A genetic linkage map of papaya based on randomly amplified polymorphic DNA markers

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Abstract A genetic linkage map of papaya (Carica papaya L.) was constructed using randomly amplified polymorphic DNA (RAPD) markers and a F2 population derived from a University of Hawaii UH breeding line 356 × ‘Sunrise’ cross. A total of 596 10-mer primers were screened, and 96 polymorphisms were detected. At LOD 4.0, 62 of these markers mapped to 11 linkage groups comprising 999.3 cM. About 80% of the markers conformed to expected Mendelian segregation ratios. We have mapped the locus that determines sex to a 14-cM region flanked by RAPD markers. The results demonstrate the usefulness of RAPD markers for developing a basic genetic linkage map in papaya.

Key words Carica papaya · RAPD · Sex-determination · Linkage map · Hermaphrodite

Introduction

Papaya (Carica papaya L.) is a popular fruit crop in Hawaii and other tropical regions. C. papaya is a polygamous species with both unisexual and bisexual tree types, although hermaphrodite plants are preferred for commercial cultivation. Sex expression and fruit development are greatly influenced by environmental (Awada 1958; Awada and Ikeda 1957) as well as genetic factors (Storey 1976). Early attempts to identify markers that co-inherit with sex led to discovery of a loose linkage between sex and flower and petiole color (Hofmeyr 1939). This is the only previous report involving genetic markers in papaya.

Development of a detailed linkage map for papaya will enhance our understanding of papaya genetics and improve the efficiency of crop improvement programs, especially those involving quantitative traits. Also, the simple segregation of sex forms in cultivated papaya provides considerable leverage for locating the factor involved in sex determination and investigating its mode of action.

The discovery of DNA-based genetic markers, initially as restriction fragment length polymorphisms (RFLP) (Grodziker et al. 1974), provides a tool that offers a potentially unlimited number of genetic markers (Helentjaris et al. 1985) that can be used to map and characterize entire genomes (Botstein et al. 1980). Within a span of 10 years, RFLP-based linkage maps have been constructed for several economically important crops including maize (Helentjaris et al. 1986), tomato (Bernatzky and Tanksley 1986), lettuce (Landry et al. 1987), potato (Gebhardt et al. 1989), rice (McCouch et al. 1988) and soybean (Tingey et al. 1989). The utility of a saturated linkage map for understanding the complex nature of the inheritance of quantitative traits has already been demonstrated in tomato (Paterson et al. 1988).

Recently, a new method for producing DNA polymorphisms, randomly amplified polymorphic DNA (RAPD), has been developed (Williams et al. 1990; Welsh and McClelland 1990). This approach is based on the polymerase chain reaction (PCR) (Saiki et al. 1988) amplification of template DNA using short, synthetic deoxyribonucleotides of random sequence as primers. Each primer can direct the amplification of several unrelated regions of the genome. RAPD technology is much faster and requires fewer resources than RFLP technology. An added advantage of RAPD is its ability to detect more polymorphism than RFLP analysis (Williams et al. 1990; Foolad et al. 1993). This is especially important in papaya where genetic variability in the available breeding lines is limited (Stiles et al. 1993). Mendelian segregation of RAPD mark-
Fig. 1 Segregation of RAPD marker OPO10 in the F₂ population. An ethidium bromide-stained 1.5% agarose gel showing separation of the amplified fragments from the RAPD/PCR reaction run as described in the methods.

Figures have been demonstrated in soybean (Williams et al. 1990), conifers (Carlson et al. 1991) and alfalfa (Echt et al. 1992). RAPD-based linkage maps are available in pine (Chaparro et al. 1992), Arabidopsis (Reiter et al. 1992) and faba bean (Torres et al. 1993). The major limitation of RAPD technology is that most markers are dominant as opposed to the codominant nature of RFLP markers. Despite this disadvantage, due to its speed and ease, RAPD analysis has found applications in population studies (Welsh et al. 1991; Hu and Quiros 1991), biosystematics (Stiles et al. 1993), gene tagging (Klein-Lankhorst et al. 1991; Martin et al. 1991) and especially genetic mapping.

In this report we present a genetic linkage map for papaya based on RAPD markers as a first step towards understanding the papaya genome. We also investigated the genetics of sex determination in papaya.

Materials and methods

Mapping population

The segregating F₂ population of a cross between the Hawaiian cultivar ‘Sunrise’ and UH breeding line 356, derived from an introduction from Florida, was used for the present study. Two populations from the same cross were grown; one at the University of Hawaii Poamoho Experiment Station (153 plants) and the other at the Waimanalo Experiment Station (100 plants). ‘Sunrise’, inbred for over 25 generations, was used as the male parent and UH breeding line 356, derived from the third sib-mated generation, was used as the female parent (Zee 1985). Morphologically, the parents are distinct.

DNA isolation

DNA extraction, purification and quantification was performed as previously described (Stiles et al. 1993). DNA was extracted from young leaves, generally once from each plant unless additional DNA was required. A total of 596 decamer primers were used for PCR amplification. Five hundred primers were obtained from Operon Technologies (Alameda, Calif.) (kits A through Y), and 96 primers were synthesized at the University of Hawaii Biotechnology Instrumentation Facility. Each potential polymorphism was confirmed at least three times. Ninety-six polymorphisms were scored in the F₂ population.

DNA amplification

The PCR procedure described by Williams et al. (1990) was followed with minor modifications. Amplification reactions were carried out in 25 µl containing 0.2 µM primer, 150 µM of each deoxyribonucleotide triphosphate, 2 mM MgCl₂, 10 mM TRIS-HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 15–25 ng template DNA and 0.75–1.25 U Taq DNA polymerase. Reaction conditions consisted of 45 cycles of 1 min at 95°C, 1 min at 35°C and 2 min at 72°C in a Coy model 50/60 thermocycler. The PCR reaction was concluded by a 5-min extension at 72°C. Products were separated by electrophoresis at 50 V for 6–8 h in 1.5% agarose gels, stained with ethidium bromide and photographed under UV light. Figure 1 shows the results of a typical gel. Lanes containing DNA from specific plants were scored for presence or absence of segregating bands. Absence of a band was confirmed by repetition.

Data analysis

Goodness-of-fit to the expected segregation of 3:1 (dominant), 2:1 (sex) or 1:2:1 (codominant) for the F₂ population was tested by chi-square analysis. The linkage map was constructed using MAPMAK-