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An interspecific (*Capsicum annuum*×*C. chinese*) F_2 linkage map in pepper using RFLP and AFLP markers

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Abstract We have constructed a molecular linkage map of pepper (*Capsicum* spp.) in an interspecific F_2 population of 107 plants with 150 RFLP and 430 AFLP markers. The resulting linkage map consists of 11 large (206–60.3 cM) and 5 small (32.6–10.3 cM) linkage groups covering 1,320 cM with an average map distance between framework markers of 7.5 cM. Most (80%) of the RFLP markers were pepper-derived clones, and these markers were evenly distributed across the genome. By using 30 primer combinations, we were able to generate 444 AFLP markers in the F_2 population. The majority of the AFLP markers clustered in each linkage group, although *Pst*I/*Mse*I markers were more evenly distributed than *Eco*RI/*Mse*I markers within the linkage groups. Genes for the biosynthesis of carotenoids and capsaicinoids were mapped on our linkage map. This map will provide the basis of studying secondary metabolites in pepper.

Keywords Amplified fragment length polymorphism (AFLP) · *Capsicum* · Linkage map · Restriction fragment length polymorphism (RFLP)

Introduction

Pepper fruits are consumed as food additives for their unique color, pungency, and aroma in many regions of the world, particularly in Asia and South and Central America. Five species of *Capsicum* peppers, including *C. annuum*, *C. chinense*, *C. baccatum*, *C. frutescens,* and *C. pubescens,* are cultivated in different parts of the world. Of these, *C. annuum* is most widely grown in both Asia and worldwide (Pickersgill 1997). This species includes most of the Mexican chili peppers, most of the hot peppers of Africa and Asia, and various cultivars of sweet peppers grown in temperate regions of Europe and North America.

During the last decade, the construction of molecular linkage maps has become an essential tool for plant molecular genetics and breeding research. Although pepper genome research is being conducted by only a small number of research groups worldwide, the development of a linkage map in *Capsicum* has been greatly aided by the use of tomato-derived restriction fragment length polymorphism (RFLP) probes. All published genetic maps of *Capsicum* so far have been based on either interspecific populations (Tanksley et al. 1988; Prince et al. 1993) or intraspecific populations (Lefebvre et al. 1997) with the use of tomato-derived RFLP probes. Recently, Livingstone et al. (1999) published another pepper genetic map containing nearly a thousand DNA markers. Nevertheless, the linkage map is only moderately saturated, and many markers were distinctly clustered. Prince et al. (1993) suggested that sparsely mapped genomic regions may correspond to regions of the pepper genome which have diverged more rapidly from tomato and so are not detectable with tomato probes. Therefore, current pepper genetic maps still need to be completed using pepper-derived probes for a comprehensive understanding of pepper genome structure.

Here we report the construction of a molecular linkage map of pepper using mainly pepper-derived probes based on a population of 107 interspecific F_2 individuals. In addition to random cDNA and genomic DNA clones as RFLP markers, we mapped several genes associated with biosynthetic pathways for carotenoids and capsaicinoids. To saturate the linkage map we also used amplified fragment length polymorphism (AFLP) markers.

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Materials and methods

Plant materials and DNA isolation

Pepper accessions *Capsicum annuum* cv. TF68 and *C. chinense* cv. Habanero, both available freely to scientists, were obtained from Hung-Nong Seed Company. TF68 is a southern Asian-type cultivar bearing long, slim, red, and nonpungent fruits. Habanero originated from South America and bears campanulate, orange-colored fruits that are very well known for their extremely pungent and aromatic flavors. An F_2 population of 107 plants was constructed by selfing an F_1 hybrid between the lines with TF68 as the female and Habanero as the male of the F_1 to serve as the mapping population (Nahm et al. 1997).

Genomic DNA extraction

Genomic DNA extraction procedures were as previously described (Prince et al. 1997). Young and healthy pepper leaves were ground to a fine powder with a mortar and pestle well-chilled with liquid nitrogen. About 15 ml of frozen powder was aliquoted into pre-labeled 50-ml polypropylene tubes chilled with liquid nitrogen. Twenty-five milliliters of hot (65°C) extraction buffer (7 *M* urea, 0.35 *M* NaCl, 0.05 *M* Tris-HCl pH 8.0, 0.02 *M* EDTA, pH 8.0, 0.25% sarkosyl, 5% phenol, and 0.2% sodium bisulfite) was added to each tube of frozen powdered tissue, followed by 0.75 ml of 20% SDS. The tubes were incubated at 65°C for 30 min, with inversion every 10 min to mix the contents. Chloroform:isoamyl alcohol (24:1) was then added to the tubes and the tubes subsequently centrifuged for 15 min at 5,000 *g*. DNA was precipitated by adding an equal volume of ice-cold isopropanol. The precipitated DNA was hooked out with a Pasteur pipette, rinsed with 70% ethanol, and transferred to a sterile 1.5-ml microtube. The precipitated DNA was extracted three times in 600–700 μl of TE by incubation in a 65°C water bath for 1 h each time.

