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Comparative Mapping of Consensus SSR Markers in an Intraspecific F_8 Recombinant Inbred Line Population in *Capsicum*

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Abstract. A saturated intraspecific genetic map is critical for studying QTLs associated with *Phytophthora* root rot resistance in pepper. The map was constructed using a population of 126 F_8 recombinant inbred lines derived from a cross between YCM334 (resistant to *Phytophthora* root rot) and the susceptible local variety, Tean. To identify a set of consensus markers for mapping, 67 anchor SSR markers were selected from the reference map Pepper-FAO3 and 130 from SNU3. Polymorphic rates were low: 43 out of 197 were polymorphic. In addition, 1,667 EST-SSR primers were used. Given 11% of polymorphism rate was enough to frame, but not to saturate the map. To saturate the map, 66 AFLP primer combinations were also used. Among the 454 markers used, 281 AFLPs, 101 EST-SSRs, 37 consensus SSRs and 1 CAPS marker were mapped and distributed in 19 linkage groups (LGs). Based on distribution of the consensus markers, 14 linkage groups were assigned into 12 chromosomes of pepper. The map covered 2177.5 cM with an average of 5.2 cM. Distribution and order of consensus markers in the present linkage map were consistent with the previously developed maps. The map will become a useful tool for analyzing QTLs of the mapping population.

***Additional key words*:** *Capsicum annuum*, genetic mapping, linkage analysis, recombinant inbred line, RIL

Introduction

Pepper is a member of the family *Solanaceae*, which is one of the largest families in the plant kingdom and includes more than 3,000 species (Knapp, 2002). The *Solanaceae* family includes important crops, such as pepper, tomato, tobacco, potato, and eggplant and has been highly cultivated over the years for human nutrition and health. *Capsicum* species are valued and consumed worldwide because of their unique color, pungency, and aroma. *Capsicum* peppers include *C. annuum*, *C. chinense*, *C. baccatum*, *C. frutescens*, and *C. pubescens* are cultivated in different parts of the world. Of these, the varieties of the chili pepper plant species *C. annuum* are the most heavily consumed due to their nutritional value and spicy taste (Govindarajan and Sathyanarayana, 1991), while the other four spices are used to produce spice or used as genetic resources for disease resistance genes (Caranta et al., 2002; Pickersgill, 1997).

The development of a detailed genetic map, on which markers associated with desirable traits are identified, is a valuable tool to improve breeding efficiency. Genetic maps have been developed in almost all the agricultural crops (O'Brien, 1993). In order to maximize the polymorphism for map construction, interspecific mapping populations have previously been constructed in pepper (Kang et al., 2001; Lee et al., 2004; Lee et al., 2009; Paran et al., 2004; Wu et al., 2009; Yi et al., 2006). However, maps based on interspecific populations may not represent the true recombination distances of the cultivated species (Causse et al., 1994; Lefebvre et al., 1995). Also, reduced recombination or chromosomal rearrangements between species within an interspecific cross may lead to segregation distortion (Tadmor et al., 1987). Molecular maps developed from crosses between cultivars are the most useful for breeding applications as they identify polymorphic markers within the cultivated gene pool and are therefore more likely to be present in crosses involving other cultivated genotypes (Menéndez et al., 1997). Crossing within the cultivated species may also negate the problem of linkage drag often encountered in crosses derived from wild species (Saliba-Colombani et al., 2000). Genetic maps based on intraspecific crosses have also been recommended for the mapping of quantitative trait loci due to less segregation distortion (Havey and Muehlbauer, 1989). Till date, several linkage maps of pepper based on intraspecific mapping populations have been reported. However, those maps have traditionally been performed using F_2 populations (Barchi et al., 2007; Kim et al., 2008; Minamiyama et al., 2006; Ogundiwin et al., 2005; Sugita et al., 2005). In those cross-progeny types, linkage disequilibrium (caused by physical linkage between loci) is used to detect (Tanksley, 1993).

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Additionally, it is not fully used for practical purposes by breeders or geneticist. In species where inbreeding is possible, mapping populations can be derived by self-pollinating F_2 progeny to yield lines that are essentially homozygous at all loci (i.e., recombinant inbred line or RIL). RIL has many advantages over the other population that are used for genetic mapping and quantitative trait locus (QTL) analysis. RIL can serve as a permanent mapping resource that will permit replicated trials in multiple environments or evaluating with different strains of the pathogen. Using RIL is especially powerful for analyzing quantitative traits because replicated trials can be analyzed using identical genetic materials (Burr and Burr, 1991). Although codominant markers are preferred over dominant markers for genetic mapping using F_2 plants, dominant and codominant marker systems provide equivalent information in RIL analysis (Reiter et al., 1992; Staub et al., 1996).

Comparative mapping using consensus markers can be used to combine genetic information from related species. One of the prerequisites for comparative mapping is a genetic linkage map for each species. Another requirement for comparative mapping is a set of markers that can be used to evaluate homoeology and conservation of linkage groups. Comparative mappings using RFLP and SSR markers as anchor markers has been conducted in interspecific cross (Kang et al., 2001; Lee et al., 2004; Lee et al., 2009; Rao et al., 2003). However, no comparative mappings between intraspecific and interspecific crosses have been conducted so far. The main objectives of this study were to (i) identify and characterize a set of SSR markers that would be useful for comparative mapping among *Capsicum* spp., (ii) develop an intraspecific genetic map of the pepper genome using recombinant inbred line population derived from a cross between a *C. annuum* line YCM334 and a local variety 'Tean', and (iii) complete delineation of the linkage groups to compare with pepper chromosomes that the map can be used to elucidate the loci governing desirable traits that segregate in the population and gene discovery.

Materials and methods

Plant materials and DNA extraction

A cross of *C. annuum* line YCM334 (resistance to *Phytophthora capsici*), an F_6 line derived from a cross between Yolo Wonder and CM334 in 1992 at AVRDC-The World Vegetable Center, Taiwan, and local variety 'Tean' (highly susceptible to *Phytophthora capsici*) was made in 2000 at National Institute of Horticultural and Herbal Science (NIHHS), Rural Development Administration (RDA), Korea. A mapping population of 200 F_8 recombinant inbred lines (RILs) obtained by single seed descent method was advanced in 2008. A collection of 126 from the 200 RILs and the 2 parents was selected as a mapping population. Genomic DNA of the 126 individuals and parents was extracted from young leaves of greenhouse-grown plants following the protocol described by Raz and Ecker (1997).

AFLP analysis

The AFLP assay was performed as described by Vos et al. (1995) with minor modifications. Genomic DNA (250 ng) was digested with 8 U of *Eco*RI and 6 U of *Mse*I (New England Biolabs, UK) and incubated at 37℃ for 4 hours. Digestion solution was ligated to the two adaptors for *Eco*RI and *Mse*I cutting sites and then preamplified with *Eco*RI (E) and *Mse*I (M) primers with single nucleotides (E +A or C or G, M + T or C). Selective amplification was done using various combinations of E primers with 2, 3 and 4 selective nucleotides and M primers with 3 selective nucleotides (Table 1). The amplification products were analyzed in parallel in a 5% denaturing polyacrylamide gel (19:1 acrylamide-bisacrylamide, 7.5 M urea) in 0.5 X TBE buffer (25 mM Tris, 25 M boric acid, 0.5 mM EDTA, pH 8.0) using a S3S T-Rex™ Aluminum Backed Sequencer and visualized by silver staining. Silver staining and developing was done according to Promega's DNA Silver Staining System.

