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The Study of Genetic Diversity and Relationships on *Carica* sp. by Means of Random Amplified Polymorphic DNA (RAPD) Analysis

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ABSTRACT

The genus of *Carica* comprises 21 species, among them three species have been domesticated and cultivated. They are *Carica papaya, Carica candamarcencis* and *Carica monoica*. In Indonesia, two species have been cultivated viz. *Carica papaya* and *Carica candamarcencis*. In this study, four accessions of *Carica papaya* species and one accession of *Carica candamarcencis* species were analyzed using RAPD technique. We successfully amplified a total of 40 fragments from these DNA genome by using 6 random primers with GC bases content ≥ 60% of each primer. The number of fragments of each primer ranged from 5 to 8 averaged 6.7 fragments per primer. Out of total fragments, 90% showed as polymorphic ranged from 5 to 8 on average 6 fragments per primer. A dendogram based on the UPGMA-link method using Nei and Li similarity and Principal Component Analysis (PCA) plot separated the accessions into two main groups, the *Carica papaya* species on the one side and the *Carica candamarcencis* species on the other side. The result demonstrated that RAPD analysis was able to reveal genetic difference between *Carica papaya* and *Carica candamarcencis* species, as well as genetic diversity in *Carica papaya* species.

Key words: Carica papaya, Carica candamarcencis, Random Amplified Polymorphic DNA (RAPD), Genetic diversity

INTRODUCTION

The genus *Carica* comprise 21 species, and among them, three species have been domesticated and cultivated. Those are viz. *Carica papaya* L., *Carica candamarcensis* Hook and *Carica monoica* (Nakasone and Paull, 1999). Among the three species, only the common papaya, *Carica papaya* L. has high economic value.

Indonesia is very rich in *Carica* germplasm, spreading to almost all over Indonesian archipelago and cultivated as landraces (Pusat Kajian Buah-buahan Tropika, 2000), however, information on the characterization of *Carica* is lack behind. The characterization and quantification of genetic diversity have long been a major goal in evolutionary biology. In plant breeding programs, information on genetic diversity within and among closely related crops species is essential for rational use of genetic resources and particularly useful in characterizing individual accessions and cultivars, for detecting duplications of genetic materials in germplasm collections, and as a general guide in parent's assessment for crop improvement programs as well as developing informative mapping populations for genome mapping.

Molecular markers provide a quick and reliable method for estimating genetic relationships among genotypes of any organism (Thormann *et al.*, 1994). Random amplified polymorphic DNA (RAPD) analysis (Williams *et al.*, 1990) has been used for diversity analysis in a vast array of crops, widely used for the determination of genotypes (Hasizume *et al.*, 1993), gene mapping (Ohmori et al., 1996) and QTL analysis (Grandillo and Tanskley, 1996). This approach is based on the polymerase chain reaction (PCR) (Saiki *et al.*, 1988) amplification of template DNA genome using short, synthetic deoxyribonucleotides of random sequence as primers. Each primer can direct the amplification of several unrelated regions of the genome (Sondur *et al.*, 1996). The resolving power of RAPD technique is several folds higher than visual and protein markers and is much simpler and technically less demanding than RFLP and other similar techniques (Williams *et al.*, 1990).

In this study, we employed RAPD analysis to analyse genetic diversity and relationships between

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Carica papaya species and *Carica candamarcencis* species, and among genotypes of *Carica papaya* species.

MATERIALS AND METHODS

Plant Materials

Four accessions of *Carica papaya* species (GM, NM203, KD and DTM) and one accession of *Carica candamarcencis* species (Dieng) were used in this study. The accessions are part of Center for Tropical Fruit Studies germplasm collection.

DNA Isolation

Total DNA from each accession was isolated using CTAB method (Doyle and Doyle, 1987) with slight modification. Three hundred mg of leave tissue was frozen in liquid nitrogen and ground into a fine powder using a mortar and pestle. The pulverized materials were transferred to a microtube and 600 µl of extraction buffer [100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1400 mM NaCl, 2% (w/v) cetyl trimethylammonium bromide (CTAB), and 0.2% (v/v) mercaptoethanol] solution was added. The tubes were vortexed for a few seconds and incubated at 60 ^{0}C for 20 minutes. Following incubation, 570 µl of chloroform: isoamylalcohol (24:1) (v/v) was added and mixture was shaken vigorously. The extracts were centrifuged for 15 min at 12.000 rpm. The supernatant transferred to a fresh microtube and then 600 µl of cold isopropanol was added. DNA fibers became visible upon gentle swirling. The DNA was transferred to a fresh tube, rinsed with 70% ethanol and dissolved in 50-100 µl of sterilized deionized water. DNA concentration was measured with UV-spectrophotometer (Shimadzu Corporation, Japan) at wavelengths of 260 and 280 nm.