RFLP analysis

DNA probes

Most of the DNA probes used in this study were prepared from the TF68 (*C. annuum*) cDNA libraries (10^8 pfu/μg DNA) and the size-selected (0.5–2.0 kb) *Pst*I genomic library (10^5 colonies/μg DNA). cDNA clones derived from the mRNA of shoot tips, including leaves, flowers, and small fruits, were designated as PCD2, and those from leaves were designated as DC. Random single-copy genomic clones from *C. annuum* AC2258 were designated as PST. Sixty hot pepper EST clones (Hong et al. 1998) were also used and designated as CAN. Sixty tomato clones (TG, CD, and CT clones provided from S.D. Tanksley of Cornell University, USA) were selected to represent all chromosomes and used to compare our map with others. Clones of several known genes were also used in this study, as listed in Table 1. In total, 550 pepper clones (320 cDNA and 230 genomic DNA) and 108 tomato clones were assayed for polymorphism on Southern blot survey filters.

Southern hybridization

The survey filters were prepared from parental DNA digested with five different restriction enzymes, *Eco*RI, *Dra*I, *Eco*RV, *Hin*dIII, and *Xba*I. Restriction digestion was carried out with 0.5–1 U restriction enzyme per microgram of DNA. Approximately 20 μg of pepper DNA was loaded per lane and separated on 0.8% agarose gels in 0.5×TBE buffer for 12 h at 40 V. Progeny filters with parents and F_2 progeny DNA were created with the same method. The filters were prehybridized for at least 2 h. Probes were labeled with α-[^{32}P]dCTP using the random hexamer procedure (Promega, Madison, Wis.). Labeled probes were denatured by alkali treatment with 0.2 *M* NaOH, and then added to filters in 40 ml of hybridization buffer (6×SSC, 0.5% SDS, 5×Denhardt's reagent, and 100 μg/ml salmon sperm DNA). Hybridization was carried out at 65°C for 24 h. Filters were washed at low stringency (2×SSC) and then at high stringency (1 or 0.5×SSC) and placed on Agfa CP-BU film (European Communities) for 1–5 days, depending on the strength of the signal.

Table 1 Pepper EST, genes, and cDNA and genomic DNA clones mapped by RFLP analysis

Clone	Linkage group	Identity	Accession number	References
CAN7	10	Putative RNA directed RNA polymerase of pepper mild mottle virus (P29098)	AA840687	Hong et al. (1998)
CAN12	4	Unknown	AA840697	Hong et al. (1998)
CAN15	14	NTP303 homolog (P29162)	AA840702	Hong et al. (1998)
CAN21	5	RAB1X homolog (Z73935)	AA840710	Hong et al. (1998)
CCS	4	Capsanthin-capsorubin synthase	X76165	Bouvier et al. (1994)
Ca4H	4	Cinnamic acid 4-hydroxylase	AF088847	
CaSIG19	6	Defense-related gene		
COMT	2	Caffeic acid o-methyl transferase	AF081214	
CRTHYD	4	β-carotene hydroxylase	Y09225	Bouvier et al. (1998a)
GGPS	7	Geranylgeranyl pyrophosphate synthase	X80267	Badillo et al. (1995)
LCY	10	Lycopene β-cyclase	X86221	Hugueney et al. (1995a)
MADSP10	4	MADS-like gene		
PAL	2	Phenylalanine ammonia lyase	AF081215	
PDS	2	Phytoene desaturase	X68058	Hugueney et al. (1992)
PFTF	6	Plastid fusion and/or translocation factor	X80755	Hugueney et al. (1995b)
PSY	7	Phytoene synthase	X68017	Romer et al. (1993)
TK2	1	Transketolase 2	Y15782	Bouvier et al. (1998b)
RDNA5 S	6	5 S ribosomal DNA		
RDNA25 S	10	25 S ribosomal DNA		
DC[a]		cDNA clones from *C. annuum* TF68 leaf mRNA		
PCD2[a]		cDNA clones from *C. annuum* TF68 total tissue		
PST[a]		Genomic DNA clones from *C. annuum* AC2258		
CD, CT, TG		Tomato genomic and cDNA clones		

[a] Their sequences have not yet been identified, and they have simply been used as DNA markers for linkage map construction