SSR and CAPS analysis

PCR was performed in a 15-μl volume containing 10-25 ng of genomic DNA as templates, miscosatellite primers, 0.8 U of *Taq* DNA polymerase (Genet Bio, Korea) and 10 X buffer solution. All amplifications were performed on an Eppendorf Mastercycler Gradient. After 5 min at 95℃, 35 cycles were performed for 1 min at 94℃, 1 min at 55℃, 1 min at 72℃ and a final extension step for 5 min at 72℃. All primers were from previously published literature or database and were synthesized from Bioneer, Korea. PCR products amplified by SSR primer were analyzed on 6% denaturing sequencing gel using a S3S T-Rex™ Aluminum Backed Sequencer and visualized by silver staining according to Promega's DNA Silver Staining System. Amplicon product amplified by CAPS primers were resolved on 2% agarose gel in 0.5 X TBE buffer and stained with ethidium bromide.

Screening of polymorphism, marker scoring, and nomenclature

For each marker type, polymorphisms were surveyed using parental lines and 6 RILs which were selected randomly among 126 RILs. Polymorphic markers were visually scored. AFLPs were scored as dominant markers and SSRs and CAPS were scored as codominant markers. Band presence or absence associated with the YCM334 allele was coded as 1; band presence or absence associated with the 'Tean' allele was coded as 2, and those bands with both parents were

Table 1. List of selected AFLP primer combinations used for genotyping.

Code of primer combination	Primer combination	Code of primer combination	Primer combination
2	EcoRI+AAC/MseI+TGC	83	EcoRI+AGG/MseI+CAC
4	EcoRI+ACT/MseI+TGC	86	EcoRI+AGC/MseI+CAC
5	EcoRI+ACG/MseI+TGC	94	EcoRI+GTGA/MseI+CAC
7	EcoRI+AGG/MseI+TGC	96	EcoRI+AAG/MseI+CTA
10	EcoRI+AGC/MseI+TGC	97	EcoRI+AAC/MseI+CTA
11	EcoRI+AG/MseI+TGC	99	EcoRI+ACT/MseI+CTA
15	EcoRI+GT/MseI+TGC	100	EcoRI+ACG/MseI+CTA
16	EcoRI+GG/MseI+TGC	101	EcoRI+ACC/MseI+CTA
17	EcoRI+GGAT/MseI+TGC	102	EcoRI+AGG/MseI+CTA
18	EcoRI+GTGA/MseI+TGC	105	EcoRI+AGC/MseI+CTA
29	EcoRI+AGC/MseI+CAA	111	EcoRI+GG/MseI+CTA
31	EcoRI+AC/MseI+CAA	112	EcoRI+GGAT/MseI+CTA
35	EcoRI+GG/MseI+CAA	113	EcoRI+GTGA/MseI+CTA
36	EcoRI+GGAT/MseI+CAA	114	EcoRI+CAG/MseI+CTA
37	EcoRI+GTGA/MseI+CAA	119	EcoRI+ACG/MseI+CTT
38	EcoRI+CAG/MseI+CAA	124	EcoRI+AGC/MseI+CTT
42	EcoRI+ACT/MseI+CAT	130	EcoRI+GG/MseI+CTT
43	EcoRI+ACG/MseI+CAT	131	EcoRI+GGAT/MseI+CTT
48	EcoRI+AGC/MseI+CAT	132	EcoRI+GTGA/MseI+CTT
53	EcoRI+GT/MseI+CAT	133	EcoRI+CAG/MseI+CTT
54	EcoRI+GG/MseI+CAT	134	EcoRI+AAG/MseI+CTG
55	EcoRI+GGAT/MseI+CAT	137	EcoRI+ACT/MseI+CTG
56	EcoRI+GTGA/MseI+CAT	149	EcoRI+GG/MseI+CTG
57	EcoRI+CAG/MseI+CAT	150	EcoRI+GGAT/MseI+CTG
59	EcoRI+AAC/MseI+CAG	151	EcoRI+GTGA/MseI+CTG
63	EcoRI+ACC/MseI+CAG	152	EcoRI+CAG/MseI+CTG
65	EcoRI+AGC/MseI+CAG	154	EcoRI+AAC/MseI+CTC
68	EcoRI+AG/MseI+CAG	157	EcoRI+ACG/MseI+CTC
71	EcoRI+AA/MseI+CAG	158	EcoRI+ACC/MseI+CTC
72	EcoRI+GT/MseI+CAG	159	EcoRI+AGG/MseI+CTC
73	EcoRI+GG/MseI+CAG	169	EcoRI+GGAT/MseI+CTC
78	EcoRI+AAC/MseI+CAC	170	EcoRI+GTGA/MseI+CTC
82	EcoRI+ACC/MseI+CAC	171	EcoRI+CAG/MseI+CTC

coded as 3 for heterozygote. Ambiguous bands were considered as missing data for map construction purposes. Each AFLP markers was assigned a name consisting of 1 letter as “a” and following was primer combination code and number of polymorphic bands generated by its primer combination. SSR markers originated from Korea Research Institute of Bioscience and Biotechnology (KRIBB) were named consisting of 2 letters as “ca” or “cs” and serial numbers followed by the letters. SSR markers selected from Sol Genomics Network (SGN) (Mueller et al., 2005), which was so-called Pepper-FAO3 map, and the map with expressed sequence tags (EST) (Yi et al., 2006), which was so-called SNU3 map, were named as their origin names.

Map construction and comparison of linkage groups

Linkage analysis was performed with MAPMAKER/EXP 3.0 (Lander et al., 1987). The “tripple error detection” feature was used to recognize the circumstance when an event was more probably the result of error than recombination. This feature avoids map expansion (Cervera et al., 2001). Linkage groups were established at a LOD score of 7.0 and a recombination fraction of 0.30 by two-point analysis using the “group” command. The best marker order of the linkage group having eight or fewer markers was identified using the “compare” command, whereas the order of the groups with more than eight markers was identified using the “order” and

Table 2. Molecular markers used for construction of the genetic linkage map.

Marker type	No. of primers/primer pairs screened	No. of polymorphic primers/primer pairs	No. of polymorphic primers/primer pairs used	No. of polymorphic markers	No. of mapped markers
AFLP	171	130	66	302	281
EST-SSR	1667	183	109	111	104
SSR-SNU3 map	135	18	15	15	12
SSR-Pepper-FAO3 map	67	25	25	25	22
CAPS	4	1	1	1	1
Total	2044	357	216	454	420

"try" commands. The marker order of each linkage group was verified using the "ripple" command. The Kosambi mapping function (Kosambi, 1944) was used to convert the recombination fractions into additive genetic distance (centiMorgans or cM). Linkage groups were drawn with the MAPCHART 2.2 program (Voorrips, 2002).

The map can be compared if consensus markers exist. The term "consensus markers" stands for the markers, placed on the map, published by SGN (Mueller et al., 2005) and the map with expressed sequence tags (EST) (Yi et al., 2006). The marker order can be changed using option "fixed orders". However, if the order is significantly inconsistent, the marker introduction into specific group doesn't proceed.

Results

A mapping population of 126 F_8 recombinant inbred lines (RILs) was derived from the *C. annuum* line YCM334 (resistance to *Phytophthora capsici*), an F_6 line derived from a cross between Yolo Wonder, and CM334 and local variety 'Tean' (highly susceptible to *Phytophthora capsici*). A genetic map was constructed for this population, with AFLP, SSR, EST-SSR and CAPS markers.

