Theor Appl Genet (1996) 93: 547–553

S. N. Sondur · R. M. Manshardt · J. I. Stiles

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

Received: 8 March 1995 / Accepted: 26 January 1996

Abstract A genetic linkage map of papaya (*Carica papaya* L.) was constructed using randomly amplified polymorphic DNA (RAPD) markers and a F_2 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 then 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-

Journal series No. 4146 of the Hawaii Institute of Tropical Agriculture and Human Resources

Communicated by G. E. Hart

S. N. Sondur · R. M. Manshardt
Department of Horticulture,
College of Tropical Agriculture and Human Resources,
University of Hawaii, Honolulu, Hawaii, 96822, USA

S. N. Sondur · J. I. Stiles (✉)
Department of Plant Molecular Physiology,
College of Tropical Agriculture and Human Resources,
University of Hawaii, Manoa, Honolulu, Hawaii, 96822, USA

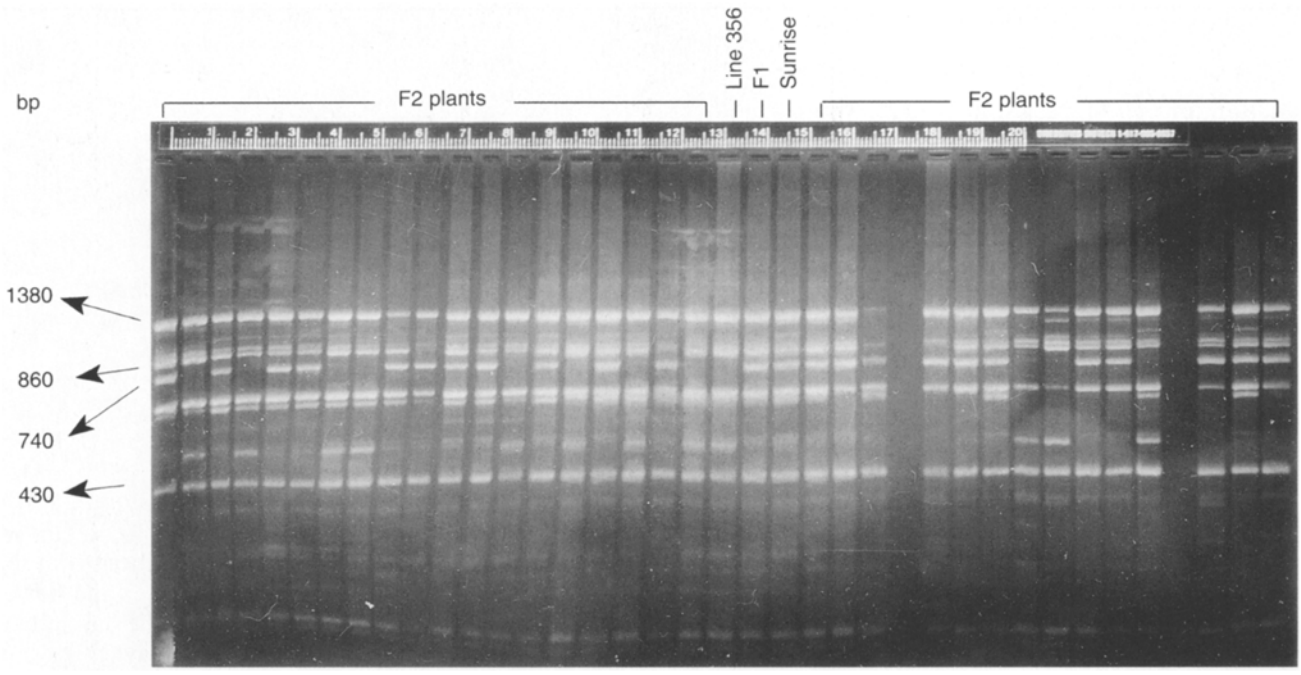

Fig. 1 Segregation of RAPD marker OPO10 in the F_2 population. An ethidium bromide-stained 1.5% agarose gel showing separation of the amplified fragments from the RAPD/PCR reaction run as described in the methods

ers has 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 F_2 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. 'Sunrise' is a gynodioecious, tall, late-bearing commercial cultivar; UH breeding line 356 is a dioecious, semi-dwarf, early-bearing selection. The gynodioecious F_2 population segregated for pistillate and hermaphrodite plants that are readily distinguishable on the basis of floral reproductive morphology.