Table 2 List of primers and adapters used in the AFLP analysis

Primers/adapters	Sequence
*Mse*I adapter	5´-GACGATGAGTCCTGAG-3´ 3´-TACTCAGGACTCAT-5´
*Mse*I+1 primer	GATGAGTCCTGAGTAA+C
*Mse*I+3 primers	+CAA(M1)+CAC(M2)+CAG(M3)+CAT(M4) +CTA(M5)+CTC(M6)+CTG(M7)+CTT(M8) +CGA(M9)+CGC(M10)+CGG(M11)+CGT(M12) +CCA(M13)+CCC(M14)+CCG(M15)+CCT(M16)
*Eco*RI adapter	5´-CTGTAGACTGCGTACC-3´ 3´-CTGACGCATGGTTAA-5´
*Eco*RI+1 primer	GACTGCGTACCAATTC+A
*Eco*RI+3 primers	+AAC(E1)+AAG(E2)+AAT(E3)+AAA(E4) +ACA(E5)+ACT(E6)+ACC(E7)+ACG(E8) +AGC(E9)+AGG(E10)+AGT(E11)+AGA(E12) +ATC(E13)+ATG(E14)+ATT(E15)+ATA(E16)
*Pst*I adapter	5´-CTCGTAGACGTACATGCA-3´ 3´-CATCTGACGCATGT-5´
*Pst*I+1 primer	GACTGCGTACATGCAGGA+G
*Pst*I+3 primers	+GGA(P1)+GGT(P2)+GGG(P3)+GGC(P4) +GCA(P5)+GCT(P6)+GCG(P7)+GCC(P8)

AFLP analysis

The AFLP protocol was essentially the same as that described by Vos et al. (1995) with only minor modifications. Adapters, *Mse*I site primers, *Eco*RI site primers, and *Pst*I site primers used in this study were synthesized by Bioneer (Chungwon, Korea) and are listed in Table 2. *Taq* DNA polymerase was from Boehringer Mannheim (Germany) and γ-[^{32}P]ATP was from Amersham Pharmacia Biotech (N.J.).

Sixteen *Eco*RI primers and 16 *Mse*I primers, each with three selective nucleotides, were used for surveying the AFLP polymorphism between the two parental lines, TF68 and Habanero. To compare the number of amplified DNA fragments with *Eco*RI/*Mse*I primer combinations and polymorphism rate, we also used 70 primer combinations from 8 *Pst*I primers and 10 *Mse*I primers.

For template preparation, approximately 0.5 µg DNA was digested with 5 U of two restriction enzyme sets (*Eco*RI/*Mse*I or *Pst*I/*Mse*I). The fragments were then ligated with *Eco*RI (or *Pst*I) and *Mse*I adapters in the same tube as the restriction digestion was performed. For amplification of the restriction fragments, a two-step protocol was followed. The first step included the selective preamplification of adapter-ligated DNA using polymerase chain reaction (PCR) with primers having one selective nucleotide. In the second step, selective amplification of preamplified DNA was performed with primers having three selective nucleotides. PCR products were mixed with an equal volume of formamide dye (98% formamide, 10 m*M* EDTA, 0.005% bromophenol blue, and xylene cyanol), denatured at 90°C, and separated by electrophoresis on 6% denaturing acrylamide gels in 1×TBE buffer. The gels were pre-run for 20 min before 3–5 µ l of the mix was loaded. Gels were run at 40 W for 3 h, vacuum-dried, and exposed to CP-BU X-ray film (Agfa, European Communities).

Based on the concept that the most useful primer combination would give the higher polymorphism rate and generate a reasonable number of total bands for unambiguous scoring, 11 *Pst*I/*Mse*I primer combinations (P1/M2, P1/M3, P1/M4, P1/M5, P1/M7, P3/M1, P3/M4, P3/M8, P4/M1, P4/M3, P4M4) and 18 *Eco*RI/*Mse*I primer combinations (E1/M2, E1/M3, E1/M6, E3/M12, E3/M13, E4/M12, E4/M13, E5/M2, E5/M3, E5/M7, E6/M3, E7/M2, E7/M3, E11/M10, E12/M14, E13/M12, E16/M10, E16/M12) were selected by primer screening. From these primer combinations a total of 444 markers were scored, 125 and 319 from the *Pst*I/*Mse*I and *Eco*RI/*Mse*I primer combinations, respectively. All the AFLP markers were scored as either presence or absence of a polymorphic band (Fig. 1). For some markers intensity differences (putative codominant loci where only a single allele is observed) were discernible, but this information was not used because of the difficulty in scoring. Data were obtained by visually