Polymorphic markers for mapping

In total, 171 AFLP primer pairs were tested on parents (YCM334 and Tean), and 6 RILs (YT4, YT11, YT40, YT64, YT66, YT91). Of these, 130 primer combinations (76%) showed polymorphism; however, 66 combinations were selected to genotype the RIL population (Table 1). These produced 346 scorable and segregating markers. Fourty-four of these were nearly monomorphic in the F_8 RIL population and were excluded from further analysis. Thus, calculations of the presented frame work maps of *C. annuum* started with 302 AFLP markers. On average, 4.6 segregating markers were produced per primer pair, with a range of 1-15. A total of 1,667 SSR primers derived from EST were selected from a database of KRIBB and screened on the two parents. Of these, 183 primers showed polymorphism. These polymorphic primers were confirmed by screening with six RILs and the parents and 109 were selected to genotype the whole RIL population. Of the 135 SSR primers selected from SNU3 map (Yi et al., 2006) and 67 from the Pepper-FAO3 map (Mueller et al., 2005), 18 and 25 primers, respectively, showed polymorphism between YCM334 and 'Tean'. Of the 18 polymorphic primers from SNU3 map, 15 were used to genotype. Thus, 149 SSR primers were used (Table 2), which collectively produced 151 clear and reproducible markers for mapping. Sequences of the SSR primers are listed in Table 3. Due to published anchor SSR markers were not available for chromosome 9 in the Pepper-FAO3 map, 5 RFLP markers were selected and converted into PCR-based markers using high-resolution melting (HRM) analysis, but none were polymorphic and similar with SCAR markers (data not shown). Out of the four CAPS markers screened, only one marker showed polymorphism. The size of the markers varied from approximately 50 bp to 1000 bp.

Construction of the linkage map

A total of 454 markers (302 AFLPs, 151 SSRs and 1 CAPS) were used for linkage map construction (Table 2). Of these, 420 markers which included 281 AFLPs, 138 SSRs and 1 CAPS were mapped and split into 19 linkage groups (LGs) using LOD scores of 7.0 and maximum recombination value of 0.30. The analysis revealed 19 linkage groups varying in length from 11.1 cM to 202.8 cM (Fig. 1). The number of loci per linkage group ranged from 4 to 48 (Table 4). The map spanned a total length of 2177.5 cM with an average marker density of 5.2 cM between adjacent markers.

AFLP and SSR markers were well distributed throughout all linkage groups of the genome. However, SSR markers were not mapped on LGa and LGc. AFLP markers were only clustered in two regions of LG9 and the clusters were 0.4 cM apart.

Seven of the linkage groups (LG2, LG4, LG5, LG6, LG8, LG8a, and LG11) contained gaps between adjacent markers of more than 20 cM, the maximum distance being 31.3 cM. LG9 was the densest with 48 markers and an average marker density of 4.0 cM. The sparsest linkage group (LGa) comprised 14 markers, with an average density of 8.5 cM.

Segregation of the markers was observed in analyzed mapping population. Chi-square analysis ($P<0.05$) revealed 68 markers (43 AFLPs and 25 SSRs), which did not segregate according to expected Mendelian ratio of 1:1. Large number

Table 3. List of SSR markers mapped in the genetic linkage map of pepper.

Marker	Chromosome/ Linkage group	Forward primer	Reverse primer	Position	Reference
ca07096	1	TCACAAAGATGGAGAAGGGAA	TCCAAAGGAGCACATTCACA	0.0	
ca15581	1	TCTAATAAAGCTAGTTCTTCAGCG	TTGACAAATTCTTCCGAGGG	18.0	
ca14771	1	CGAGCTAGGTACGTGCTTTGA	CACACTCAACGCTTTCCTCA	19.7	
ca15910a	1	CACACTGTTTCTTGCCTT	TTCTTCGTCTTGGTCATCCC	43.7	
cs170141	1	GGTCCTTTCATGCTGGGTAA	AGAACTTTCCTGCCCATCCT	44.5	
cs20006	1	TCCAGATTTTGCACTCGCTA	TGCTTCCACAACAAAATCCA	50.1	
ca11565	1	AACAAAACGCGCTAAAATGG	ACAAGTCATGGGAGAATGGC	55.7	
cs13023	1	AAAGAGGGGAGTTATGGCGT	GGTCAGAAGCAAAAGGGTCA	63.6	
AF39662	1	CCCCCTCGTCTCTCTTTATTT	TTGCAAATCTTTTGTCAATTTTT	67.9	Mueller et al., 2005
HpmsE004	1	TGGGAAGAGAAATTGTGAAAGCA	CAATGCCAACAATGGCATCCTA	69.6	Yi et al., 2006
ca18386	1	GCTCCTCATTAGTAGCCCCC	TGGACTTGGACAACCAATCA	69.6	
ca12144	1	ATTTTGATGCGTTGCTTTCC	CCACAAAAAGGGTGTTTGCT	72.6	
CP10061	1	ATCCCAAAAGGCAAAATC	CCCTTCCACATTCAGTCA	78.2	Mueller et al., 2005
ca12352	1	TTGTTCGGGAGTTCTCTTGG	ACAGCGAAAGTTGCTTCGAT	92.4	
ca14164	2	GACACGAAAAGCCGAAAGAG	TTTGGCTCGAGCTTTCACTT	0.4	
cs26050	2	ACCCTTCACTTGTGCCAATC	GTAAATTTCCTGCATGGCGT	7.9	
ca17182	2	CTCCGTTTCCGCCTTAAAAT	GGGAAAGATGGGCCATAAAT	35.6	
ca14289	2	GTCTTCTTCCATCGCTTTGC	TTCGAGGAAGTTTTCGCTGT	38.0	
ca00220	2	TCCAAAGGCAATTTCTGGAC	CTTTGGCAGTGTATCAGCGA	39.2	
ca02455	2	CAAGGCTCACACAGCATTCA	TAAATCTCCCATGGCTCCTG	40.9	
ca13319	2	TTCCCCTCCCTCTCTCTCTC	ACCCAGAACCCACAAAACAA	48.7	
ca12098a	2	TATGGCCTCATCTTCTCCCA	TTTGCAATTAAATCCTCGGC	68.4	
CA515055	2	TAATCGAGCGGTAGATTCGG	TAAGTGGAGGTGCCCTTCTG	77.8	Mueller et al., 2005
cs24043	2	ACCACTTTGGAGGAGGGAGT	CATCTGCTGTTGCTGCATTT	90.3	
GP20031	2	TGATCAGCGGACAAATCT	GGTGACACTGACCCCATA	92.2	Mueller et al., 2005
ca04782	2	ATCCCACAACAGTAGCCCAC	CCAGGGGTCTATCGAAAACA	106.9	
ca12891	2	ATTTCAAACCACCCGTTGAG	AAATCCGGAGAGGAAGGCTA	110.4	
cs24012	3	GAAGCACAACCTTCAGCCAT	GATAATTACCCGCCTGCTGA	49.7	
ca03461	3	CAAACGACCCTTCAGGGATA	CAAGAAAGTGTGCCCCAAAT	68.8	
cs26051	3	TTTGCAATGTCTTTGTTGCC	AGAATGCAACTCTTCAACTTTTT	87.8	
HpmsE005	3	TGCCTCAGTTTCCCAACCCT	ACCAACACCGTAACGCACCC	89.1	Yi et al., 2006
HpmsE053	3	TTCAAAGAATCCAGAGACTTCACA	TTCATGCAATTCCAAAGTCTCCA	99.4	Yi et al., 2006
ca07820	3	ACTGGCTGCAACTCACTCCT	TTTGACAAATAATGGTGCATGA	99.4	
cs09103	3	CTATTTGCTGCAGCCCTAGC	CCAGCTGAAGTAGTCCTCGG	100.2	
cs07014	3	TCTTGGTGGCACAAGTGAAG	TCAGCTTACGTTCACCTCCC	123.4	
BM61910	3	ATTGTGATAGCAACCCCTGG	CACAGATGAGGGCACAAATG	127.4	Mueller et al., 2005
ca07740	3	TCAGCATACGTGAAAGTCGG	CTCTCGTCCTCATCCTCGTC	128.2	
cs09087	3	ACGCCAAGAAAATCATCTCC	AGAGATGGAGACCTGAGCCA	140.1	
ca07449	3	TATGCCTACAGCGACAACCA	CCCTCAAGAATTCCCTCCAT	148.8	
ca11002	3	CTTGTTCCTTTTGTTTCGGG	AAGTCCCACACATAGCACACC	152.3	
ca14976	3	ATCTTCCACCCAATCACTCG	ACTGGGCTTGATGCTCTTGT	158.6	
ca00377	3	CAAAGTGCATCGACTTTCCA	GCTCTGTCATCTCCTGCTCC	166.6	
ca18075	3	GCCTTCTTTTTCATCTTTCCC	CTGGCAACCCAAGTCTTAGC	166.6	
ca18179	3	GTGTTTTGCTCCAATTCCGT	CCAGAGAAAACCCACAAAGC	168.8	
ca13889	3	AATGCAAAGTGGATCTTCGG	CATCCATTTACCAAAAACCAAAA	170.1	
BM62655	3	AGGAACGGCAGTCTTGCTAG	GATGCTAGGTCTGGATTCCTG	171.4	Mueller et al., 2005
HpmsE010	3	CTGTTTGCCAATCACCATCAGG	GCTATTTTCCGGCGTGTGAGAG	171.4	Yi et al., 2006

Table 3. Continued.

