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Molecular diversity analysis in F_3 intergeneric population of papaya (*Carica papaya* L.)

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ABSTRACT

Attempts were made to estimate molecular diversity present in F_3 populations of intergeneric crosses between *Carica papaya* L. (Var. Pusa Nanha and CP 50) and *Vasconcellea cauliflora*. Molecular studies revealed that PCR amplification using five ISSR primers in 40 F_3 progenies yielded 53 reproducible amplified bands. Of the 53 bands, 44 were polymorphic (83.02%). Polymorphic Information Content (PIC) value ranged between 0.90 (ISSR 807 x 810) and 0.66 (ISSR 834 x 810). Similarity coefficients based on five ISSR markers ranged from 0.05 to 0.96. Maximum similarity was observed for genotypes 1, 4 and 6 of Pusa Nanha x *Vasconcellea cauliflora* (0.96). Minimum similarity was observed between genotypes 3 and 14 of CP 50 x *Vasconcellea cauliflora* (0.04). This higher genetic diversity of papaya progenies stands to contribute to development of new varieties and, using the data, further hybridization and selection can be planned.

Key words: *Carica papaya, Vasconcellea cauliflora,* intergeneric hybridization, molecular diversity

INTRODUCTION

Papaya (*Carica papaya* L.), belonging to the family Caricaceae, is one of the important fruit crops in tropical and subtropical regions due to its economic, nutritional, industrial, pharmaceutical and medicinal values, both for local and export markets. Many diseases in papaya are economically important, the most important being the papaya ring spot virus (Purcifull, 1972). Management of PRSV by roguing out infected plants, quarantine regulation for restricting plant movement, use of insecticides against insect vectors and, cross protection, have generally not been effective in controlling the disease. Naturally occurring resistance to PRSV-P has not been identified in any papaya cultivar to date. Thus, developing PRSV resistant papaya is considered to be the best strategy for long-term control of this virus. Several species from a related genus, *Vasconcellea*, exhibit complete resistance to PRSV-P, and present a valuable resource for developing new PRSV-P resistant papaya varieties.

Crossing tolerant varieties with susceptible high-yielding commercial varieties has imparted some tolerance and improved yield under infectious conditions (Chan, 2004). Numerous efforts have been made to incorporate resistance genes from other genera present in the Caricaceae family, namely, *Vasconcellea cauliflora, V. quercifolia, V. stipulata* and *V. pubescens*. Intergeneric hybrids between papaya and PRSV resistant species have been produced by a number of investigators with the aid of embryo rescue techniques (Horovitz and Jimenez, 1967; Khuspe *et al*, 1980). However, not much progress is evident in this direction. Therefore, intergeneric hybridization was initiated by us between *Carica papaya* and *Vasconcellea cauliflora* to incorporate resistance gene from the latter into cultivars of papaya. Molecular markers are a useful complement to morphological and physiological characterization of cultivars as these are plentiful, independent of tissue, age or environmental effects, and allow for cultivar identification early in plant development. Thus, for genetic assessment, molecular markers are often considered advantageous over morphological markers. ISSRs are a versatile tool and are used extensively in plant breeding and evolutionary studies because of their high fidelity for showing diversity among cultivars (Levi and Roland, 1997).

In the present study, attempts were made to assess molecular diversity in F_3 populations of intergeneric hybrids of *Carica papaya* and *Vasconcellea cauliflora* using ISSR markers.

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MATERIAL AND METHODS

The present study, undertaken during 2009-2010 at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India, involves evaluation of molecular diversity among F_3 generation of intergeneric populations of *Carica papaya* (vars. Pusa Nanha and CP50) and *Vasconcellea cauliflora*. Intergeneric hybridization was made using *Carica papaya* as the female and *Vasconcellea cauliflora* as the male parent, to transfer genes desirable for PRSV resistance. Sibmating was made in selected plants of the F_2 population to develop F_3 population in Pusa Nanha x *Vasconcellea cauliflora* and CP50 x *Vasconcellea cauliflora*. In all, 25 progenies of the cross Pusa Nanha x *Vasconcellea cauliflora*, and 15 progenies of CP50 x *Vasconcellea cauliflora* were used in this study. Seeds obtained from selected combinations of F_2 population and their parents were sown in nursery bags. Screening of F_3 population was done by mechanical sap inoculation, followed by observations on disease intensity as per the disease score scale developed by Dhanam (2006). Healthy seedlings showing uniform growth were planted at a spacing of 1.8 × 1.8m. Standard package of practices was followed for the period under study.