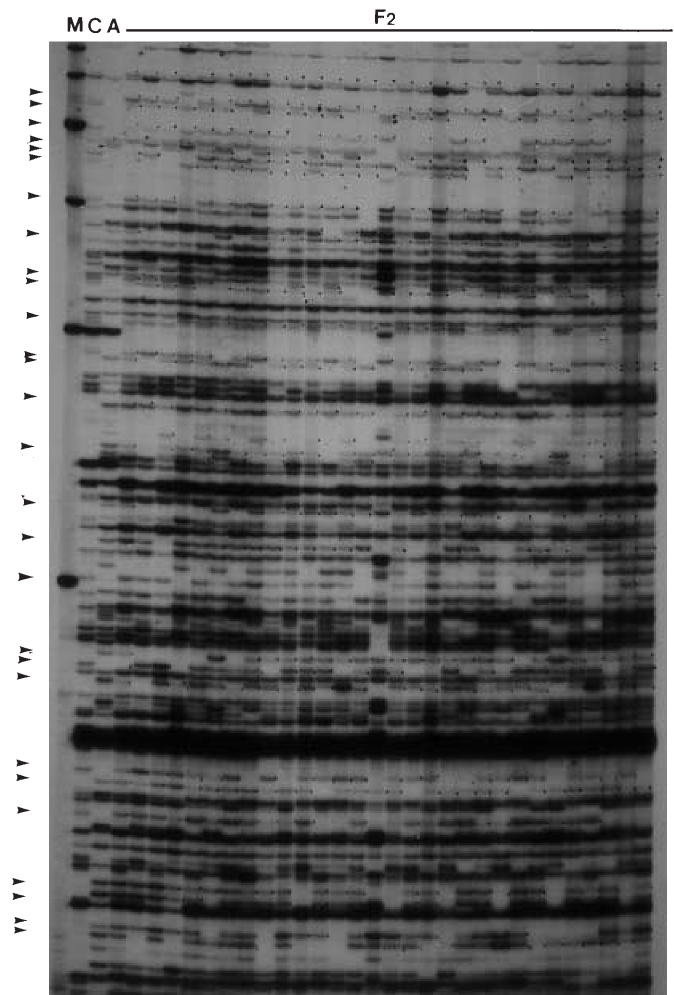

Fig. 1 Segregation of alleles revealed by AFLP markers derived from the selective amplification of restriction fragments by primer combinations E5M2. A 100-bp ladder (*M*) is arranged on the *left side* of the pepper lanes; *C* the male parent Habanero, *A* the female parent TF68, F_2 F_2 progeny