Marker	Chromosome/ Linkage group	Forward primer	Reverse primer	Position	Reference
HpmsE016	3	CCAAGTTCAGGCCCAGGAGTAA	TGCAGAGAAGACTCACCAGTCC	172.2	Yi et al., 2006
ca11558	3	CCTAACTAAGAGTGCGGGGG	CGACAGCCATACTCACGCTA	173.4	
ca06544	3a	GATATTATGGTCGTGGCGCT	TGACGTATCCGTCCAAAACA	0.0	
cs15031	3a	CACCTTTCAAAAGGGCATGT	TCAAATAGGCGGATTCTTCG	19.2	
ca00040	3a	GGGTGGTTGTGCTTGAAGAT	CGGTTCCACAATAATGGTAAA	26.3	
ca15286	3a	AGCAAGAGGATTGGGATGTG	TCAAAGAACCCAAAGGTGAAA	63.4	
ca14551	3a	CCTTCTGATTCCACCACTGC	AACAGCAACACCACCATTGA	77.5	
ca17713	3a	TGGTTGGTCAAACAAACAGG	CATGAGGAATCGCTGATTGA	81.5	
ca17316	3a	GACTCACACACAACAAAGAAATCA	GGGAATATACACTGGGCACG	82.5	
cs23047	3a	AGGAGGCAAATTTTGGGACT	CCGCTTCCTCCTCTTCTCTT	84.0	
ca05048	3a	AGGAGGCGAACCAAAATACA	GCGTGCAGTGATTTCTTCAA	86.6	
BM59622	3a	CGTCTTTCACTTGTCTTTTGTTC	AGTGGGTTCACTGACTTGGG	93.3	Mueller et al., 2005
ca13527	4	CCCAAACCTCACTTTCTTGC	CATGTGACAAACAGTAGCAGCA	15.0	
CAN13082	4	GCTAATTACTTGCTCCGTTTTG	AATGGGGGAGTTTGTTTTGG	25.6	Mueller et al., 2005
cs21036	4	TCCATCTCCCTCCTCTTCCT	GGGCCTTGATTAGCTTCCTC	48.0	
asu2	4	GGGTCTATCGGAAACAACCTTTCTAC	CTCTATGAATGGTGGGCCAGTAGTACCC	71.4	Mueller et al., 2005
ca04602	4	GCTTGTGGCCAAGGTTAAAA	AATTTTTCCGAGTTTGGCCT	89.8	
ca00635	4	TCATTTGTTGGCAGCTGTTT	CACCCCTTTAGATTCTCCTCC	212.7	
cs13070	4	GTTTAACAGAGGCGACGGAG	GAGCGAAATCAGAGAAACCG	214.0	
CB164897	5	GGGACGTATTTTCGAAGAGG	CTTCGCCTTGTTGACTAGGG	0.0	Mueller et al., 2005
HpmsE015	5	TTGTGAGGGTTTGACACTGGGA	CCGAGCTCGATGAGGATGAACT	69.2	Yi et al., 2006
ca07831	5	GTGTGGGATGTGCTTGATTG	TTTTAGACAAGCCCCCAAAA	132.2	
cs10113	5	AATTTGCAATACCAGCTCCG	AGGCTCGAGAGACTTACCGA	133.0	
ca16279	5	TCTCGATTTTGCGATCTTCA	TTCGTCCTTCCTGTTTCCAC	138.0	
CA524065	5	TCTCTCTCTACATCTCTCCGTTG	TGTCGTTCGTCGACGTACTC	185.1	Mueller et al., 2005
ca02059	5	CATTGGATCTTTTGGGTTGG	ACTGCTAATGGACATACACA	215.9	
ca00807	6	CGTCATCCATTTTTCTCAACAA	TGCTCAAATCCACTGTCTGC	0.0	
ca12797b	6	GGGAGATATGGTGGTGATGG	TACCCTCTTCAACGATTGCC	8.6	
ca05311	6	GGAATTCTGCAGGGAAATGA	CCTACGGCCCACAATAAGAA	25.3	
ca16272	6	CGAACGAATCCTTATCCACG	CGCGCTTGATGAATCTTGTA	60.0	
cs10008	6	GAATGAGTCTTCTGGTGCTGG	AGCAAGCAGGGTATGATCCA	76.8	
CA523558	6	AATCCTCCAAATCCACCCTC	ATTCGATTGCTTGCTCCTTG	86.4	Mueller et al., 2005
CA516044	6	ATCTTCTTCTCATTTCTCCCTTC	TGCTCAGCATTAACGACGTC	97.6	Mueller et al., 2005
ca01483	6	TGCACAGGACTTTTCTTCCC	CGTTAAAGCACCATTTCCGT	97.6	
cs15052	6	TTTTTGGAGCAGGATGTTCA	ATTTTGCGATCCAAACTTGC	103.2	
ca12368	6	ATATAAAAGGGCCCCACAGG	ATCCCATCCATGTGTGTGTG	107.7	
GP1102	7	GAACCCTTCATTCCTGTATGT	TTTGCCCGCATTATGTAAATC	4.5	Mueller et al., 2005
ca15531	7	GCTGGACCAAAATGGAAGAA	CAACCCATCATTTCTCTGTGG	19.1	
ca15597	7	TCATATGGGCATTTTCAATGTT	TCGAGATCTGTTTGGTGCTG	28.2	
ca12098b	7	TATGGCCTCATCTTCTCCCA	TTTGCAATTAAATCCTCGGC	85.5	
ca01678	7	ATTCCACTCAATTCAAAACT	ACTCTTCGCCGCTATTTTCA	93.2	
ca13839	7	GGAGATTTATCTTCGAACTTTCTTC	AAACTTGCGTTGTCCGATTC	110.1	
ca17522	7	TGTGCAGATGGAATTACCCA	TGCTATTCCGGCTTGAAATC	117.4	
ca13629	7	AGGGTTTTGATTTTGCATCG	ATCGGAGTGCGTTCCATTAG	122.4	
cs24046	7	AGGTGGGTACGCACGATAAG	CTCGCTTCCTGATGAAGACC	123.6	
ca04384	8	GACTTTACTTTCACCTCCCTTG	TTGATTGCCCTTTTCTCACC	7.7	
CA526211	8	TTGGGGACTTCACGTCTCTC	TTGATGATAAATCCTCCCCC	112.4	Mueller et al., 2005

Table 3. Continued.