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 F_2 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 µ*M* primer, 150 µM of each deoxyribonucleotide triphosphate, 2 m*M* $MgCl_2$, 10 m*M* TRIS-HCl (pH 8.3), 50 m*M* 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 F_2 population was tested by chi-square analysis. The linkage map was constructed using MAPMAK-

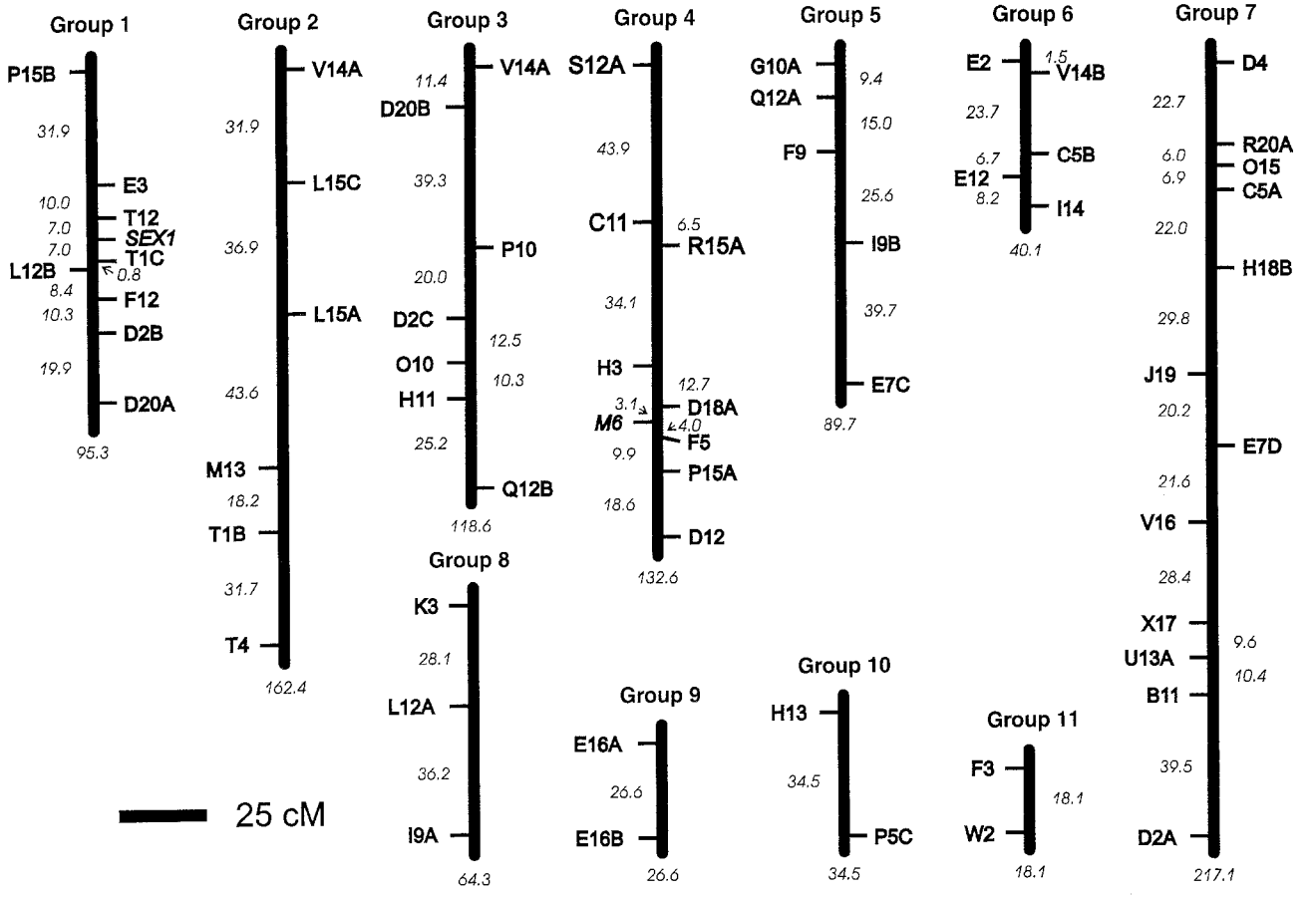

Fig. 2 Genetic linkage map of papaya. Genetic markers in *italics* are co-dominant (*SEX1* and *M6*). Markers to the *left* are dominant in UH breeding line 356; markers to the *right* are dominant in 'Sunrise'; *numbers along* the linkage groups indicate marker distance in cM; *numbers below* linkage groups indicate total size of the linkage group. The markers are the same as those in Tables 1 and 2 except that the OP or UH designation has been omitted

ER/EXP (version 3.0) (Lander et al. 1987). The approach used was that described by Lincoln et al. (1993). Polymorphic markers were grouped at LOD 4.0 and a recombination frequency (r) of 0.35. Within a group, a LOD threshold of 2.0 was used to order the markers using the MAPMAKER 'order' command. Marker order was confirmed using the 'ripple' command. The markers in a group with a distance less than 35 cM were ordered first, and the remaining markers in that group (with r=0.35 to 0.45) were then mapped with a minimum threshold of LOD 1.5.

