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Molecules and Cells

Construction of an Integrated Pepper Map Using RFLP, SSR, CAPS, AFLP, WRKY, rRAMP, and BAC End Sequences

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Map-based cloning to find genes of interest, marker-assisted selection (MAS), and marker-assisted breeding (MAB) all require good genetic maps with high reproducible markers. For map construction as well as chromosome assignment, development of single copy PCR-based markers and map integration process are necessary. In this study, the 132 markers (57 STS from BAC-end sequences, 13 STS from RFLP, and 62 SSR) were newly developed as single copy type PCR-based markers. They were used together with 1830 markers previously developed in our lab to construct an integrated map with the Joinmap 3.0 program. This integrated map contained 169 SSR, 354 RFLP, 23 STS from BAC-end sequences, 6 STS from RFLP, 152 AFLP, 51 WRKY, and 99 rRAMP markers on 12 chromosomes. The integrated map contained four genetic maps of two interspecific (*Capsicum annuum* 'TF68' and *C. chinense* 'Habanero') and two intraspecific (*C. annuum* 'CM334' and *C. annuum* 'Chilsungcho') populations of peppers. This constructed integrated map consisted of 805 markers (map distance of 1858 cM) in interspecific populations and 745 markers (map distance of 1892 cM) in intraspecific populations. The used pepper STS were first developed from end sequences of BAC clones from *Capsicum annuum* 'CM334'. This integrated map will provide useful information for construction of future pepper genetic maps and for assignment of linkage groups to pepper chromosomes.

INTRODUCTION

A genetic map is a basic tool necessary for marker assisted selection (MAS) in breeding and to search for genes of interest. Many pepper genetic maps have been constructed in the course of studying this plant. Up to now, reported genetic maps have been divided into two types: interspecific (Livingstone et al., 1999; Rao et al., 2003; Yi et al., 2006) and intraspecific (Barchi et al., 2007; Ben Chaim et al., 2001; Lefebvre et al., 1995). Most of the maps consist of multi-copy markers such as amplified fragment length polymorphisms (AFLPs) and random amplified polymorphic DNA (RAPD, Barchi et al., 2007; Ben Chaim et al., 2001). Multi-copy type markers generate many amplified bands which when visualized on a gel makes it difficult to precisely pinpoint the bands used for the marker and to map the markers on other maps for comparison. In contrast, a previously characterized single copy marker can be easily compared with a newly developed one even if the marker is used in different populations because it generates only a few bands on a gel. Because of the ease with which these markers can be mapped in different populations, single copy type markers such as restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), and sequence tagged sites (STSs) are generally preferred by researchers. RFLPs, a single copy type marker, is Southern blot-based, while SSR (Chiba et al., 2003; Han et al., 2006; Hearnden et al., 2007), SNP (Hayashi et al., 2004; Troggio et al., 2007), STS (Bao et al., 2006; Bowers et al., 2003), sequence-related amplified polymorphisms (SRAPs; Sun et al., 2007), and bacterial artificial chromosome (BAC) markers (Frelichowski et al., 2006) are PCR-based. The latter single copy markers have been used more extensively than multi-copy type markers in cotton, sorghum, cabbage, rice, barley, and grape, although they are still laborious, expensive, and time-consuming to develop. Single copy type markers such as SSRs (Barchi et al., 2007; Lee et al., 2004; Minamiyama et al., 2006; Yi et al., 2006), STSs (Barchi et al., 2007), SCARs (Ogundiwin et al., 2005) and CAPS (Lefebvre et al., 2002) have been developed for gene tagging and map construction.