RAPD Analysis

Six random primers of 10 bases in length with GC base content $\geq$ 60% of each primer was selected: 3 from SB series, 2 from OPH (Operon Technologies, Inc.) series and 1 from OPM (Operon Technologies, Inc.) series (Table 1). PCR reactions were carried out in a 25 µl reaction mix containing approximately 25-50 ng template DNA, 10X PCR buffer (100 mM Tris-HCl, pH 9.0 at 25 °C, 500 mM KCl, 1.0 Triton X-100), 2 mM $MgCl_2$, 0.2 mM of each dATP, dCTP, dGTP and dTTP, 0.4 pmol of a single 10-base primer, and 1 unit of Taq DNA polymerase (Gene Amp). Amplification was performed in Perkin Elmer thermal cycler programmed for 45 cycles of each of the following: 95 ^{0}C for 1 min, 35 ^{0}C for 1 min, and 72 ^{0}C for 2 min. A final elongation step of 5 min at 72 ^{0}C was included. Reaction products were mixed with 2.5 µl of loading dye (0.25% bromphenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a microfuge before loading (Sambrook *et al.*, 1989), and then separated on 6% polyacrylamide gel by adding 7 M urea (17.9% (v/v) acrylamid 40% (38% acrylamid, 2% N, N, methylenediacrylamid, w/v), 7.5% (v/v) 10X TBE, 1% (v/v) ammonium persulphate 10%, 0.1% (v/v) TEMED (N, N, N', N'-tetramethylethylenediamine) and 42% (w/v) urea) at 60 V for 5 h in TBE buffer (1 M Tris-HCl, 0.83 M boric acid, 10 mM EDTA, pH 8.3) and stained with ethidium bromide, and the gels were illuminated under UV light.

Data Analysis

Each accession product was considered to be a unit character, and scored for the presence (1) or absence (0) of a product. Genetic similarity (F) between all pairs of accessions were calculated according to Nei and Li (1979): F=2Nab/(Na+Nb), where Nab=number of bands common in a and b, Na=number of bands present in a, Nb=number of bands present in b. The similarity indices were converted into dissimilarity (D=1-F). The resulting dissimilarity matrix was subjected to hierarchical cluster analysis using the Unweighted Pair Group Method and Arithmetic Average (UPGMA) and was done by using computer program NTSYS-pc, version 1.80 (Exeter software, New York). A principal component analysis (PCA) subjected to separate among accessions and to determine the relationships based on the diversity and was conducted using computer program MINITAB release 11.12.

RESULTS AND DISCUSSIONS

Results

A total of 40 fragments were successfully generated using 6 random primers with GC bases content $\geq$ 60% of each primer. The fragment number of each primer ranging from 5 to 8, on average 6.7 fragments per primer (Table 1). Out of total fragments, 90% showed as polymorphic with ranged from 5 to 8, on average was 6 fragments per primer (Table 1).

Table 1. Sequence of RAPD primers used and number of fragment amplification products

Primer	Oligonucleotide Sequence (5' to 3')	No. of total fragments	No. of polymorphic fragments
SB-05	GGGTAACGCC	7	5
SB-06	GTCCGAACCC	7	7
SB-18	TCGGCGATAG	6	6
OPM-06	CTGGGCAACT	8	8
OPH-18	GAATCGGCCA	7	5
OPH-20	GGGAGACATC	5	5

A dendogram based on the UPGMA-link method using Nei and Li similarity (1979) and principal component analysis (PCA) plot were performed to separate and examine the relationships among the accessions (Figure 1 and Figure 2). The accessions separated into two main groups. *Carica papaya* (GM, NM203, KD and DTM) species were in one cluster and *Carica candamarcencis* (Dieng) in other group. These results indicate that *Carica papaya* and *Carica candamarcencis* have different genetic characters. KD and DTM accessions were the closest, sharing as many as 14 identical banding values. The longest distance was observed between NM203 and Dieng accessions that differed at seven loci. Among *Carica papaya* accessions, KD and DTM accessions were in one group, NM203 and GM accessions in other group.

Discussions.

The results demonstrate that RAPD analysis was able to differentiate between species and could be employed to reveal genetic diversity among individual cultivars in one species. Stiles et al. (1993), indicated that RAPD could be used to establish the relationships among closely related cultivars and even individuals in at least some cultivars in papaya. Both results show that RAPD analysis was a powerful tool technique to elucidate genetic diversity between *Carica papaya* and *Carica candamarcencis* as well as among genotypes of *Carica papaya*.

A dendogram based on UPGMA-link method using Nei and Li similarity (1979) and principal component analysis plot based on data produced from 6 primers separated the accessions into two main groups (Figure 1 and Figure 2). GM, NM203, KD and DTM accessions on the one side and Dieng accession on the other side. These results indicate that the accessions from one species have closer relationships if compared to other species.

Dieng accession is a *Carica candamarcencis* species which has different characters from *Carica papaya* species. The Dieng ripe fruits have a firm flesh, low acidity, fragrant and the cavity contains numerous seeds covered with a whitish juicy sarcotesta. This study revealed different DNA genome between *Carica papaya* species and *Carica candamarcencis* species, nevertheless, also revealed the same banding pattern presence on both species which indicated presence of genetic relationship.

Among the *Carica papaya* species group, KD and DTM accessions show closest genetic distance. Both accessions were collected from same region (Bogor), however, they show several differences in morphological characters. Compared with KD and DTM accessions, the NM203 accession has distant genetic distance. This result could be proved based on origin and the morphological characters that differentiate among the accessions. NM203 accession come from Banten and has a purple stem, petiole and peduncle. The relationship between NM203 and DTM accessions was closer than NM203 and KD accessions, since more monomorphic banding pattern were observed between NM203 and DTM accessions (Figure 3).

This study also revealed that GM accession has the farest genetic distance from other *Carica papaya* accessions. This difference could be distinctly on the morphological characters, and predictly caused by different genetic background. GM accession is a S6 inbred line variety with green stem, petiole and peduncle and has a broadly toothed leaf. As a S6 inbred line variety, GM accession already has characters with high purity genetic structure and uncontaminated by another alleles from another varieties. Whereas, the other three accessions are open pollinated genotype that have an alleles contaminated probability by other genotypes. The genetic diversity on *Carica papaya* is important genetic resources, that offer possibility to be used in the future *Carica* improvement and breeding program.

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