Marker	Chromosome/ Linkage group	Forward primer	Reverse primer	Position	Reference
ca12261	8	AGGGAGAAGCCAACAACACA	TCTTCCTCTTTTTGGAGGCA	134.5	
cs170520	8a	CTCCAGATTGTAACGCACT	CGCTCATTCTCAATGATCCTG	0.0	
CP10020	8a	GGGAAGGCCATTAGATGT	TATCGGCTACTGGGAATG	1.7	Mueller et al., 2005
ca04813	8a	AACACCCTTACACCCGAACC	GGAAAACGATCACGGAGAAA	38.7	
ca10396	9	CACTTTGCCCTTTCCACATT	CAACCCAAGAAAACCCATTG	12.9	
HpmsE082	9	TTTTTCCCACTTTGCCCTTTCC	CAACCCAAGAAAACCCATTGGA	13.3	Yi et al., 2006
ca16205	9	GCCCCAAAACAAAACACTTC	ATGGGTTATGGGGTTGTTGA	21.0	
HpmsE007	9	CCCCATTTCCCCTTCCCATA	GAGGGGTCATGTTGAAGGCAA	22.2	Yi et al., 2006
ca02136	9	ATGTAGGAGCCTTGGTGGTG	GAGGTAGCGCTATGGACTGC	140.4	
HpmsE025	9	TGAGCATCCCGTTATCTCAAATCA	CCCAATTCTTCAGGCAATCTCC	152.7	Yi et al., 2006
cs17037	10	AGACTTGAACCCGTGACCAG	TTGTTGTTTGAAAGGGAGCC	0.0	
ca12800	10	CACAAAAACGAAAACCTAGTG	ACATGATGATCCAGATGCCA	19.1	
GP20068	10	TTCCCTGTGAAAACACTG	TGTTCAACTGCTCTGAGAC	40.1	Mueller et al., 2005
ca12797a	10	GGGAGATATGGTGGTGATGG	TACCCTCTTCAACGATTGCC	50.0	
ca03308	10	ATCGATGGAGAATGGAGTGC	GCCTCTGTATAACAATTCAACGG	80.4	
ca11895	10	TCTGCACATATCGGAGCAAG	CCCGGTATTTTTACTATGTTTGC	117.7	
ca16293	10	CGATGAAATCCCACAAGTGA	GTGCCATCTGAATCGACCTT	162.6	
CA516439	10	GACAGTCTTTCAAGAACTAGAGAGAG	TGGAGCAAACACAGCAGAAC	164.3	Mueller et al., 2005
ca15660	10	TTCAAGAACTAGAGAGAGAAAACT	TGGAGCAAACACAGCAGAAC	164.3	
cs19002	10	AAAAAGAAACCTCCCTTCACG	TCTCCCTCCTCCCTCTGTTT	164.7	
HpmsE031	10	CCCTAAATCAACCCCAAATTCAA	CCCCCATTACCTGACTGCAAAA	187.2	Yi et al., 2006
ca07185	11	TCTCCTGTTTTCCGATGCTT	CGCAAAATGATTTAGGTGTGG	51.1	
CA525390	11	GGAAACTAAACACACTTTCTCTCTC	ACTGGACGCCAGTTTGATTC	95.1	Mueller et al., 2005
cs23011	11	CTATGGCCTCCAACCAGAAA	TGAAACCCACTCCCATCATT	95.1	
GP20087	11	CCCTCTCCTCAATTCACA	CCTTTACCCCTAAATTTGAT	130.2	Mueller et al., 2005
HpmsE023	11	TTTAACACCTCTCTAACCGTCACC	GCGATTTCAGCCCATCAACAAT	162.5	Yi et al., 2006
ca03079	11	AAAAACCAGGAGCAGATGGA	ACAATGGGACATCCCACATA	179.0	
ca11483a	12	TGGGGAACAGAGGAAGAAGA	TCCACTTGCATGAACTTGCT	3.0	
ca11483a	12	AGGCTTGATGAACTGTTGCC	GCATCGTAGCGCCTTTCTAC	3.0	
cs21031	12	AATGATGGCAACAACAGCAA	TATTGCAGCATTTGGACTGC	23.0	
GP1127	12	CACCACCAGTCACAAAGTTAC	CCCTTCAAATACATCCCATGC	40.3	Mueller et al., 2005
cs10102	12	TCACTGCAACCAACAATTTCA	ACCCCTTTGTGTCTGCTTTG	79.0	
GP1017	12	TTTTGATCCCTCGATAAGTCTTT	TCACACCAGACTCAGCCAATTTA	135.5	Mueller et al., 2005
ca08223	12	ATGGAGATCGCAACCTCATC	GCGGCAAGAAGATGAAAGTC	163.1	
cs16031	12	ATCTTTCATCCCTTTGTGGC	TTCGCCTCTGTTTCGATTCT	163.9	
ca14517	12	TGCTTCTTTTCTACGCCCAT	CTTTGAAAGGCAATTTGGGA	165.6	
cs240430	12	TTCATATATGCAACCGCCAA	AAACCAGGACCAAAAACACG	167.3	
ca16392	12	CATGGTTTCTGCTGACGTGT	TCCAAGAAATACCACACCCA	175.1	
ca05802	12	CTACCCAGATTCCACTGCGT	GGTTTGATCTCCCTTGTGCT	176.9	
HpmsE064	12	CCCTCCTTTTACCTCGTCAAAAA	ATGCCAAGGAGCAATGAGAACC	180.4	Yi et al., 2006
ca11907	12	TGCGGTGTGCTAAATAGTGC	GCTGTTGCTACTCGCAATGA	191.6	
ca04827	LGb	AAATTGGAATTGAAAGGGGG	TGTTGGAGCCATGTCAGAAG	16.1	
ca16104	LGc	GCTGTAGTCTTCGGTTTGCC	TTCAGACGGTATACGCACCA	20.6	
ca16955	LGd	GGAGTTGGATATTCGCGTGT	AGTGCTGCAGTTCCCAGAAT	0.0	

of markers that exhibited segregation distortion in this study could be because mapping population was selected from 200 F_8 recombinant inbred lines. Markers deviating from expected segregation ratio are generally believed to be linked to genes

Table 4. Characteristics of the intraspecific genetic linkage map of pepper.

Linkage group	Length (cM)	Number of markers	Average distance between markers (cM)
LG1	84.5	17	5.0
LG2	127.5	25	5.1
LG3	102.3	22	4.7
LG3a	158.7	39	4.1
LG4	202.8	40	5.1
LG5	200.2	25	8.0
LG6	94.2	13	7.2
LG7	113.1	20	5.7
LG8	117.1	20	5.9
LG8a	33.1	6	5.5
LG9	193.9	48	4.0
LG10	193.9	36	5.4
LG11	202.5	39	5.2
LG12	167.8	39	4.3
Lga	119	14	8.5
LGb	18.3	5	3.7
LGc	18.7	4	4.7
LGd	18.8	4	4.7
LGe	11.1	4	2.8
Total	2177.5	420	5.2

that are subjected to direct selection; for example: a lethal allele in *Populus* spp. affecting embryo development was the cause of segregation distortion of markers (Bradshaw and Stettler, 1994); markers cosegregating with the *Melampsora* resistance gene also showed a significant deviation (Cervera et al., 2001). Therefore, all distorted markers in this study should be used in the mapping process to avoid missing of parts of linkage groups. The distorted markers were not more specific to either of the parents.

