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Genetic Variability Studies in Papaya (*Carica papaya* L.)

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ABSTRACT

Papaya is one of the most widely cultivated tropical fruit tree. Pusa Nanha x Solo, Pusa Dwarf x Coorg Honeydew, Pusa Nanha x Coorg Honeydew and Solo x Coorg Honeydew were the hybrids selected for the study . Characterization of papaya hybrids was done using Random Amplified Polymorphic DNA technique, which is quick, reliable and widely applicable. Genomic DNA was successfully isolated from four papaya hybrids using CTAB (Cetyl trimethyl ammonium bromide) method. All the 10 primers yielded amplification products with the DNA of Pusa Nanha x Coorg Honeydew. Primers OPA-03, OPA-04, OPA-12, OPB-04 and OPB-17 gave 40 scorable bands, with an average of 8.0 bands per primer. A genetic similarity matrix was constructed using the Jaccard's Coefficient Method. The pair wise similarity coefficient values varied from 0.389 to 0.714. The least similarity coefficient value (0.389) was that of Pusa Dwarf x Coorg Honeydew with Solo x Coorg Honeydew. The highest value (0.714) for similarity index was obtained for Pusa Nanha x Coorg Honeydew with Pusa Dwarf x Coorg Honeydew followed by Pusa Nanha x Coorg Honeydew – Pusa Nanha x Solo pair (0.658). Results of the trial revealed that the largest cluster in dendrogram was formed by three hybrids namely Pusa Nanha x Coorg Honeydew, Pusa Dwarf x Coorg Honeydew and Pusa Nanha x Solo. The second cluster contained only one hybrid - Solo x Coorg Honeydew. The pair wise similarity coefficient values varied from 0.389 to 0.714. The minimum similarity coefficient detected in the present study was 0.389, suggesting a genetic differentiation among the papaya hybrids.

Key Words : Characterization, DNA , Dendrogram, Papaya, Primer.

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the important delicious fruit crop grown in tropical and subtropical parts of the world. Recently, DNA based molecular markers like Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified fragment length polymorphism (AFLP) have been widely used for genetic characterization, as they are not affected by environmental conditions. Among these RAPD (Random Amplified Polymorphic DNA) marker technique is quick, reliable and widely applicable. It is a Polymerase chain reaction (PCR) based technique for identifying genetic variation. It involves the use of a single arbitrary primer in a Polymerase chain reaction , resulting in the amplification of many discrete DNA products. This procedure detects nucleotide sequence polymorphisms in a DNA amplification-based assay using only a single primer of arbitrary nucleotide . The polymorphisms between individuals result from sequence differences in one or both of the primer binding sites, and are visible as the presence or absence of a particular Random Amplified Polymorphic DNA band. Such polymorphisms thus behave as dominant genetic markers. Random Amplified Polymorphic DNA approach has been applied for the estimation of genetic relationships in many plant species (Matsumoto *et al*, 2010). These markers are decamer DNA fragments from Polymerase chain reaction amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence and able to differentiate between genetically distinct individuals, although not necessarily in a reproducible way. It is used to analyse the genetic

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Table 1. Quality and quantity of DNA isolated from different papaya hybrids using CTAB method.

Papaya hybrid	A_{260}	A_{280}	A_{260}/A_{280}	DNA yield (μg/ml)
Pusa Nanha x Coorg Honeydew	0.028	0.015	1.86	840
Pusa Dwarf x Coorg Honeydew	0.025	0.014	1.78	750
Solo x Coorg Honeydew	0.018	0.010	1.80	540
Pusa Nanha x Solo	0.020	0.011	1.80	600

diversity of an individual by using random primers. Random Amplified Polymorphic DNA requires only one primer for amplification. Unlike traditional Polymerase chain reaction analysis, Random Amplified Polymorphic DNA does not require any specific knowledge of the DNA sequence of the target organism: the identical 10-mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the primers' sequence. The present study was initiated for the characterization of papaya hybrids using Random Amplified Polymorphic DNA technique.