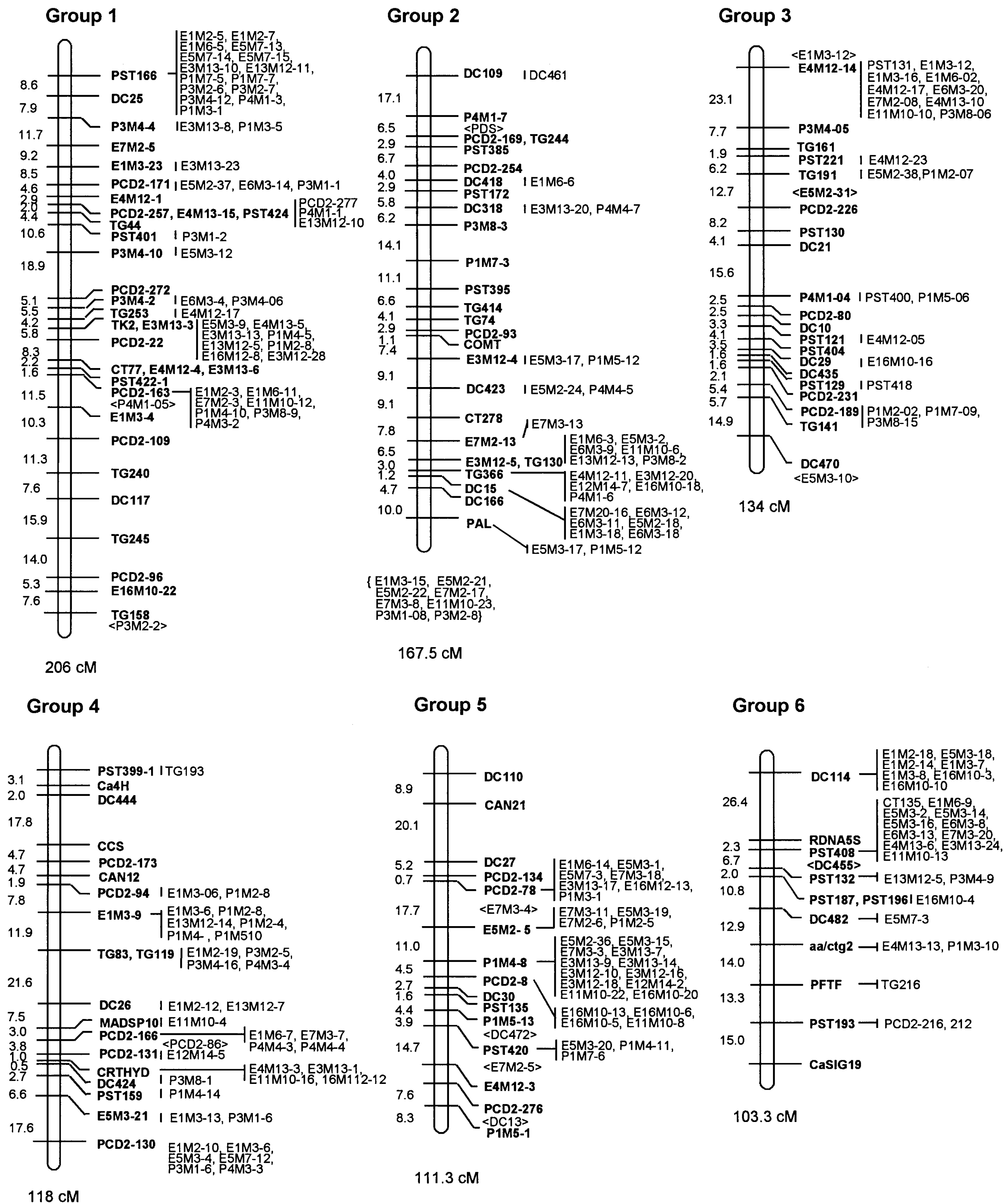

Fig. 2 A combined linkage map of hot pepper using 150 RFLP and 430 AFLP markers. The 16 linkage groups were arbitrarily labeled according to the total map distance of each linkage group. On the *left* of the *vertical double lanes* are map distances in centiMorgans (cM) calculated by the Kosambi function, and on the *right* are DNA markers with identification numbers and names. AFLP markers are designated by the code for the *Eco*RI (or *Pst*I) and *Mse*I selective primers followed by the *numbers* given in descending order of molecular weights. Framework markers, in *bold*, were ordered at LOD>3.0. Markers following a comma are cosegregating markers. Markers to the *right* of *vertical bars* were mapped to either side of the closest framework markers at LOD>2.0. Markers in *parenthesis* were placed between framework markers at 2<LOD<3. Markers listed *below* the linkage group showed linkage to that linkage group, but a specific map position could not be ascertained. Some details on the RFLP markers used for map construction are described in Table 1