Since most RAPD markers are dominant and thus provide less information per individual (especially in repulsion phase), two separate maps (not shown) with markers in coupling phase were constructed to confirm the linear order of the markers in the combined map shown in Fig. 2. The map distances were reported in centiMorgans (cM) using the Haldane correction.

Because of the unusual situation of linkage group 1, where the dominant homozygous class (SEX1-H/SEX1-H) is missing, the mapping data for markers tightly linked to SEX1 was reexamined, and q was calculated with an adjustment for the missing class. Figure 3 shows the expected genotypes and 'RAPD phenotype' for RAPD markers in coupling phase with SEX1-H. Above each gamete class is the probability of that class, either (1-q)/2 for parental gametes or q/2 for recombinant gametes. The probability of each class is the product of the probability of the gametes making up that class. The total probability for the apparent lethal classes (shaded in Fig. 3) is:

$[(1-\theta)/2]^2 + 2(\theta/2)[(1-\theta)/2] + (\theta/2)^2 = 1/4$

The probabilities of the recombinant classes are:

RAPD marker +, female plant

$\{2[(1-\theta)/2](\theta/2) + (\theta/2)^2\} \div 3/4 = 2/3\theta + 1/3\theta^2$

RAPD marker –, hermaphrodite plant

$\{2(\theta/2)[(1-\theta)/2]\} \div 3/4 = 2/3\theta - 2/3\theta^2$

Total recombinants $4/3\theta - \theta^2$

The probabilities of the parental classes are:

RAPD marker –, female plant

$\{[(1-\theta)/2]^2\} \div 3/4 = 1/3 - 2/3\theta + 1/3\theta^2$

RAPD marker +, hermaphrodite plant

$\{2[(1-\theta)/2](\theta/2) + 2[(1-\theta)/2]^2 + 2(\theta/2)^2\} \div 3/4 = 2/3 - 2/3\theta + 2/3\theta^2$

Results

Polymorphisms

The parents, 'Sunrise' and UH breeding line 356, were screened with 596 10-base primers. An average of eight major fragments, ranging from 200 bp to 2500 bp in size, were amplified per primer, although about 15% of the primers did not give any amplification product. Sixty of the primers detected a total of 96 polymorphisms of which 61 satisfied the mapping criteria of linkage at LOD 4.0 or greater in the F_2 mapping population (Table 1). Only 1 of the 96 polymorphisms (OPM6) was inherited in codominant fashion. Sixty percent of the polymorphic markers gave a single polymorphism per primer, 37% gave 2 poly-

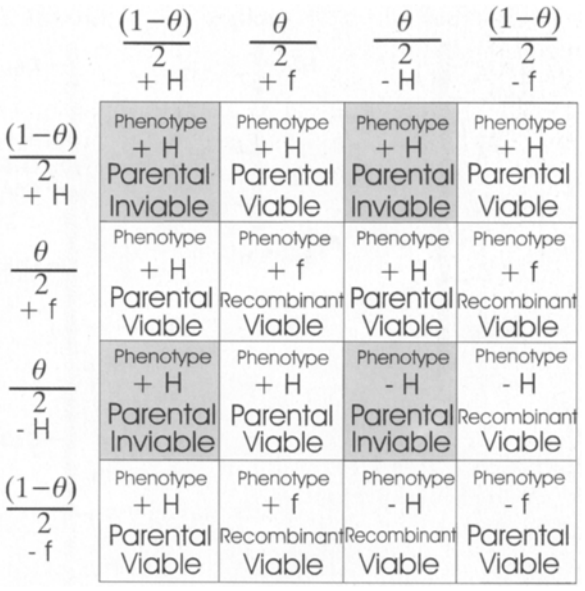

Fig. 3 Phenotype of individuals in the F_2 population. The phenotype, recombination status and viability of various classes from a self of the F_1. *Shaded* classes are inviable. The probability of each gamete, either $(1-\theta)/2$ for parental gametes or $\theta/2$ for recombinant gametes, is given with each gamete class