Integrated maps have been constructed in several crops such as cucumber, lettuce, and rose (Bradeen et al., 2001; Truco et al., 2007; Yan et al., 2005). The integrated map can provide comprehensive marker information on several different maps through comparison of anchor markers located on those maps. An integrated map (Paran et al., 2004) in pepper was built using six mapping populations and contained 440 RFLP, 288 RAPD, and 1528 AFLP markers. In order to effectively apply the information from this integrated pepper map to other

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mapping populations, more PCR-based single copy type markers are needed. In this study, four genetic maps containing AFLP, RFLP, reverse random amplified microsatellite polymorphism (rRAMP, Min et al., 2008), SSR, STS from BAC-end sequences, STS from RFLP, and WRKY markers were used to construct an integrated map. This new integrated map contains a larger number of single copy type markers than any previously reported pepper map. The markers developed for an integrated map represent good common markers that can be used for different mapping populations, and the integrated map will be useful as a reference pepper map.

MATERIALS AND METHODS

Four maps and their mapping populations

To construct an integrated map, we used four individual maps: SNU3 containing SNU2, SNU4, SNU5, and SNU6. SNU2 (Lee et al., 2004) and SNU3 (Yi et al., 2006) maps were constructed using the 107 F_2 from a cross between *C. annuum* 'TF68' and *C. chinense* 'Habanero'. The SNU4 map (Kim et al., 2008a) was built using the 100 F_2 individuals from a cross between *C. annuum* 'CM334' and *C. annuum* 'Chilsungcho'. The SNU5 map (Kim et al., 2008b) was constructed using 100 F_2 individuals from a cross between *C. annuum* 'CM334' and *C. annuum* 'Chilsungcho'. The SNU6 map (unpublished data) was constructed using 93 F_2 individuals from a cross between *C. annuum* 'TF68' and *C. chinense* 'Habanero'. Mapping populations for SNU2, 3, 4, and 5 were grown in a greenhouse at Seoul National University, Suwon, South Korea, and the population for the SNU6 map was grown at National Horticultural Research Institute (NHRI), Suwon, South Korea. Genomic DNA was extracted from young leaf tissues of each parent, F_1, and F_2 plant as previously described (Bruce et al., 1997).

AFLP

AFLP was performed as previously described by Vos et al. (1995) with minor modifications in the adaptor ligation and the visualization of PCR bands. The modifications were done as previously described (Kim et al., 2008b).

RFLP

CAN, CD, CT, DC, PCD2, PST, and TG markers were used as previously described (Kang et al., 2001). Disease-related EST clones of tobacco and pepper were provided by D. Choi (KRIBB, Daejeon, Korea), and markers from the probes were designated as Tob and CDI, respectively. Potato clones adjacent to *Phytophthora* resistance loci in potato were amplified with reported primers (Collins et al., 1999; Oberhagemann et al., 1999) to obtain GP and StPto markers. Thirty-three homologous clones of NBS-LRR were isolated from 'CM334' using degenerate PCR with primers designed from conserved sequences of NBS and LRR regions (Kanazin et al., 1996; Wenkai et al., 2006).

rRAMP

The $(AG)_8$ and $(AAG)_6$ SSR motifs were used as core sequences in anchored primers which had all possible variations of dinucleotides at the 3′ end. All possible combinations of 32 anchored primers and 103 UBC primers were used to screen parental DNAs and 93 primer pairs were finally selected for further use based upon both the reproducibility of amplified bands and the detection of more than six polymorphic bands. rRAMP was performed as previously described by Min et al. (2008).