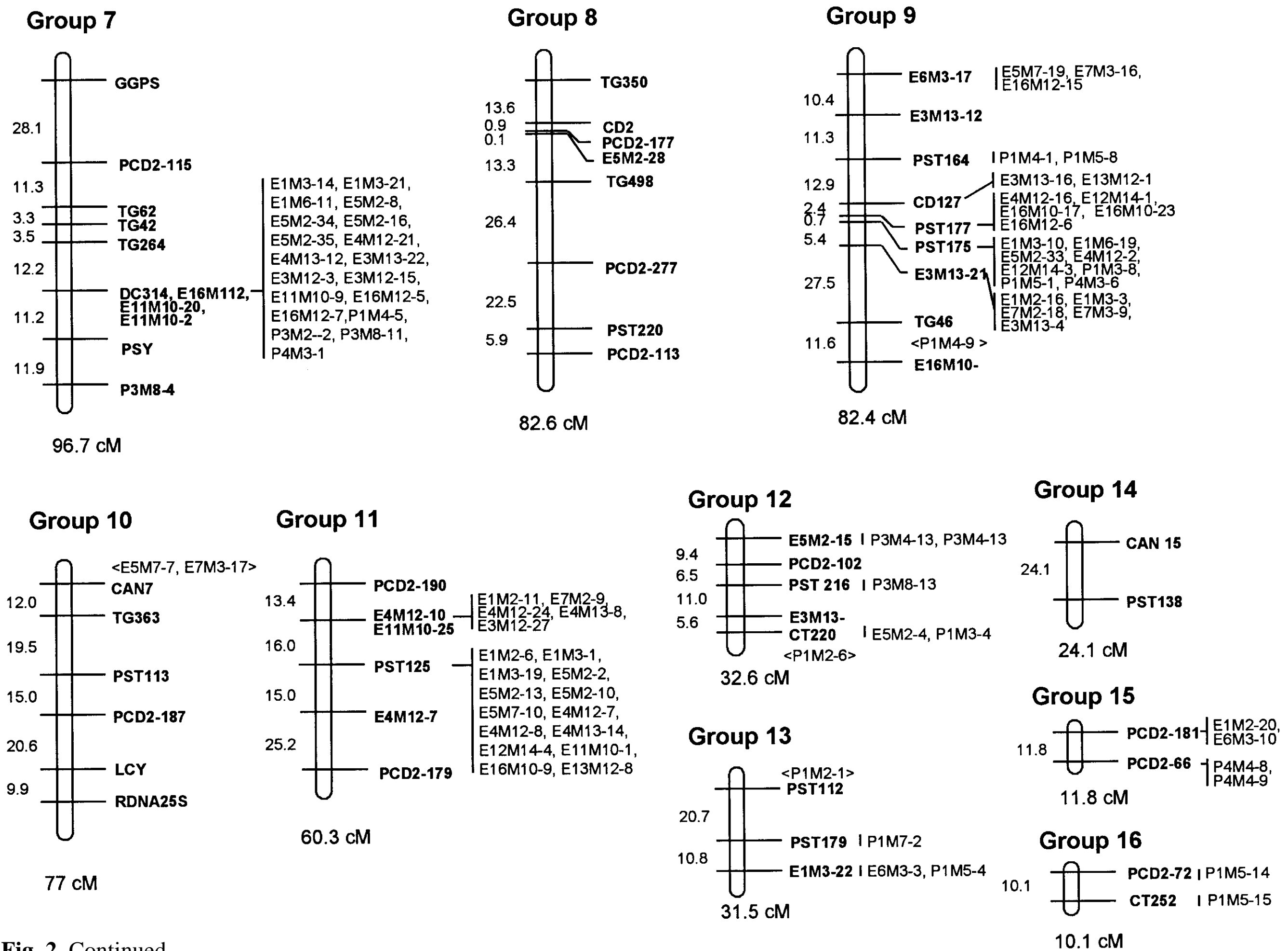

Fig. 2 Continued

scoring clear and unambiguous bands on autoradiograms and were translated into the following allele configurations: B/D codings for dominant markers contributed by the female parent (Habanero), where D stands for the presence of a band and B for the absence of a band; A/C codings for dominant markers contributed by the male parent (TF68), where C stands for the presence of a band and A for the absence of a band.

Map construction

Linkage analysis of the entire set of markers was performed using MAPMAKER 3.0/EPX (Lander et al. 1987). Since all AFLP markers were dominantly scored, we first constructed a framework map using RFLP markers, the AFLP markers were then merged into this framework map. To identify linkage groups using RFLP markers, we performed pairwise comparisons and grouping of markers using the "Group" command at a maximum recombination fraction of 25 cM and a minimum LOD score above 3.0. To establish the most likely order within each linkage group, we used the "Compare" command with the above-mentioned criteria and the exclusion threshold of 3.0 LOD score units. The ordered RFLP markers were confirmed using the "Ripple" command. AFLP markers were added into the framework map using the "Assign" and "Build" command. If the AFLP markers were placed on the framework map above an LOD value of 3.0, the markers were included in the framework map. The last of the markers were placed within the framework map using the "Place" command. Recombination fractions were converted to map distances in centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944).

Results

RFLP analysis

Out of the 550 pepper clones assayed, the number of probes which revealed RFLPs with at least one enzyme was 256 (46%). When tomato clones were assayed, 61 out of 108 clones (56%) showed polymorphism. Of the 317 polymorphic clones, only 153 were usable for RFLP linkage analysis because half of the markers which showed polymorphism on survey filters could not be scored clearly for F_2 individuals. A total of 43 markers (31.4%) out of 153 markers deviated from the normally expected F_2 ratio of 1:2:1 or 3:1. Of the 147 markers segregating 1:2:1, 22 markers were skewed toward the *C. annuum* allele, 15 markers toward the *C. chinense* allele, and 3 markers toward the heterozygote. One marker, PCD2–171, did not show skewness towards any allele; in other words, it showed an abnormal 1:1:1 segregation ratio. Among the remaining 6 dominant markers (PCD2–66, DC-114, DC478, CAN17, RDNA5 S, and RDNA25 S), 2 markers (DC-114 and RDNA5 s) showed segregation distortion toward *C. chinense*.

AFLP analysis

Variations in AFLP patterns between the two lines were analyzed. The number of bands generated by the different *Eco*RI/*Mse*I primer combinations revealed a large range of variation, from 23 bands for E8M14 (+ACT/+CCC) to 118 for E1M4 (+AAC/+CAT), with an average of 70. In contrast, the number of fragments amplified with *Pst*I/*Mse*I primers ranged between 15 for P8M10 (+GCG/+CGC) and 70 for P2M1 (+GGT/+CAA), with a mean of 40. The number of amplified bands of each primer was dependent on the sequences of the selective nucleotides. The primers having+NAA+NAT,+NTA, and+NTT (M1, M4, M5, M8, E15, E16) selective sequences amplified more bands than other primers. When polymorphism rates were compared, *Eco*RI/*Mse*I primer combinations showed a much higher polymorphism rate than *Pst*I/*Mse*I primer combinations, with an average of 59.8% and 49%, respectively.

*Eco*RI/*Mse*I primer combinations generated about twice as many scorable AFLP markers than *Pst*I/*Mse*I primer combinations. The average number of scorable markers per primer combination was 17.7 in *Eco*RI/*Mse*I combinations and 10.4 in *Pst*I/*Mse*I combinations. Although many detectable polymorphic fragments were observed, a large number of these were difficult to score owing to the dense and sometimes overlapping banding patterns. Of 444 AFLP markers, 232 (52.2%) were B/D (dominant markers contributed by the female parent), and 212 (48.8%) were A/C (dominant markers contributed by the male parent).

The usefulness and applicability of the AFLP markers in genetic linkage mapping was evaluated by examining all 444 markers with the χ^2 test for goodness of fit. This statistical analysis revealed that 129 markers (29%), 82 for the *Eco*RI/*Mse*I combination and 47 for *Pst*I/*Mse*I deviated from the expected Mendelian segregation ratio.

