in future improvement programmes. Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at genetic level. Molecular marker based Genetic Diversity Analysis (MMGDA) also has potential for assessing changes in genetic diversity over time and space (Duwick 1984). Genetic variation is a pre requisite for any crop improvement programme to be successful. DNA based molecular markers acted as versatile tools to study variability and diversity in different plant species. Though a range of plant characters are currently available for distinguishing between closely related individuals, their sensitivity to environment and less genome coverage hinders their usage. DNA based molecular markers clearly allow the comparison of genetic material of two individual plants avoiding any environmental

influence on gene expression. Presently, many kinds of DNA based molecular markers such as RFLP, RAPD and AFLP etc., are available which detect polymorphism at the DNA level.

The present study employed RAPD technique to assess genetic polymorphism. The major advantages of the RAPD technique is that it does not need sequence information to start with. The polymorphism among genotypes can be detected by using random primer variation in the banding pattern of the amplification products which occur because of variation in the length of DNA sequences flanked by the primers.

MATERIALS AND METHODS

In the present investigation, Genotypes were selected during the field survey of papaya growing districts of Gujarat. After preliminary testing in field condition at main vegetable Research Station, Anand Agricultural University, Anand and based on better performance on yield, fruit size, dwarfing, fruit shape and colour they were selected as Gujarat Anand Papaya (GAP). The average concentration of DNA extracted from disease free fresh young leaves of papaya was 1860.6 ng/µl, quantified on Nanodrop spectrophotometer (Table 1). The quality of DNA was determined at 260/280 and 260/230 ratio and was further confirmed on agarose gel electrophoresis. DNA thus extracted was of good quality and was utilized for molecular markers study through RAPD markers.

Randomly Amplified Polymorphic DNA (RAPD)

Amplification of RAPD fragments was performed according to Choudhury *et al.* (2008) with some modifications using decamer arbitrary primers (MWG Biotech, Germany).

RESULTS AND DISCUSSION

Total 20 primers were screened for molecular characterization of papaya, out of which all 20 primers were found polymorphic. These 20 primers were used for RAPD analysis of twelve papaya genotypes (Table 2).

The 20 RAPD primers amplified a total of 91 bands. The RAPD marker OPA 8 produced maximum number of 65 bands, while OPE 12 produced minimum number of 8 bands. The average of allele amplified by 20 markers was 26. The highest PIC (Polymorphic Information Content) value obtained was 0.97 by marker OPE 7, while the lowest value was 0.28 by marker OPA 18. High PIC values can be attributed to the use of more informative markers.

It was also found that higher the PIC value of a locus the higher the number of alleles detected. The PIC values indicated that OPE-7 might be the best marker for diversity analysis of papaya genotypes followed by OPA-8, OPA-3, OPA-14 and OPA-20, while OPA-15 and OPE-12 were likely the least powerful markers.

Table 1: DNA quantification and preparation of working solution of papaya genotypes

Sl. No.	Genotype	Concentration of Stock solution (ng/µl)	Preparation of working solution (20 ng/µl, 100µl)	
			Stock solution taken (µl)	Water (Nuclease free) added (µl)
1	Pusa Nanha	2879.5	0.69	99.31
2	Pusa Dwarf	2060.9	0.97	99.03
3	Pune Selection 3	2784.6	0.72	99.28
4	Madhu Bindu	1538.6	1.30	98.70
5	CO 8	2577.6	0.78	99.22
6	GAP 6	1750.8	1.14	98.86
7	GAP 7	1347.7	1.48	98.52
8	GAP 10	1496.1	1.34	98.66
9	GAP 12	1456.3	1.37	98.63
10	GAP 29	1251.3	1.60	98.40
11	GAP 30	1703.1	1.17	98.83
12	GAP 31	1480.9	1.35	98.65

Table 2: Analysis of RAPD patterns generated using 20 primers for 12 papaya genotypes