STS from BAC-end sequences

A BAC library (Yoo et al., 2003) containing 12X genome equivalents from *C. annuum* 'CM334' was used for development of STS from BAC-end sequences. For the STS from BAC-end sequences, 52 BAC clones were obtained from a BAC library screened with an mRNA probe from pepper flower, and 22 BAC clones were randomly selected. Supercoiled BAC plasmid was isolated using the alkaline lysis procedure (Sambrook et al., 1989) from 6 ml of *E. coli* culture which was grown overnight in 2 × LB liquid media with 12.5 μg/ml chloramphenicol at 37°C. The DNA pellet was dissolved in 30 ml sterile water supplemented with 100 μg/ml RNase. BAC end-sequencing from the T7 end of the BAC plasmid was performed using an ABI Prism 3730 XL DNA Analyzer (PE Applied Biosystems, USA) at NICEM (Seoul National University, South Korea). To obtain sequences greater than 1.5 Kb in length, BAC plasmids were sequenced two more times. After removing vector sequences, the remaining BAC insertion sequences were analyzed using the pepper EST database (http://plant.pdrc.re.kr/sol) and the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/). SSR repeat motifs were identified using RepeatMasker (http://www.repeatmasker.org). To develop STS from BAC-end sequences, primers were designed by Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) from the BAC end-sequences. PCR amplification was carried out in a 20 μl reaction volume containing 20 ng genomic DNA, 0.5 μM each primer, 1 mM dNTPs, 0.5 unit Taq DNA polymerase (Takara, Japan) and 1× buffer solution pH 8.3 (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl). All amplifications were performed in a Mycycler (BIO-RAD, USA) and a Peltier thermal cycler PTC-200 (MJ Research, USA) as follows: denaturation for 3 min at 94°C; 40 cycles of 30 s at 94°C, 45 s at 55, 58, or 60°C, 1 min 30 s at 72°C and a final elongation for 10 min at 72°C. The monomorphic PCR fragments amplified from *C. annuum* 'CM334', 'Chilsungcho', 'TF68', and *C. chinense* 'Habanero' were digested by 30 restriction enzymes (NEB, USA; Roche, Germany). The enzyme digestion occurred in a total reaction volume of 10 μl containing 5 μl PCR product, 1× reaction buffer, and 1 unit restriction enzyme, and the reaction was incubated for 8 hours at 37°C. The enzyme-treated PCR products were separated on 1% agarose gels. PCR primers producing polymorphic bands between parent lines were applied to the four F_2 populations.

STS from RFLP

A *Pst*I genomic library was constructed using Uni-ZAP II vector kit (Stratagene, USA) and PST clones (0.5-2.0 kb) were sequenced at NICEM (Seoul National University, South Korea). All PST amplifications with primers designed from the clone sequences were performed in a Peltier thermal cycler PTC-200 (MJ Research, USA). An initial denaturation for 5 min at 94°C was followed by 40 cycles of 30 s at 94°C, 1 min at 57°C, 2 min 30 s at 72°C and a final extension step for 10 min at 72°C. The amplified monomorphic fragments between parents were digested by 30 restriction enzymes (NEB, USA) at 37°C for 16 h. Each digestion reaction was in a total volume of 15 μl with 5 μl PCR product, 1.5× reaction buffer, and 0.5 units restriction enzyme. All PCR products were separated on 1% agarose gels.

SSR and WRKY markers

One hundred forty-five SSR markers (Lee et al., 2004; Yi et al., 2006) and 109 SSR markers (Minamiyama et al., 2006; 2007) were generated as previously reported (Minamiyama et al., 2006; Yi et al., 2006). The newly developed HpmsF, HpmsG, and HpmsK SSR markers were from primer sets which did not shown polymorphic bands for the parents in the SNU3 map (Yi et al., 2006).

The conserved sequences of the WRKY domain of each group (I, II, and III) were amplified to develop WRKY markers (Kim et al., 2008b).

Map construction

Linkage analysis of molecular markers was performed using the Joinmap 3.0 program (Van Ooijen and Voorrips, 2001). The Chi-square test was carried out with goodness of fit to the segregation ratio (1:2:1 or 3:1) at significance $P < 0.05$ with F_2 populations. The Kosambi map function (Kosambi, 1944) was used for converting recombination frequency into genetic distance with a LOD score of 6 and recombination rate of 0.4. Highly distorted markers ($P < 1.00E-4$) were discarded from further study. Linkage groups containing more than two common markers in each map were selected and integrated using the "Combine the Groups for Map Integration" function. The Haldane map function in the integrated map was used with the same parameters as previously mentioned for the Kosambi map function. All linkage maps were drawn and the order of markers was compared using the MapChart 2.2 program (Voorrips, 2002).