Table 1 Sequence of primers from the 5′ prime end

Name	Sequence	Name	Sequence
UHC5	'GAGTTCCGCA'	UHC11	'AGCAAAGGCC'
UHD4	'TTGGGACAGT'	UHD12	'TGGCGTTGCT'
UHE12	'ACCATCCCCA'	UHF3	'ATCTGTGTGG'
UHF5	'CACAGGTTCT'	UHF9	'GCATCTCAGT'
UHG12	'CCCTAGCTGT'	UHH11	'TTAGGGCCTC'
OPB11	'GTAGACCCGT'	OPD2	'GGACCCAACC'
OPD18	'GAGAGCCAAC'	OPD20	'ACCCGGTCAC'
OPE2	'GGTGCGGGAA'	OPE7	'AGATGCAGGCC'
OPE16	'GGTGACTGTG'	OPF12	'ACGGTACCAG'
OPG10	'AGGGCCGTCT'	OPH3	'AGACGTCCAC'
OPH13	'GACGCCACAC'	OPH18	'GAATCGGCCA'
OPI9	'TGGAGAGCAG'	OPI14	'TGACGGCGGT'
OPJ19	'GGACACCACT'	OPK3	'CCAGCTTAGG'
OPL12	'GGGCGGTACT'	OPL15	'AAGAGAGGGG'
OPM6	'CTGGGCAACT'	OPM13	'GGTGGTCAAG'
OPO10	'TCAGAGCGCC'	OPO15	'TGGCGTCCTT'
OPP15	'GGAAGCCAAC'	OPP5	'CCCCGGTAAC'
OPQ12	'AGTAGGGCAC'	OPR15	'GGACAACGAG'
OPR20	'ACGGCAAGGA'	OPS12	'CTGGGTGAGT'
OPT1	'GGGCCACTCA'	OPT4	'CACAGAGGGA'
OPT12	'GGGTGTGTAG'	OPU13	'GGCTGGTTCC'
OPV14	'ACCCCCTGAA'	OPV16	'ACACCCCACA'
OPW2	'ACCCGCCAA'	OPX17	'GAGACGGACC'

morphisms per primer, and 3% gave 3 polymorphisms per primer. Three polymorphic markers did not segregate in the F_2 population (i.e. were present in all F_2 plants). Two of these markers (OPA4 and UHE5) were dominant in the maternal parent, the UH breeding line 356, and 1 (OPL8) was dominant in the paternal parent 'Sunrise'.

Segregation analysis

Chi-square analysis was performed to check for goodness-of-fit to the expected Mendelian segregation (Table 2). Inheritance of sex and 48 RAPD markers showed the expected Mendelian segregation in the F_2 population. Of the 13 markers that did not follow the expected segregation 8 exhibited strong deviations from expected ratios (significant at $P<0.01$).

Table 2 Segregation of RAPD markers in F_2 population[a]

Primer	Parent	Present	Absent	Expected ratio	Chi-square
UHC5A	SR	183	62	3 : 1	0.00
UHC5B	SR	189	54	3 : 1	0.85
UHC11	356	187	60	3 : 1	0.03
UHD4	SR	176	70	3 : 1	1.39
UHD12	SR	163	35	3 : 1	5.27*
UHE12	356	196	46	3 : 1	4.36*
UHF3	SR	183	63	3 : 1	0.02
UHF5	SR	183	62	3 : 1	0.00
UHF9	356	176	66	3 : 1	0.55
UHG12	356	195	57	3 : 1	0.64
UHH11	356	176	74	3 : 1	2.58
OPB11	356	140	37	3 : 1	1.07
OPD2A	356	156	97	3 : 1	23.29**
OPD2B	SR	187	60	3 : 1	0.03
OPD2C	356	176	72	3 : 1	1.93
OPD18A	SR	160	80	3 : 1	8.44**
OPD20A	SR	161	29	3 : 1	9.09**
OPD20B	356	128	57	3 : 1	3.02
OPE2	356	146	43	3 : 1	0.38
OPE3	SR	141	59	3 : 1	1.95
OPE7C	SR	157	30	3 : 1	7.53**
OPE7D	SR	125	40	3 : 1	0.03
OPE16A	356	134	109	3 : 1	49.94**
OPE16B	356	183	60	3 : 1	0.00
OPF12	SR	140	46	3 : 1	0.00
OPG10A	356	198	50	3 : 1	2.84
OPH3	356	198	51	3 : 1	2.36
OPH13	SR	192	56	3 : 1	0.64
OPH18B	SR	162	86	3 : 1	11.86**
OPI9A	356	187	58	3 : 1	0.16
OPI9B	SR	200	45	3 : 1	5.93*
OPI14	SR	187	62	3 : 1	0.01
OPJ9	356	196	50	3 : 1	2.61
OPK3	356	139	55	3 : 1	0.90
OPL12A	356	150	43	3 : 1	0.61
OPL12B	356	177	10	3 : 1[b]	37.46**
OPL15A	SR	116	78	3 : 1	23.12**
OPL15C	SR	143	42	3 : 1	0.40
OPM6	Codominant	43 95	58	1 : 2 : 1	2.73
OPM13	356	152	45	3 : 1	0.37
OPP5C	SR	163	33	3 : 1	5.27
OPO10	356	147	51	3 : 1	0.03
OPO15	SR	159	51	3 : 1	0.01
OPP10	SR	155	34	3 : 1	4.59*
OPP15A	SR	157	38	3 : 1	2.87
OPP15B	356	146	40	3 : 1	1.02
OPQ12A	356	149	51	3 : 1	0.01
OPQ12B	SR	156	42	3 :	1.30
OPR15A	SR	150	50	3 : 1	0.01
OPR20A	SR	143	44	3 : 1	0.14
OPS12A	356	127	58	3 : 1	3.61