MATERIALS AND METHODS

Papaya hybrids used for the study were Pusa Nanha x Solo, Pusa Dwarf x Coorg Honeydew, Pusa Nanha x Coorg Honeydew and Solo x Coorg Honeydew. The quality of DNA was assessed from the ratio of the OD (optical density) values recorded at 260 nm and 280 nm. Agarose gel electrophoresis was carried out in horizontal gel electrophoresis unit. Agarose concentration used was 0.8 per cent for visualizing genomic DNA and 1.4 per cent for visualizing amplified products.

Random Amplified Polymorphic DNA analysis was performed using the selected primers to amplify the DNA of all the four papaya hybrids . Photographs of the amplification profile obtained were taken with the help of gel documentation system. The Random Amplified Polymorphic DNA bands were represented as '+' (plus) for presence and '-' (minus) for absence and recorded Polymerase chain reaction was repeated twice inorder to assess the reproducibility. Reproducible bands were scored for the presence (+) or absence (-) for all the four papaya hybrids. A genetic similarity matrix was

Table 2. Primer associated banding patterns with the DNA of papaya hybrid Pusa Nanha x Coorg Honeydew using 10 primers.

Sr. No.	Primers	Number of faint bands	Number of intense bands	Total number of bands
1	OPA-03	3	6	9
2	OPA-04	2	2	4
3	OPA-12	7	2	9
4	OPA-13	0	1	1
5	OPA-14	1	1	2
6	OPA-15	1	2	3
7	OPB-04	2	2	4
8	OPB-08	0	1	1
9	OPB-10	1	1	1
10	OPB-17	2	3	5

constructed using the Jaccard's coefficient method. Based on the similarity coefficient a dendrogram was constructed by following the nearest neighbour (single link) method. Association between the hybrids was obtained from the dendrogram.

RESULTS AND DISCUSSION

DNA yield of different papaya hybrids ranged from 540 µg/ml (Solo x Coorg Honeydew) to 840 µg/ml (Pusa Nanha x Coorg Honeydew) (Table1). The ratio of A_{260}/A_{280} ranged from 1.78 (Pusa Dwarf x Coorg Honeydew) to 1.86 (Pusa Nanha x Coorg Honeydew). Jaime *et al* (2007) observed that the yield of DNA of different papaya varieties ranged from 600 µg/ml to 8035 µg/ml and purity ratio of 1.65 to 2.2.

The Polymerase chain reaction amplification was carried out using ten primers of Kit A and Kit B with the DNA of papaya hybrid Pusa Nanha x Coorg Honeydew. All the 10 primers yielded amplification products with the DNA of Pusa Nanha x Coorg Honeydew. Total number of bands, number of faint bands and number of intense bands produced by primers were recorded (Table 2).

Total of 39 bands (average 3.9 bands per primer) were generated, of which 36 bands (92.31 %) were polymorphic. This accounts to an average of 3.6 bands per primer. Monomorphic bands were produced by primers OPA-13, OPB-8 and OPB-10. The highest number of Random Amplified Polymorphic DNAs (9 each) were produced by the primers OPA-3 and OPA-12 . Of these primers, the highest number of intense bands (6 bands) was produced by OPA-3. Total of five bands was produced by OPB-17. The primers OPA-4 and OPB-4 produced four bands each. The primers OPA-15 and OPA-14 produced three and two bands respectively.

Five primers which produced the highest number of bands as well as the highest number of intense bands were selected for amplifying DNA from four papaya hybrids. Muthulakshmi *et al* (2007) obtained 40 scorable bands with an average of ten bands per primer in papaya. OPA-12, OPB-04, OPB-17) yielded 40 scorable bands with an average of 8.0 bands per primer. The number of bands resolved per amplification was primer dependent and varied from four to twelve .