Sl. No.	Marker	Primer Sequence (5′ 3′)	Total no. of bands	PIC value	% Polymorphism
1	OPA-1	CAGGCCCTTC	9	0.49	49.38
2	OPA-3	AGTCAGCCAC	35	0.83	82.68
3	OPA-6	GGTCCCTGAC	21	0.77	76.64
4	OPA-8	GTGACGTAGG	65	0.84	83.60
5	OPA-9	GGGTAACGCC	16	0.41	40.62
6	OPA-13	CAGCACCCAC	27	0.77	77.36
7	OPA-15	TTCCGAACCC	12	0.00	0.00
8	OPA-17	GACCGCTTGT	15	0.59	58.66
9	OPA-18	AGGTGACCGT	12	0.28	27.77
10	OPA-20	GTTGCGATCC	43	0.81	81.12
11	OPA-10	GTGATCGCAG	13	0.80	73.79
12	OPA-14	TCTGTGCTGG	34	0.82	81.66
13	OPE-1	CCCAAGGTCC	38	0.77	76.73
14	OPE-3	CCAGATGCAC	36	0.73	73.14
15	OPE-6	AAGACCCCTC	30	0.69	69.11
16	OPE-9	CTTCACCCGA	31	0.64	63.90
17	OPE-10	CACCAGGTGA	24	0.76	75.69
18	OPE-11	GAGTCTCAGG	9	0.79	79.01
19	OPE-12	TTATCGCCCC	8	0.00	0.00
20	OPE-7	AGATGCAGCC	42	0.97	97.22
Total			**520**	—	—
Average			**26**	—	—

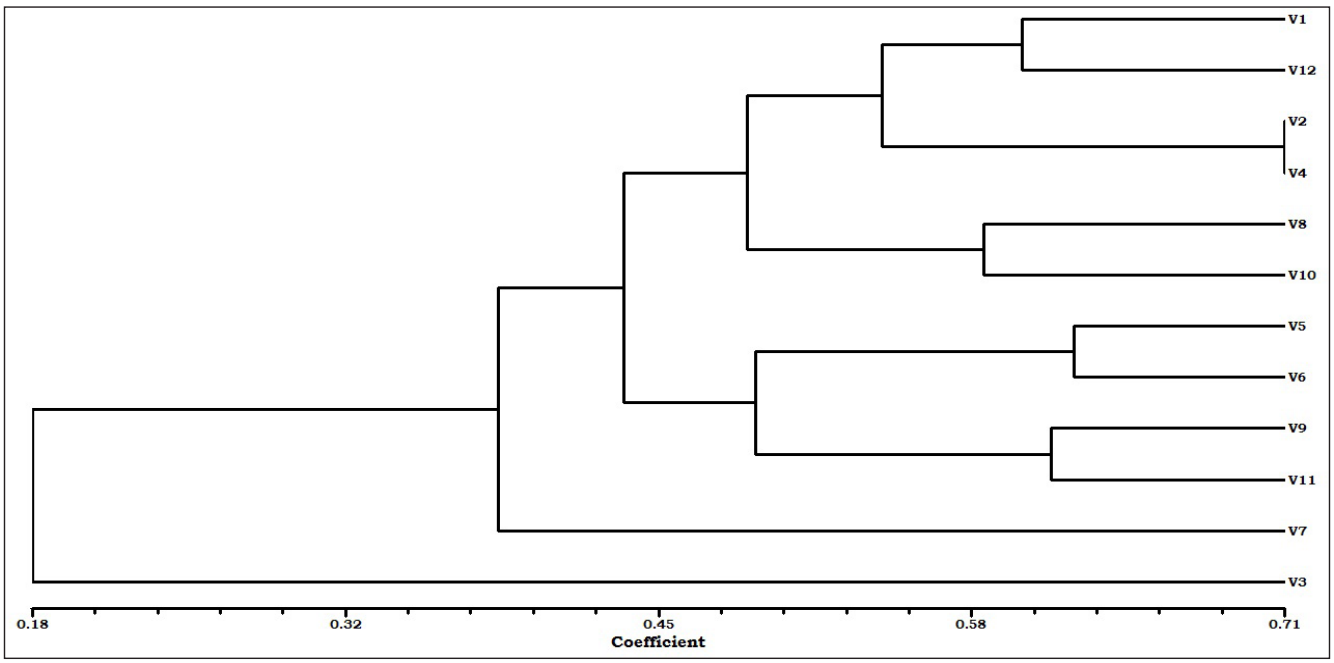

Fig. 1: Cluster analysis (Dendrogram) of genetic relationship among 12 genotypes based on RAPD analysis