Consensus SSR makers and linkage groups

A total of 37 consensus markers were placed in the linkage map. Of these, 22 anchor SSR markers from the Pepper-FAO3 map (Mueller et al., 2005) were distributed into 13 linkage groups except LG9, LGa, LGb, LGc, LGd, and LGe. The order and distribution of the most anchor markers were consistent with the reference map (Fig. 2). Thus, these linkage groups were assigned into 11 chromosomes of pepper except chromosome 9. The alignment was successful for all chromosomes; however, grouping of more than one LG of the intraspecific map with single LG of the interspecific map was still observed (Fig. 2). The linkage groups LG3 and LG8 in the present map were split into 2 LGs each. Linkage group LG3 was grouped along with LG3a, and LG8 along with LG8a. This might be due to subsequent resolution of the sequences that joined the linkage groups in the interspecific mapping populations. By developing intraspecific maps for *C. annum* using consensus SSR markers and comparing them might provide the molecular insight of the likely chromosomal rearrangements that led to the evolution of *C. annuum*. Thirteen consensus SSR markers from the SNU3 map were distributed into 8 linkage groups (LG1, LG3, LG5, LG7, LG9, LG10, LG11, and LG12). The dispersion of the markers in the linkage groups was consistent with 8 chromosomes (1, 3, 5, 7, 9, 10, 11 and 12) in the reference map. Though there was no anchor SSR marker on chromosome 9, 3 consensus SSR markers, which belonged to chromosome 9 in the SNU3 map, were placed on LG9 in the present linkage map. This could demonstrate that LG9 belongs to chromosome 9 of pepper. The orders of these SSR markers on each linkage group were the same as those in the Pepper-FAO3 and the SNU3 maps except for some minor differences for some markers. Thus, 12 chromosomes of pepper were assigned. Because there were no consensus markers in the other 5 linkage groups, they were named LGa, LGb, LGc, LGd, and LGe. We expect that the small linkage groups will merge into larger linkage groups when more markers are assigned.

Discussion

Recombinant inbred line population is particularly useful in genetic mapping studies and quantitative trait locus analysis. It is the basis for Mendel's first genetic experiments and continues to be the key to the study of genes, heredity, and genetic variation today. Improving of precision linkage mapping using such population for further QTL analysis of interested traits is needed.

Polymorphism between *C. annuum* YCM334 and 'Tean'

Low levels of DNA marker polymorphism in crops is an obstacle to apply molecular marker technology in breeding programs. In *Solanaceae* crops, levels of polymorphic loci are generally low within each species (Minamiyama et al., 2006; Nunome et al., 2001; Terzopoulos et al., 2008). Therefore, degree of marker polymorphism in an intraspecific population is lower than in an interspecific population. In the present study, 67 SSR markers selected from the Pepper-FA03 map (Mueller et al., 2005) was used for screening polymorphism between *C. annuum* YCM334 and 'Tean', but only 37.3% were polymorphic. A lower polymorphism rate (11%) was also observed in a set of 135 SSR primers from the SNU3 map (Yi et al., 2006), and 1667 EST-derived SSR primers from KRIBB. Minamiyama et al. (2006) have observed the low levels of polymorphism within *C. annuum*. To overcome this low polymorphism, 171 AFLP primer combinations were used. Level of polymorphism (76%) in this study was higher

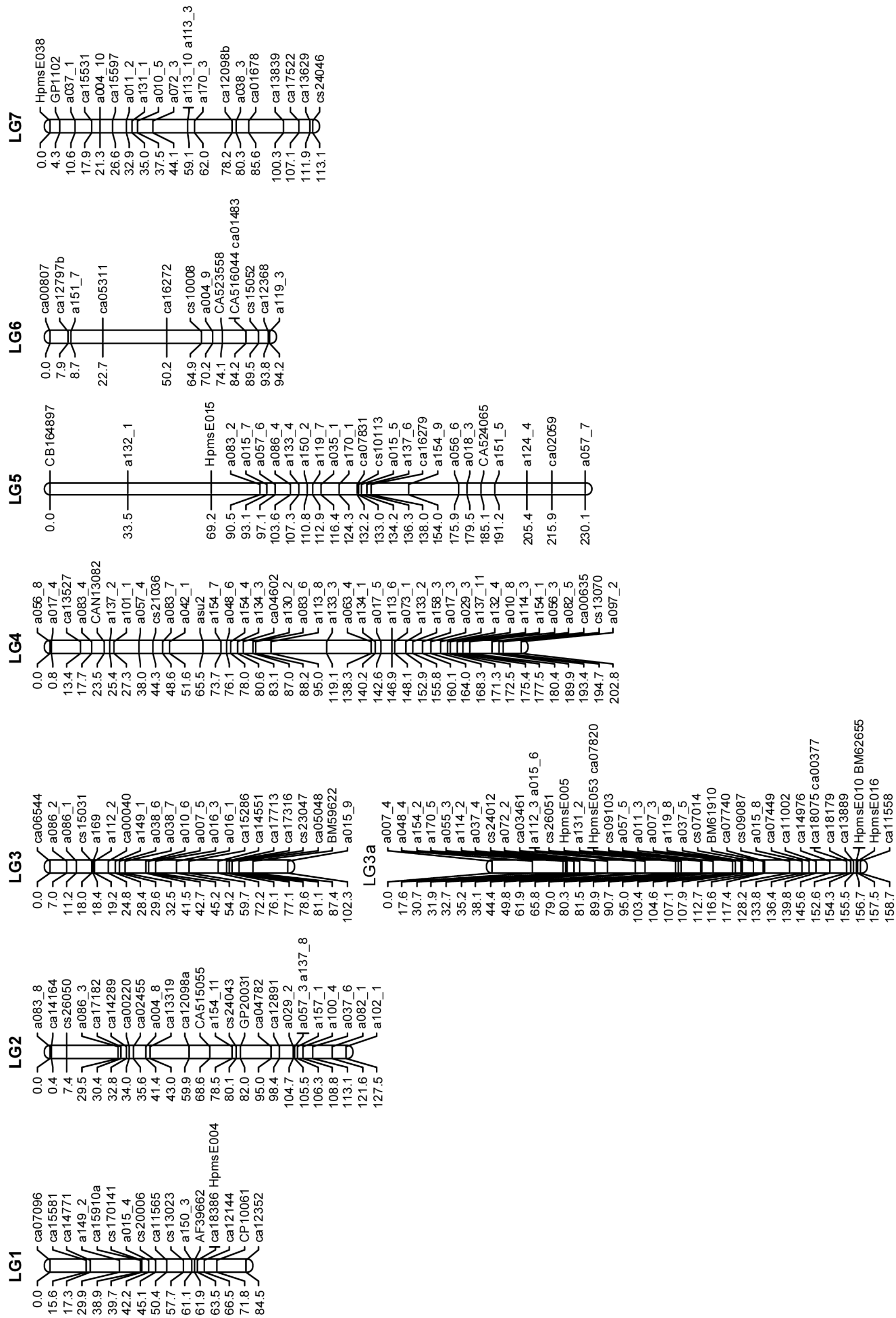

Fig. 1. A genetic linkage map of pepper on an intraspecific RIL population derived from a cross between YCM334 and 'Tean' at a LOD score of 7. Loci names are indicated on the right side of the vertical bars and genetic distance in centiMorgans (Kosambi function) are on the left side of the vertical bars. AFLP markers were named consisting of 1 letter as "a" and following was primer combination code and number of polymorphic bands generated by its primer combination. EST-SSR markers were named consisting of 2 letters as "ca" or "cs" and serial numbers followed by the letters. SSRs-Pepper-FAO3 map (Mueller et al., 2005) and SSRs-SNU3 map (Yi et al., 2006) were named as their origin names.