The hybrids Solo x Coorg Honeydew and Pusa Nanha x Solo yielded three specific bands, which was absent in other hybrids with primer OPA-04. Using primer OPA-12 papaya hybrid Solo x Coorg Honeydew yielded one specific band which is absent in other hybrids. With primer OPB-17, papaya hybrid Pusa Nanha x Coorg Honeydew yielded three specific bands, which was absent in all other papaya hybrids selected for the present study.

A genetic similarity matrix was constructed using the Jaccard's Coefficient Method (Table 3). The pair wise similarity coefficient values varied from 0.389 to 0.714. Madarbokus *et al* (2012) reported pair wise similarity coefficient values in papaya ranged from 0.4 to 0.833. The least similarity

Table 3. Similarity matrix of four papaya hybrids based on Jacquard's similarity index.

Pusa Nanha x Coorg Honeydew	1.000			
Pusa Dwarf x Coorg Honeydew	0.714	1.000		
Solo x Coorg Honeydew	0.459	0.389	1.000	
Pusa Nanha x Solo	0.658	0.639	0.514	1.000
	Pusa Nanha x Coorg Honeydew	Pusa Dwarf x Coorg Honeydew	Solo x Coorg Honeydew	Pusa Nanha x Solo

coefficient value (0.389) was that of Pusa Dwarf x Coorg Honeydew with Solo x Coorg Honeydew. The highest value (0.714) for similarity index was obtained for Pusa Nanha x Coorg Honeydew with Pusa Dwarf x Coorg Honeydew followed by Pusa Nanha x Coorg Honeydew – Pusa Nanha x Solo pair (0.658).

Based on similarity coefficient, a dendrogram was constructed. In the dendrogram along the point corresponding to the similarity coefficient 0.58, all the four hybrids were grouped into two clusters. Cluster one contained three hybrids – Pusa Nanha x Coorg Honeydew, Pusa Dwarf x Coorg Honeydew and Pusa Nanha x Solo. Cluster two contained the hybrid Solo x Coorg Honeydew alone. The result of the present experiment was in conformity with findings of Niu *et al* (2011), who applied Random Amplified Polymorphic DNA to analyse the relationship among ten cultivars of *Carica papaya* and he grouped seven out of the 10 cultivars to one cluster of dendrogram.

CONCLUSION

Genomic DNA was successfully isolated from four papaya hybrids using CTAB method. DNA yield of different papaya hybrids ranged from 540 µg/ml (Solo x Coorg Honeydew) to 840 µg/ml (Pusa Nanha x Coorg Honeydew) . The ratio of A_{260}/A_{280} ranged from 1.78 (Pusa Dwarf x Coorg Honeydew) to 1.86 (Pusa Nanha x Coorg Honeydew). Agarose gel electrophoresis was used for analyzing the genomic DNA isolated from different papaya hybrids as well as for the Random Amplified Polymorphic DNA analysis. Agarose 0.8 per cent was found optimum for genomic DNA and 1.4 per cent for RAPD analysis. Primers OPA-03, OPA-04, OPA-12, OPB-04 and OPB-17 gave 40 scorable bands, with an average of 8.0 bands per primer. The highest number of Random Amplified Polymorphic DNAs (9 each) were produced by the primers OPA-3 and OPA-12. A genetic similarity matrix was constructed using the Jaccard's Coefficient Method. The pair wise similarity coefficient values varied from 0.389 to 0.714 The least similarity coefficient value (0.389) was that of Pusa Dwarf x Coorg Honeydew with Solo x Coorg Honeydew. The highest value (0.714) for similarity index was obtained for Pusa Nanha x Coorg Honeydew with Pusa Dwarf x Coorg Honeydew followed by Pusa Nanha x Coorg Honeydew – Pusa Nanha x Solo pair (0.658). The largest cluster in dendrogram was formed by three hybrids - Pusa Nanha x Coorg Honeydew, Pusa Dwarf x Coorg Honeydew and Pusa Nanha x Solo. The second cluster contained only one hybrid - Solo x Coorg Honeydew. The minimum similarity coefficient detected in the present study was 0.389, suggesting a genetic differentiation among the papaya hybrids

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