Table 3: Similarity matrix of simple match coefficient for papaya genotypes based on bands amplified with 20 RAPD primers

	Pusa Nanha	Pusa Dwarf	Pune Selection 3	Madhu Bindu	CO 8	GAP 6	GAP 7	GAP 10	GAP 12	GAP 29	GAP 30	GAP 31
Pusa Nanha	1.000											
Pusa Dwarf	0.540	1.000										
Pune Selection 3	0.257	0.235	1.000									
Madhu Bindu	0.561	**0.714**	0.123	1.000								
CO 8	0.528	0.441	**0.113**	0.474	1.000							
GAP 6	0.500	0.403	0.180	0.426	0.625	1.000						
GAP 7	0.328	0.324	0.211	0.355	0.348	0.327	1.000					
GAP 10	0.407	0.351	0.127	0.509	0.422	0.426	0.488	1.000				
GAP 12	0.464	0.375	0.186	0.417	0.537	0.435	0.432	0.477	1.000			
GAP 29	0.452	0.529	0.164	0.603	0.449	0.397	0.479	0.587	0.500	1.000		
GAP 30	0.446	0.380	0.190	0.377	0.512	0.477	0.319	0.391	0.615	0.479	1.000	
GAP 31	0.603	0.475	0.236	0.600	0.439	0.466	0.414	0.527	0.361	0.517	0.390	1.000

RAPD Cluster Analysis

Based on the RAPD data, cluster analysis was performed using genetic similarity values and a dendogram was generated showing genetic relationships among these genotypes. The similarity coefficient ranged from 0.11 to 0.71. The highest similarity index value of 0.714 was found between Pusa Dwarf and Madhu Bindu, while the lowest similarity index value was observed between (0.113) CO-8 and Pune Selection 3 (Table 3). The Cluster analysis divided the genotypes into two major groups A and B. The first major group (A) included Pusa Nanha, GAP 31, Pusa Dwarf, Madhu Bindu, GAP 10, GAP 29, CO-8, GAP 6, GAP 12, GAP 30 and GAP 7.

In this group Pusa Dwarf and Madhu Bindu showed the highest genetic similarity. First cluster (A) was further sub-divided into two sub clusters and the first minor cluster (A1) comprised of Pusa Nanha, GAP 31, Pusa Dwarf, Madhu Bindu, GAP 10, GAP 29, CO-8, GAP 6, GAP 12 and GAP 30, whereas the second minor cluster (A2) included GAP 7. The second major cluster (B) contained Pune Selection 3 (Fig. 1). Highest genetic variation was observed between Pune Selection 3 and Pusa Nanha, which is in accordance to our morphological data.

CONCLUSION

Molecular characterization by RAPD markers showed genetic divergence among different genotypes. Genotypes Pusa Dwarf and Madhu Bindu showed the highest genetic similarity, while the lowest genetic similarity was observed between Pune Selection 3 and Pusa Nanha.

REFERENCES

Badillo, V.M. 2001. Nota corrective *Vasconcella*st. Hilly no *VasconcellaCaricaceae*. *Ernstia,* **11**: 75-76.

Choudhury, P.R., Singh, I.P., George, B., Verma, A.K. and Singh, N.P. 2008. Assessment of genetic diversity of pigeonpea cultivars using RAPD analysis. *Biologia Plantarum,* **52**(4): 648-653.

Duwick, D.N. 1984. Genetic diversity in major farm crops on the farm and reserve. *Econ. Bot*., **32**: 161-178.

Frankel, O.H., Brown, A.D.H. and Burdon, J.J. 1995. The conservation of plant biodiversity. Cambridge university press, Cambridge, UK, pp. 299.

Kim, M.S., Moore, P.H. and Zee, F. 2002. Genetic and molecular characterization of *Carica papaya* L. *Genomes,* **45**: 503-512.