Table 2 (Continued)

Primer	Parent	Present	Absent	Expected ratio	Chi-square
OPT1B	356	155	42	3 : 1	1.23
OPT1C	SR	145	52	3 : 1	0.12
OPT4	356	154	40	3 : 1	1.75
OPT12	SR	135	47	3 : 1	0.03
OPU13A	356	153	53	3 : 1	0.82
OPV14A	SR	139	51	3 : 1	0.10
OPV14B	SR	152	39	3 : 1	1.89
OPV16	356	136	33	3 : 1	2.41
OPW2	356	158	34	3 : 1	5.15*
OPX17	356	142	50	3 : 1	0.05
Sex[c]		173 (H)	80 (♀)	2 : 1	0.21

* $P<0.05$, ** $P<0.01$

[a] For 25 markers, a total of 253 plants were scored and for the remaining markers, 200 plants were scored. Those polymorphisms which could not be scored unambiguously or could not be repeated were reported as missing.

[b] This marker shows linkage to the *Sex1-f* allele from UH breeding UH breeding line 356 and thus should be present in all plants unless a recombination event has taken place

[c] Female allele is from UH breeding 356; hermaphrodite allele is from 'Sunrise'. H, hermaphrodite plants; ♀, female plants

Genetic linkage map

A total of 72 markers were assigned to linkage groups using a minimum LOD score of 4.0. Ten of these markers, those that could not be ordered unambiguously and that did not meet the set criteria, were discarded. Hence, the genetic linkage map consists of 61 RAPD markers and 1 morphological marker in 11 linkage groups comprising a total of 999.3 cM (Fig. 2). About 57% (29) of the intervals are less than 20 cM, 20% (10) are between 21 cM and 30 cM, 20% (10) are between 31 cM and 40 cM and 3% (2) are between 41 cM and 45 cM.

Linkage group 1, with 9 loci, included the *SEX1* locus. The markers flanking *SEX1*, OPT12 and OPT1C, are inherited in a dominant fashion (3:1), while the sex locus itself segregated in a ratio of two hermaphrodite plants to one female due to the lethality of the dominant *SEX1-H/SEX1-H* class. N-point mapping using MAPMAKER placed OPT12 and OPT1C on opposite sides of *SEX1*, each at a distance of 7 cM. Because of the unusual 2:1 segregation of *SEX1* and the 3:1 segregation of these flanking markers, a more detailed analysis was carried out. For OPT1C the recombinant class was 0.078 (15 recombinants in 193 plants). When the lethal class is taken into account, the actual θ is 0.061. Likewise, the total recombinant class for OPT12 is 0.071 (12 recombinants in 169 plants), giving a θ of 0.056. From a comparison of q values calculated for total recombinants and for individual classes, it is evident that both the recombinant and parental classes that contain dominant RAPD markers are over-represented in both OPT1C and OPT12.

OPL12B that is in coupling phase and linked with *sex1-f* (i.e. dominant in UH breeding line 356, the female parent) is present in 95% of the plants. θ values were calculated taking into account the missing classes in a manner analogous to that done for the RAPD markers linked and in coupling phase with *SEX1-H*. In this case the total recombinants (10 in 190 plants) gave a θ value of 0.082 ($r = 2/3\theta - 1/3\theta^2$). On the basis of θ equal to 0.082 and a population of 190 plants, the RAPD – hermaphrodite class ($r = 2/3\theta - 2/3\theta^2$) should contain 9.5 individuals, and 10 individuals were observed in this class. The RAPD – female class ($r = 1/3\theta^2$) should contain 0.4 individuals, and we observed no individuals in this class.