The linkage map

A total of 597 markers (444 AFLP and 153 RFLP markers) were used for linkage map construction. Out of the total 597 markers, 585 were placed in 16 groups using a LOD score of 3.0 and maximum recombination value of 0.25 (Fig. 2). Within each linkage group markers which can be ordered at 3.0 LOD or above were used as framework markers. Out of 597 markers 177 were positioned as framework markers. The map contains 150 RFLP and 430 AFLP markers. The resulting linkage map consists of 11 large (206–60.3 cM) and 5 small (32.6–10.3) linkage groups (LGs) covering 1,320 cM with an average map distance between framework markers of 7.5 cM.

In contrast to other pepper maps that were based on tomato-derived probes (Prince et al. 1993; Livingstone et al. 1999), the RFLP markers were evenly distributed within each linkage group; this inconsistency is likely due to the difference in the origins of RFLP probes. Our map was mainly based on pepper-derived probes.

The AFLP markers were well distributed over the linkage groups except LG 8 and LG 10. Many of the AFLP markers were clustered in one region of each linkage group (LGs 2, 3, 5, 6, 7, 9, and 11). The *Pst*I/*Mse*I markers were more evenly distributed than the *Eco*RI/*Mse*I markers. There was significant clustering of skewed markers. The middle of LG 1, between PCD2–257 and PCD2–109, was skewed toward the *C. annuum* allele as was the upper middle of LG 2, between PST172 and COMT. All of LG 6 was distorted in favor of the *C. chinense* allele as was the middle of LG 9, between PST164 and PST 175. The distorted regions corresponding to LGs 1, 2 and 6 were the same as those on the other pepper maps (Prince et al. 1993; Livingstone et al. 1999).

Discussion

Map construction based on AFLP and RFLP

We have constructed a combined molecular linkage map using an interspecific F_2 population derived from an interspecific cross between TF68 (*C. annuum*) and Habanero (*C. chinense*) using 153 RFLP and 444 AFLP markers. The random distribution of RFLP markers within the linkage group was in contrast with other reports (Prince et al. 1993; Livingstone et al. 1999). Since we used pepper-derived cDNA and genomic clones, this indicates that we can construct more saturated pepper genetic map if we use pepper-derived clones. We constructed a more saturated framework map, the Seoul National University (SNU) map, with an average map distance of 7.5 cM, using only 153 RFLP markers in contrast to the Cornell map (Livingstone et al. 1999), which used 460 RFLP markers mostly derived from tomato.

In contrast to the majority of RFLP markers, which were randomly distributed within linkage groups, the majority of the AFLP markers showed distinct clustering, especially *Eco*RI/*Mse*I primer combinations. The clustering of AFLP markers with the *Eco*RI/*Mse*I restriction enzymes has been reported for other plant AFLP linkage maps such as barley (Qi and Lindhout 1998), soybean (Keim et al. 1997), Arabidopsis (Alonso-Blanco et al. 1998) and pepper (Livingstone et al. 1999). Since most of the clustered regions were near centromeres, we presume that the clusters of AFLP markers in our linkage map are putative centromeres. The clustering of AFLP markers reduces the number of useful markers in genomic regions other than the centromere. We had to screen nearly 512 *Eco*RI (+ANN) and *Mse*I (+CNN) primer combinations to obtain 3 AFLP markers within 2 cM of the *Bs3* gene, a resistance gene against *Xanthomonas campestris* pv. *vesicatoria* (data not shown).

Usefulness of AFLP as a marker system

AFLP analysis has only recently been developed as a PCR-based method for detecting differences in restric-

tion fragments (Vos et al. 1995), and AFLPs have been used for marker selection, linkage map construction, and QTL analysis (Becker et al. 1995; Cervera et al. 1996; Cnops et al. 1996 ; Alonso-Blanco et al. 1998; Bradshaw et al. 1998; Parker et al. 1998). We previously evaluated the reliablity and reproducibilty of AFLP analysis in pepper and found the technique to be both reliable and efficient for marker selection and mapping (Kang et al. 1997) in hot pepper.

The efficiency of molecular markers for genetic mapping depends on their ability to detect polymorphism. In this experiment, the total number of bands and the polymorphism rates were analyzed with 128 and 70 combinations of *Eco*RI/*Mse*I and *Pst*I/*Mse*I primers, respectively. As shown, different primer combinations exhibited large differences in the level of polymorphism detected and in band number. Therefore, the proper selection of primer combinations is necessary for the efficiency of AFLPs. On average, *Eco*RI/*Mse*I primer combinations revealed more amplified bands and a higher polymorphism rate, with an average of 59.8%, than *Pst*I/*Mse*I with 49%. These rates are comparable to the those of rice (65%) (Mackill et al. 1996) and higher than those of soybean (37%) (Maughan et al. 1996). The methylation sensitivity of *Pst*I likely resulted in reduced polymorphism in the AFLP reactions. However, this assumption needs to be tested since we used different selective nucleotide sets for the *Pst*I primer (+GNN) than for the *Eco*RI (+ANN) primer. Too many bands is undesirable due to the difficulty in scoring. In this study, AFLP primers having+NAT or+NAA sequences generated more bands than other selective nucleotide sequences. This result is consistant with those obtained in barley (Qi and Lindhout 1997) and may be due to an abundance of simple tandem repeats of $(AT)_n$ in higher plants (Morgante and Olivieri 1993).