Leaf samples of intergeneric hybrids were collected from the experimental field of College Orchard, Horticultural College and Research Institute, Coimbatore. Leaf samples collected were stored at -80°C for DNA extraction.

Genomic DNA was isolated from young leaves using Hexadecyl Trimethyl - Ammonium Bromide (CTAB) (Aitchitt *et al*, 1993). Quality and quantity of genomic DNA extracted was determined using gel electrophoresis (0.7% agarose gel). DNA concentration for PCR amplification was estimated by comparing band intensity of a sample with band intensity of known dilutions of Lambda DNA/ *Eco*RI+*Hind*III Marker (Fermentas, #SM0191). Based on the band intensity, DNA was further diluted to required concentration (25-50ng) using double-distilled water.

PCR reaction was performed using five ISSR primers, viz., UBC 807, UBC 808, UBC 810, UBC 817 and UBC 834. PCR reaction was carried out in a total volume of 10µl in 96-tube PCR plates. The Master Mix of solutions for each reaction included 1.0µl of 10X Taq buffer + $MgCl_2$ (15mM), 1.0µl of dNTP (2mM), 1.0µl (0.5µl each for combination) Primers 10µM, 0.1µl of Taq polymerase (3 IU / µl), 4.9µl of sterile double-distilled water and 2µl of Template DNA10ng/µl. Touchdown protocol was followed for all the primers; cycling profile was: initial denaturation at 94°C for 3 min, denaturation at 94°C for 30 Sec (-0.5°C), annealing (19 cycles) at 63°C for 30 Sec, extension at 72°C for 1 min, denaturation at 94°C for 15 Sec, annealing (19 cycles) at 55°C for 30 Sec, extension at 72°C for 1 min, final Extension at 72°C for 10 min, and final hold at 4°C.

Electrophoresis was performed in 1.5% agarose at 120V for 2 hours. PAGE electrophoresis was carried out using an improved staining method, a combination of different steps proposed by Benbouza *et al* (2006).

Data generated for 40 genotypes with five ISSR primers were subjected to statistical analysis. Polymorphic bands were scored visually for presence or absence for each primer. Scores were obtained in the form of a matrix with '1' and '0', which indicates presence or absence of the bands in each genotype, respectively. It is the sum total of polymorphism information content (PIC) values for all the markers generated by a particular primer. PIC value was calculated using the formula $PIC = 1-\Sigma pi^2$, where pi is frequency of the i^{th} allele (Smith *et al*, 1997). Binary data scoring was used for constructing a dendrogram. Genetic association between accessions was evaluated by calculating Jaccard's similarity coefficient for-pair wise comparisons based on the proportion of shared bands produced by primers (Jaccard, 1908). Similarity, the matrix was generated using SIMQUAL programme of NTSYS-pc software, version 2.02 (Rohlf, 2000). These similarity coefficients were used for cluster analysis, and the dendrogram was constructed using Unweighted Pair-Group Method (UPGMA) (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Molecular markers are a useful complement to morphological and physiological characterization of cultivars as these are plentiful, independent of tissue, age or environmental effects, and allow for cultivar identification early in plant development.

Thus, for genetic assessment, molecular markers are often considered advantageous over morphological markers.

Molecular markers based on differences in DNA sequence between individuals generally detect more polymorphisms than morphological and protein based markers, and constitute a new generation of genetic markers (Mignouna *et al*, 1998; Tanksley *et al*, 1989). Amplification of Inter Simple Sequence Repeats (ISSR) is a relatively

recent technique and has proved to be a powerful, rapid, simple, reproducible and inexpensive way for assessing genetic diversity or to identify closely-related cultivars in several species. It is a dominant marker, though occasionally exhibiting codominance. This marker reveals a far larger number of fragments per primer than does RAPD analysis (Bajpai *et al*, 2008).

In the present investigation, 40 F_3 genotypes of Pusa Nanha x *Vasconcellea cauliflora* (25 genotypes) and CP50 x *Vasconcellea cauliflora* (15 genotypes) were used for identifying closely-related progenies, using five ISSR primers. PCR amplification using these five primers in 40 F_3 progenies yielded 53 reproducible amplified bands. The number of bands amplified varied from 5 (ISSR 834) to 16 (ISSR 807x810). Of the 53 bands, 44 were polymorphic (83.02%). Average number of bands and number of polymorphic bands per primer was 10.6 and 8.8, respectively. Polymorphism information content (PIC) values were calculated for ISSR markers to characterize the capacity of each primer for revealing or detecting polymorphic loci among genotypes. PIC value, as a relative measure of level of polymorphism ranged between 0.90 (ISSR 807 x 810) and 0.66 (ISSR 834 x 810). A higher PIC value indicated informativeness of the primer. Among the primers used in our study, two primers, viz., 808 and 807x810, exhibited PIC value ranging from 0.90 to 0.87. These primers can provide a basis for papaya DNA profile system (Table 1).