Linkage group 7 is the largest group (217.1 cM), with 12 loci accounting for over one-fifth of the total distance covered by the map. Of the 11 linkage groups, 7 have 5 or more loci.

Discussion

Polymorphisms and segregation

We have screened a total of 596 10-base primers and detected 96 polymorphisms between the parents 'Sunrise' and UH breeding line 356. The observed frequency of 0.16 polymorphisms/primer is lower than the frequency of polymorphisms observed in inter-varietal crosses of faba bean (Torres et al. 1993), alfalfa (Echt et al. 1992) and *Arabidopsis thaliana* (Reiter et al. 1992). The parents in the present study were chosen to maximize polymorphisms by selecting unrelated and morphologically distinct lines. However, the magnitude of the morphological differences between the parents does not seem to correlate well with differences at the molecular level. This observation is consistent with the findings of Stiles et al. (1993) who reported a narrow genetic diversity among ten domesticated papaya cultivars, including the parents used for the present study. This lack of polymorphic DNA may be magnified by the relatively small genome size of papaya (Armuganathan and Earle 1991), which would limit the amount of the generally more polymorphic repetitive DNA.

Mendelian segregation of 80% of the RAPD loci observed in the F_2 segregating population is higher than that reported in *Betula alleghaniensis* (Roy et al. 1992), alfalfa (Echt et al. 1992) and conifers (Carlson et al. 1991). The larger size of the F_2 population and the use of an intra-specific cross may have contributed to the reduced level of segregation distortion observed in the present investigation.

The lack of segregation for two polymorphisms having dominant markers in UH breeding line 356 can be attributed to very tight linkage to the *sex1-f* allele or to maternal inheritance of plastids. The uniparental inheritance in the F_2 population of the marker (OPL8), which is dominant in the male parent 'Sunrise', is not clear. However, we have previously shown that RAPD markers are still segregating in the progeny from third-generation sib-mated UH breeding line 356 (Stiles et al. 1993). It is possible that the plant of UH breeding line 356 used as the female par-

ent for this cross may have been heterozygous or homozygous dominant for this marker.

One other marker, OPL12B, that shows significant deviation from the expected 3:1 segregation can be explained by its linkage, in coupling phase, to *sex1-f*. Since every plant should have at least one copy of *sex1-f*, any marker linked in coupling should also be present except when uncoupled by recombination. MAPMAKER places OPL12B 7.8 cM from *SEX1*, which is close to the θ value of 0.082 calculated from the total recombinant class. For this θ and population size (190 plants), the OPL12B null-hermaphrodite class should contain 9.5 individuals and the OPL12B null-female class should contain 0.4 individuals. The observed class sizes of 10 and 0 are close to the expected values.

Sex determination

Sex in papaya is determined by a single gene with multiple alleles (Storey 1938; Hofmeyr 1938). The alleles for male *SEX1-M* and hermaphrodite *SEX1-H* are dominant over the female *sex-f* allele. It has also been hypothesized that this simply inherited locus in fact represents a complex of many tightly linked genes affecting secondary sex characters (flower number, peduncle length), cross-over suppression ('C') and lethality ('L') (Storey 1953). The zygotic lethal factor ('L') is responsible for the death of the homozygous dominant types *(SEX1-M/SEX1-M, SEX1-M/SEX1-H, SEX1-H/SEX1-H)* during early seed development. This gives rise to a 2:1 segregation for sex type rather than the normal 3:1 ratio for a single autosomal dominant gene.

Alternatively, Hofmeyr's (1967) genic balance theory of sex determination in papaya proposes that the male and hermaphrodite regions are inert, which accounts for the zygotic lethality in the homozygous condition. Based on the more frequent occurrence of sex reversal in hermaphrodites and males than in female plants, he proposed that a greater concentration of genes affecting femaleness are concentrated on sex chromosomes while those affecting maleness are distributed all over the autosomal chromosomes. The interaction between these factors determines the sex of the plant.