We were unable to place most of AFLP markers precisely (multiple equivalent LOD scores) within the framework map. This may be due to incomplete information on the homozygous and heterozygous genotypes of the AFLP markers in the F_2 mapping population. To utilize the full power of the AFLP technique in linkage map construction, we should use doubled haploid (DH) or recombinant inbred lines (RIL) instead of the F_2 population. We are currently developing an F_7 RIL population derived from a cross between TF68 and Habanero (Kim et al. 1997), and the materials will be used for precisely ordering AFLP markers.

Map comparison

Although pepper has 12 chromosomes, the 597 markers fell into 11 major linkage groups and 5 minor groups in this study. We expect that the small linkage groups will be merged into larger linkage groups when more markers are assigned. Complete delineation of the linkage groups with pepper chromosomes, however, would be hard to achieve with an interspecific (*C. annuum* and *C. chinense*) mapping population alone due to incompleteness of the homologous recombination as discussed by Livingstone et al. (1999). In contrast, an intraspecific, DH mapping population was used by Lefebvre et al. (1995, 1997). It is generally believed that the degree of marker polymorphism is lower in an intraspecific population than in an interspecific population. Primary trisomics and fluorescence *in situ* hybridization (FISH) could be other useful tools for chromosome assignment. Lefebvre et al. (1995, 1997) attempted to assign linkage groups using 12 trisomic sets out of which 5 sets were successfully assigned to 5 linkage groups of the INRA pepper map. However, direct comparison of our SNU map with the INRA map is not possible at the moment because there are few markers in common and the trisomic plants are no longer available. Recently, we tested the FISH technique in hot pepper, and we are now planning to assign linkage groups to the pepper chromosomes using this technique.

In an attempt to coordinate our SNU map with other pepper maps, we used 61 tomato RFLP markers; 25 were placed in our framework map. Ten markers revealed straight-forward homologies both in marker orders and distance between 4 linkage groups (LGs 1, 2, 6, and 8) of the SNU map and 4 linkage groups (P1, P3, P7, and P12) of the Cornell map (Livingstone et al. 1999). Three markers located in LG 3 mapped to distal ends of 2 different Cornell linkage groups (P3 and P7). Two markers in the LG 8 mapped to 2 different Cornell linkage groups (P4 and 12), also on distal ends. More comprehensive coordination among the three *Capsicum* maps would be helpful for pepper genetics and breeding.

Total genome length of the pepper was estimated to be between 1,498 cM and 2,268 cM (Lefebvre et al. 1995). This value is approximately three to four times larger than the tomato genome (Tanksley et al. 1988). Prince et al. (1993) reported a linkage map covering 720 cM, and Lefebvre et al. (1995) reported an integrated map covering 822.9 cM. In this study a molecular linkage map was constructed with a total of 1,320 cM in map length. The resultant map coverage is similar to the recently reported map (Livingstone et al. 1999).

Map application

The most immediate application of the molecular linkage map is to locate markers linked to genes of economic and scientific interest on the map. About 110 phenotypic genes have been characterized in pepper (Dasksalov and Poulos 1994). Only 3 morphological traits (*fc*, *up*, *Mf*) and 3 disease resistance traits have been placed on the molecular linkage maps (Caranta et al. 1997; Lefebvre and Palloix 1996; Murphy et al. 1998). With regard to fruit quality traits such fruit color, pungency, and nutritional value, little is known about their structural and regulatory genes. Our map is particularly useful in studying genes related to the biosynthetic pathways of carotenoids and capsaicinoids. Since we used two pepper

lines with different fruit colors (red and orange) and pungency levels, we could obtain an F_2 population segregating for fruit color and pungency. In the case of carotenoids, we found that red color is determined by a single dominant gene (Huh et al. 2001). Most of the genes leading to the biosynthesis of carotenoid (*FPS*, *GGPS*, *PSY*, *PDS*, *LCY*, *CCS, GPS, TK2, CRTHYD*, and *PFTF*) and some of the upstream genes (*PAL*, *Ca4H*, and *COMT*) for capsaicin biosynthesis were positioned on our SNU linkage map (Table 1).

Placing expressed cDNA clones (EST clones) for tissue-specific expressed genes on a molecular linkage map is an important tool for clarifying the organization of the plant genome. Furthermore, the EST markers make it possible for direct comparison of gene distributions among different plant genomes. Although we used about 61 EST clones derived from the anther-specific cDNAs of hot pepper, only 5 clones could be mapped on this map. To obtain more detailed information we are currently adding more EST clones of cDNAs derived not only from the anther but also from other tissues like fruits and leaves.

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