Presence of 44 polymorphic bands in papaya progenies indicated a presence of genetic polymorphism in these progenies, which may be used in planning hybridization in papaya. Moreover, occurrence of specific bands only in some of the progenies indicates presence of specific loci in the progenies.

Molecular data for 40 F_3 progenies were analyzed using Sequential Hierarchial and Nested (SAHN) clustering methods of NTSYS-pc program version 2.02 (Rohlf, 2000) based on Jaccard's similarity coefficient, with Unweighted Pair Group Method with Arithmetic average (UPGMA). Maximum similarity was observed in genotypes 1, 4 and 6 of Pusa Nanha x *Vasconcellea cauliflora* (0.96). Minimum similarity was observed between genotypes 3 and 14 of CP50 x *Vasconcellea cauliflora* (0.04).

Based on the similarity index, a dendrogram was constructed for 40 F_3 genotypes which grouped into seven clusters at 0.56 coefficients (Fig.1). Cluster I was found to be the largest, with 27 genotypes. Cluster II was the second largest, with four genotypes. Clusters III and IV contained both genotypes and were observed to have close similarity. Clusters V and VII had only one genotype. Cluster VI contained three genotypes. The present study indicates clearly that wider variability was created using the crosses Pusa Nanha x *Vasconcellea cauliflora* and CP50 x *Vasconcellea cauliflora* in F_3 populations. The present study revealed an average genetic similarity of 56% in F_3

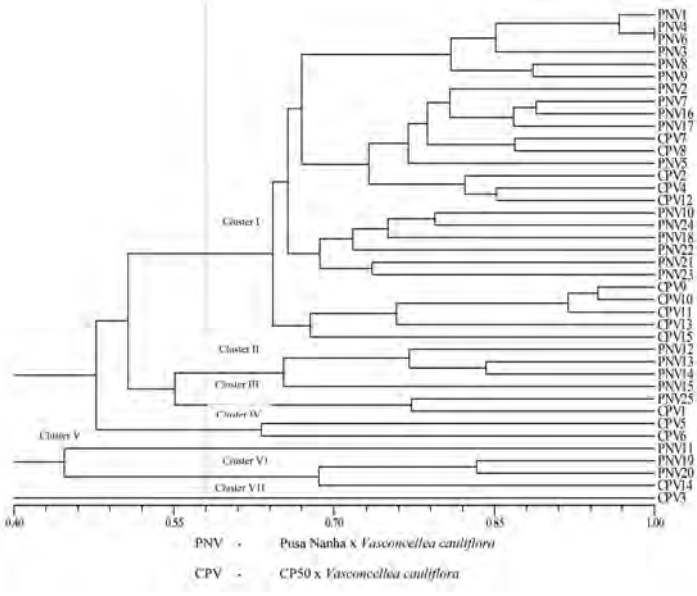

Fig 1. Molecular diversity study among F_3 progenies of Pusa Nanha x *Vasconcellea cauliflora* and CP 50 x *Vasconcellea cauliflora* using ISSR markers

Table 1. Per cent polymorphism and Polymorphic Information Content (PIC) value for ISSR primers

S. No.	ISSR Primer No. (UBC code)	Sequence (5'-3')	Total number of bands	Number of polymorphic bands	PIC value
1	808	AGA GAG AGA GAG AGA GT	15	14	0.87
2	834	AGA GAG AGA GAG AGA GC	5	5	0.78
3	807 vs 810	GAG AGA GAG AGA GAG AT	16	15	0.90
4	834 vs 807	CAC ACA CAC ACA CAC AA	8	4	0.72
5	834 vs 810	AGA GAG AGA GAG AGA GYT	9	6	0.66
Total no. of bands			53	44	-
Average number of bands per primer			10.6	8.8	-

progenies, indicating a presence of greater genetic difference among these populations. Using DNA markers of different nature could help differentiate papaya progenies, and their genetic variation can be evaluated. Molecular data can provide more information and clear discrimination between progenies. In addition, interspecific hybridization showed greater diversity and distinctness, providing information for PRSV resistance breeding programmes for inducing genetic variation in the progeny.

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