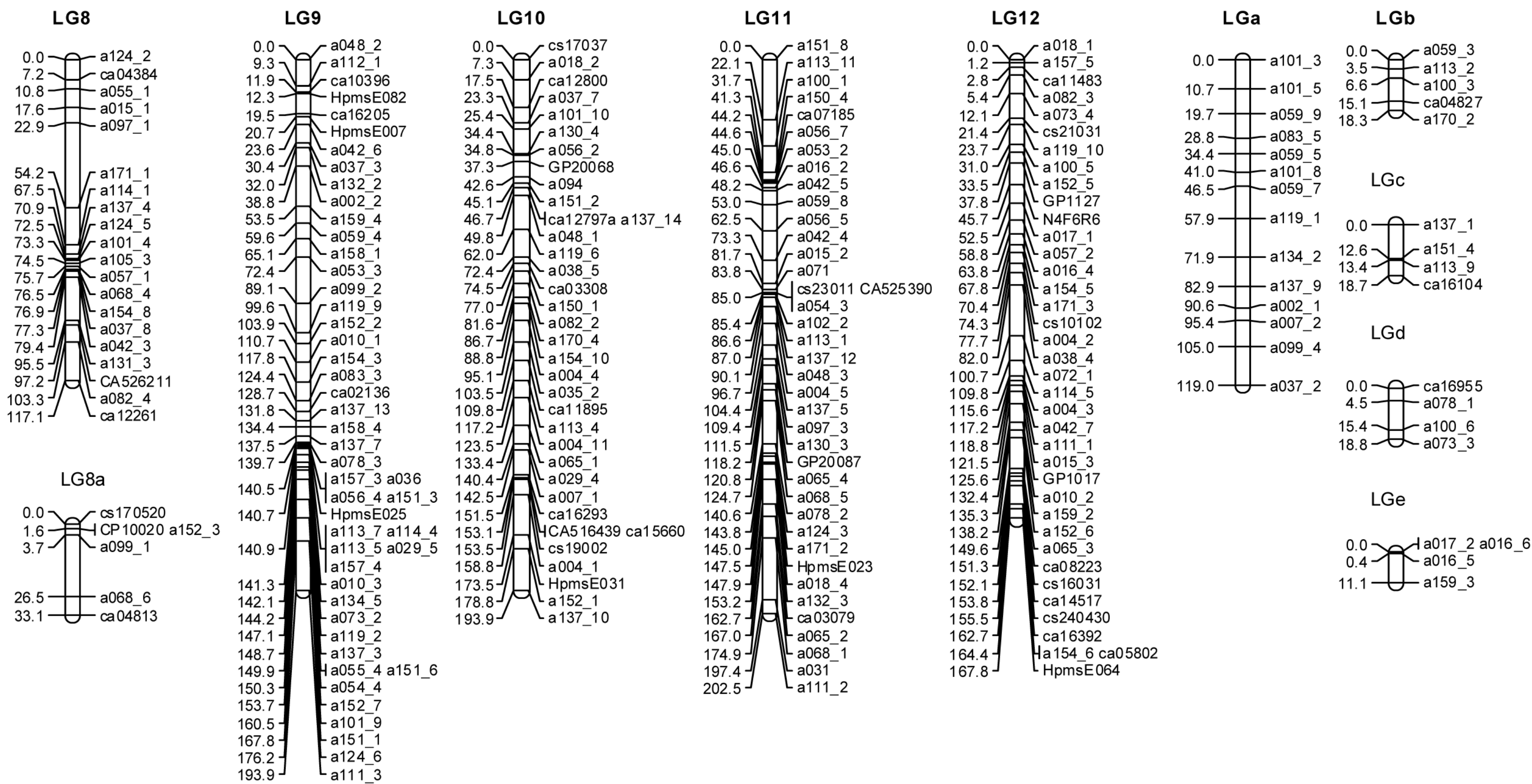

Fig. 1. Continued.

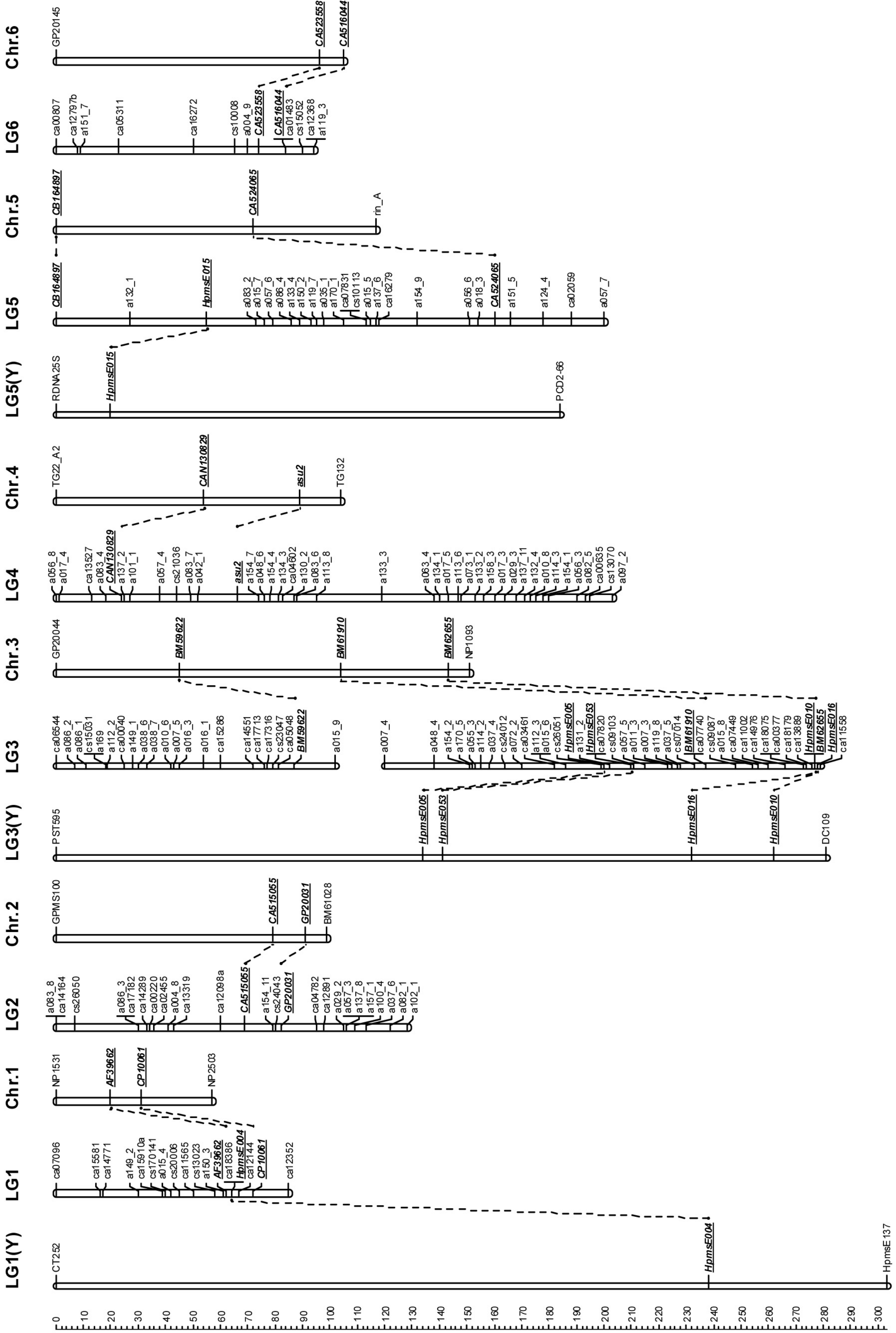

Fig. 2. Comparisons of distribution and order of the consensus markers of the 12 linkage groups (LG1-LG12) with those on chromosomes of pepper from Pepper-FAO3 map (Chr.) (Mueller et al., 2005) and SNU3 map (LG(Y)) (Yi et al., 2006).