On the basis of our current understanding of the regulation of floral development and its control through *trans*-acting regulatory proteins (e.g., see review by Gasser 1991), it is reasonable to hypothesize that *SEX1-M* encodes a *trans*-acting regulatory factor that induces male floral parts while inhibiting carpel development. The dominant *SEX1-H* allele is intermediate, it has the ability to induce male structures but only reduces carpel size. Functional carpels remain in *SEX1-H/sex1-f* plants. The *sex1-f* allele is incapable of inducing male structures and could be a null allele. The lethality of zygotes lacking at least one copy of *sex1-f* could result from an additional required function for the *SEX1* locus that is present in the *sex1-f* allele but lacking in both *SEX1-M* and *SEX1-H*. Alternatively, a chromosomal aberration, perhaps a deletion, that inactivates a required function gene may be tightly linked to the *SEX1-M* and *SEX1-H* alleles.

Linkage map

We have developed a primary genetic linkage map of papaya using 61 RAPD markers (60 dominant and 1 codominant) and 1 codominant morphological locus *(SEX1)*. The map is comprised of 11 linkage groups with a total mapped distance of 999.3 cM. *C. papaya* is a diploid species with 2n=18 (C=9) (Meurman 1925). On the basis of a normal chromosomal size range of 50–150 cM (Kashi 1985), the papaya genome is expected to be around 1350 cM. The present map covers a significant portion of the papaya genome. Although the map distances may be inflated due to the use of mostly dominant markers, this is a major improvement over the previous map that comprised 3 markers and covered only 41 cM (Hofmeyr 1939). At present, we can not assign any linkage group to a chromosome as the chromosomes have not been characterized morphologically.

We have mapped the locus that determines flower sex, *SEX1*, to a region on linkage group 1 flanked by markers in coupling phase. These loci, *T12* and *T1C*, are both 7 cM from *SEX1* but appear to segregate in a normal 3:1 ratio (Table 2), while *SEX1* segregates 2:1 due to the lethality of the homozygous dominant class.

The map surrounding *SEX1* was constructed by automated n-point analysis using the 'order' routine of MAPMAKER. Because of the unusual segregation in this region as a result of the missing and presumed lethal *SEX1-H/SEX1-H* class (that should include one-fourth of the individuals) and the over-abundance of the flanking dominant RAPD alleles, θ for the various classes was calculated. The combined recombinant q values for OPT1C and OPT12 were not significantly different from those determined by MAPMAKER. However, both the recombinant and parental classes that contained the dominant RAPD markers were over represented. This accounts for the 3:1 segregation of the RAPD markers surrounding the 2:1-segregating *SEX1* locus. The reason for the additional RAPD markers is not clear. However, this over-representation is not a general problem since the majority of the RAPD markers showed the expected pattern of dominant Mendelian segregation. We are currently cloning OPT1C and OPT12 so that they can be used as co-dominant markers to map this region in more detail.

This papaya linkage map, having 51 intervals, with a mean distance between markers of 19.6 cM, with over 75% of the intervals shorter than 30 cM, is adequate for genetic analysis of quantitative traits (Sondur et al. 1995). The results of our study confirms the utility of RAPD markers in constructing genetic linkage maps.

Acknowledgements We would like to thank an anonymous reviewer for suggestions concerning analysis of mapping data surrounding the *SEX1* locus. This research was supported by the USDA Special Grants Program for Tropical and Subtropical Agriculture Research.

References

Armuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Awada M (1958) Relationships of minimum temperature and growth rate with sex expression of papaya plants (*Carica papaya* L.). Hawaii Agric Exp Sta Tech Bull No 38

Awada M, Ikeda W (1957) Effects of water and nitrogen on composition, growth, sugar in fruits yield and sex expression of the papaya plants (*Carica papaya* L.). Hawaii Agric Exp Sta Tech Bull No 33

Bernatzky R, Tanksley SD (1986) Majority of random cDNA clones correspond to single loci in the tomato genome. Mol Gen Genet 203:8–14

Botstein D, White RL, Skolnicks MH, Davis RW (1980) Construction of genetic linkage map in man using restriction fragment length polymorphism. Am J Hum Genet 2:314–331

Carlson JE, Tulsieram LK, Glaubitz JC, Luk V, Kauffeldt C, Rutledge R (1991) Segregation of randomly amplified DNA markers in F_1 progeny of conifers. Theor Appl Genet 83:194–200

Chaparro J, Wilcox P, Grattapaglia P, O'Malley D, McCord S, Sederoff R, McIntyre L, Whetten R (1992) Genetic mapping of pine using RAPD markers. Construction of a 191 marker map and development of half-sib genetic analysis. In: Whelan WJ, Ahmad F, Bialy H, Black S, King ML, Rabin MB, Solomonsen LP and Vasil IK (eds) Advances in gene technology: feeding the world in the 21st century. (Miami Winter Symposium.) ILR Press New York, pp 129

Echt CS, Erdahl LA, McCoy TJ (1992) Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. Genome 35:84–87

Foolad MR, Jones RA, Rodriguez RL (1993) RAPD markers for constructing intra specific tomato genetic maps. Plant Cell Rep 12:293–297

Gasser CS (1991) Molecular studies on the differentiation of floral organs. Annu Rev Plant Physiol Plant Mol Biol 42:621–649

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Uhrig H, Salamini F (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78:65–75

Grodzieker T, Williams J, Sharp P, Sambrook J (1974) Physical mapping of temperature sensitive mutations of adenovirus. Cold Spring Harbor Symp Quant Biol 39:439–446

Helentjaris T, King G, Slocum M, Sidenstrang C, Wegman S (1985) Restriction fragment polymorphism as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol Biol 5:109–116

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72:761–769

Hofmeyr JDJ (1938) Genetical studies of *Carica papaya* L. S Afr J Sci 35:300–304

Hofmeyr JDJ (1939) Sex-linked inheritance in *Carica papaya* L. S Afr J Sci 36:283–258

Hofmeyr JDJ (1967) Some genetic breeding aspects of *Carica papaya* L. Agron Trop 17:345–351

Hu J, Quiros CF (1991) Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10:505–511

Kashi Y (1985) Restriction fragment length polymorphisms in cattle. MSc thesis. The Hebrew University, Jerusalem, Israel

Klein-Lankhorst R, Rietveld P, Machiels B, Verkerk R, Weide R, Gebhardt C, Koornneef M, Zabel P (1991) RFLP markers linked to root knot nematode resistance gene *Mi* in tomato. Theor Appl Genet 81:661–667

Lander E, Green P, Abrahamson J, Barlow A, Daley M, Lincolin S, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural population. Genomics 1:174–181

Landry BS, Keseseli RV, Farrara B, Michelmore RW (1987) A genetic linkage map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116:331–337

Lincoln SE, Daly MJ, Lander ES (1993) Constructing Genetic Linkage Maps with MAPMAKER/EXP version 3.0: a tutorial and reference manual, 3rd edn. Whitehead Institute for Biomedical Research Technical Report, Cambridge, Mass.

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

McCouch SR, Kochert G, Yu ZH, Wang ZY, Kush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Meurman O (1925) The chromosome behavior of some dioecious plants and their relatives with special reference to the sex chromosomes. Soc Sci Fenn Comm Biol 2:105

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincolin SF, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of RFLP. Nature 335:721–726

Reiter RS, Williams J, Feldmann, KA, Rafalski JA, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89:1477–1481

Roy A, Frascaria N, Mackay J, Bousquet J (1992) Segregating random amplified polymorphic DNAs (RAPDs) in *Betula alleghaniensis*. Theor Appl Genet 85:173–180

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sondur SN, Manshardt RM, Stiles JI (1995) Genetics of growth rate and flowering time in papaya (*Carica papaya* L.) J Quant Trait Loci 1 (http://probe.nalusda.gov:8000/otherdocs/jqtl/jqtl1995-04/jq18r2.html)

Stiles JI, Lemme C, Sondur S, Morshidi MB, Manshardt R (1993) Using randomly amplified polymorphic DNA for evaluating genetic relationships among papaya cultivars, Theor Appl Genet 85:697–701.

Storey WB (1938) Segregation of sex types in solo papaya and their application to the selection of seed. Am Soc Hortic Sci 35:83–85

Storey WB (1953) Genetics of papaya. J Hered 44:70–78

Storey WB (1976) Papaya. In: Simmonds NW (ed) Evolution of crop plants. Longman, San Francisco, pp 21–24

Tingey S, Sebestian S, Rafalski AH (1989) A RFLP map of the soybean genome. Agron Abstr, p 180

Tingey SV, del Tufo P (1993) Genetic analysis with random amplified polymorphic DNA markers. Plant Physiol 101:349–352

Torres AM, Weeden NF, Martin A (1993) Linkage among isozyme, RFLP and RAPD markers in *Vicia faba*. Theor Appl Genet 85:937–945

Welsh J, McClelland M (1990) Finger printing genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Welsh J, Petersen G, McClelland M (1991) Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. Nucleic Acids Res 19:303–306

Williams JGK, Kubelik AR, Lavak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 19:6531–6535

Zee F (1985) Breeding of papaya virus tolerance in solo papayas, *Carica papaya* L. PhD thesis, University of Hawaii, Honolulu