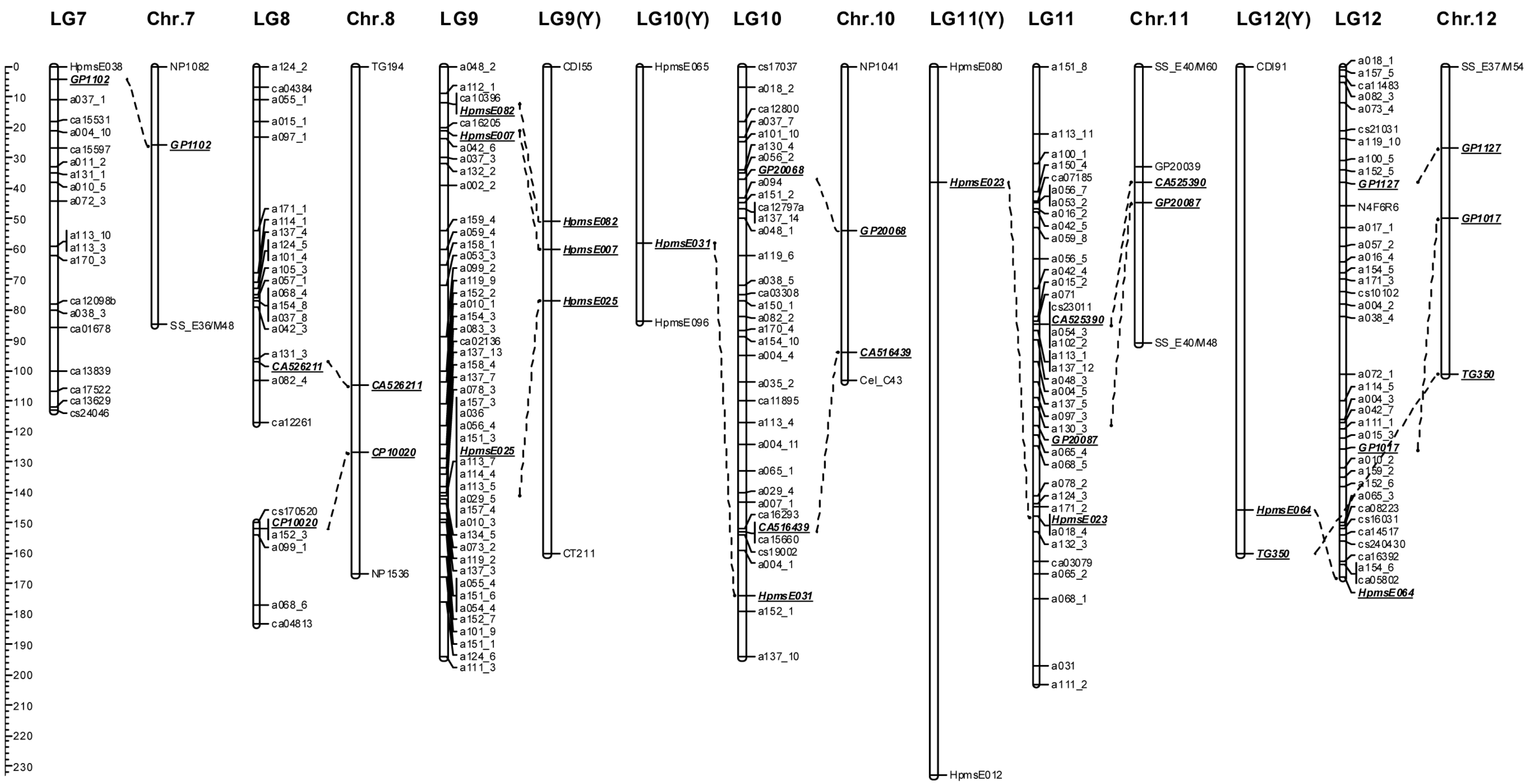

Fig. 2. Continued.

than previous study using an interspecific cross (Kang et al., 2001), indicating the diversity of restriction sites in YCM334 and 'Tean'. One explanation is that YCM334 were derived from a cross between non-pungent and pungent pepper; during evolution and plant breeding process, genetic recombination occurrence resulted more diversity. In addition, YCM334 is a western-style pepper with bell-shape fruit, whereas 'Tean' is a Korean local variety with long fruit shape and a very spicy flavor. Thus, genetic background of these two parents is a major factor of the polymorphism observed. Therefore, this should be a good source for breeding towards improving resistance to *Phytophthora* root rot and other horticulture traits in pepper.

Comparative mappings in pepper

In this study, we mainly compared the present map with two published maps: SNU3 (Yi et al., 2006) and Pepper-FAO3 (Mueller et al., 2005), which conducted using interspecific mapping populations. The composite map developed in this study consisted of 420 markers distributed over 19 LGs and covered a total genetic distance of 2177.5 cM. Comparison of the present intraspecific map with the interspecific maps developed by Yi et al. (2006) and Mueller et al. (2005) revealed high linkage conservation in at least four linkage groups. While 11 of the 14 LGs in the SNU3 map were determined 11 chromosomes and chromosome 8 was not assigned (Yi et al., 2006), 14 LGs of the present linkage map were assigned into 12 chromosomes of pepper. However, the map distances differed. While the lengths of LG1 and LG7 were very close to the lengths of chromosomes 1 and 7, respectively, in the SNU3 map, they were about of 60 cM and 210 cM shorter, respectively, in the Pepper-FA03 map (Fig. 2). In the contrary, the lengths of LG5, LG9, LG11 and LG12 were similar to the length of chromosomes 5, 9, 11, and 12 in the SNU3 map respectively; but they were more or less 100 cM longer than in the Pepper-FA03 map. These differences could be possibly due to the intraspecific nature of our mapping population. Within *C. annuum* genome, the present linkage map was slightly longer than those previous maps (Barchi et al., 2007; Kim et al., 2008; Lefebvre et al., 1995; Minamiyama et al., 2006; Ogundiwin et al., 2005). This can be explained by the dramatic increase of marker numbers in the present mapping population. The larger number of markers within one linkage group may enlarge the genetic intercrossing value between markers. Additionally, the small size of the F_8 population (126 individuals) compared with 176 DH individuals in Sugita et al. (2005) may not be enough for allele segregation and cause allele partial distribution. The five linkage groups such LGa, LGb, LGc, LGd, and LGe could be merged into chromosomes when more markers are assigned.

The distances between 2 anchor SSR markers on chromosomes 1, 2, 3, 4, and 6 in the Pepper-FAO3 map were similar with those in LG1, LG2, LG3a, LG4, and LG6, respectively (Fig. 2), indicating those markers are highly conserved across *Capsicum* species. However, distances between anchor markers in the LG10, LG11 and LG12 were greater than of those on chromosomes 10, 11, and 12, respectively. Two anchor markers, which were 20 cM apart, on chromosome 8 in the Pepper-FAO3 map were mapped into 2 linkage groups, one was on the distal end and one was on the top. Three markers on chromosome 3 were mapped to 2 linkage groups (LG3 and LG3a). More comprehensive coordination among the *Capsicum* maps would be helpful for pepper genetics and breeding. The order and distribution of the consensus SSR markers in the present linkage map was consistent with those in the SNU3 map except some minor differences.

In pepper, total genome length was estimated to be between 1,498 cM and 2,268 cM (Lefebvre et al., 1995). Thus, the present map was deeply covered the genome of pepper and will be useful as a reference map in *Capsicum annuum* and should facilitate quantitative trait locus analysis and the use of molecular marker in pepper breeding.

Potential applications of comparative mapping results

Comparative mapping is an important tool for integrating genetic data among related taxa. It helps to consolidate genetic maps and bridge linkage gaps. For instance, comparative mapping has helped to assign several small-unlinked groups to the larger homologous linkage groups in pepper (Barchi et al., 2007; Lee et al., 2004; Lee et al., 2008; Wu et al., 2009; Yi et al., 2006). Mapped consensus markers that consistently associated with the same QTL can be used to confirm and verify QTL, and to identify candidate genes for quantitative traits. For example: QTLs mapped on *C. annuum* (Kim et al., 2008; Ogundiwin et al., 2004) can be now compared across different populations if those consensus SSR markers were placed on different genetic maps.

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