RESULTS

Information on SNU map construction

For construction of an integrated pepper map, we used four maps (SNU3 containing SNU2, --SNU4, SNU5 and SNU6). The previously developed SNU2 map (Lee et al., 2004) consisted of 287 RFLP and 42 SSR markers from an interspecific population (*C. annuum* 'TF68' × C. *chinense* 'Habanero'). The SNU3 map (Yi et al., 2006) consisted of 150 new SSR markers together with 30 SSR and 63 RFLP markers from the SNU2 map of the same population. SNU4 was composed of 207 RFLP markers in a *C. annuum* 'CM334' × C. *annuum* 'Chilsungcho' population (Kim et al., 2008a), and the SNU5 map contained 199 AFLP, 8 RFLP, 97 rRAMP, 26 SSR and 41 WRKY markers in another *C. annuum* 'CM334' × C. *annuum* 'Chilsungcho' population (Kim et al., 2008b). The SNU6 map was constructed of 234 AFLP, 278 rRAMP, 62 SSR, and 143 WRKY markers in another *C. annuum* 'TF68' × C. *chinense* 'Habanero' population (unpublished data). In addition to these markers in four mapping populations, we developed new BAC, STS, and SSR markers.

Each published map (SNU3 containing SNU2, SNU4, SNU5 and SNU6) had been constructed by the Mapmaker or Carthagene programs, but to integrate four maps in this study, these maps were reconstructed by the Joinmap program and drawn by MapChart. These maps contained new and previously developed markers (Table 1). The SNU3 map, containing newly developed markers, was reconstructed with 567 markers by the Joinmap program with a map distance of 1470.2 cM. The 22 linkage groups containing 381 out of 567 markers were assigned to chromosomes 1, 2, 3, 4, 5, 6, 7, 10, and 11. SNU4 also contained new and previously developed markers. Two hundred eighty-three markers were used for the map, which consisted of 15 linkage groups with 217 markers and the map distance was 1081.3 cM. The linkage groups were assigned to chromosomes 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, and 12. SNU5 with 448 new and previously developed markers was reconstructed as 22 linkage groups, which were assigned to chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12. A total of 306 markers were located on the map with 1044.3 cM map distance. SNU6 had 18 linkage groups and was reconstructed using 754 markers. Three hundred seventy-six markers could be mapped to the linkage groups which were assigned to chromosomes 1, 2, 3, 5, 6, 9, and 11. The map distance was 657.6 cM.

Table 1. Information on marker types included in interspecific and intraspecific maps

Type	No. in interspecific map[a] ('TF68' × 'Habanero')		No. in intraspecific map[b] ('CM334' × 'Chilsungcho')		Total number
Map ID[c]	SNU2, 3	SNU6	SNU4	SNU5	
SSR	236	76	32	65	409
RFLP	318	0	207	13	538
BAC	13	23	4	20	60
STS	0	0	9	13	22
WRKY	0	143	31	41	215
AFLP	0	234	0	199	433
rRAMP	0	278	0	97	375

[a] The number of indicated markers in each interspecific map constructed using 'TF68' and 'Habanero'.
[b] The number of indicated markers in each intraspecific map constructed using 'CM334' and 'Chilsungcho'.
[c] The name of the map

Information on developed common markers

To construct an integrated map, several marker types shared across maps such as SSR, RFLP, STS from BAC-end sequences, STS from RFLP, and WRKY were developed. The majority of common markers were SSR and RFLP markers, and only SSR markers were mapped to all four maps (Table 2). Newly developed STS markers from BAC-end sequences and previously developed WRKY and rRAMP markers were first applied to construct an integrated pepper map. Of a total of 2052 markers used to construct an integrated map, 1029 (SSR, RFLP, STS) were single copy type markers and 1023 (AFLP, rRAMP, and WRKY) were multi-copy.

BAC end sequence analysis and STS marker development from BAC-end sequences

End sequences from a total of 74 BAC clones were successfully sequenced and had an average length of 1430 bp. Using a BlastN search (http://www.ncbi.nlm.nih.gov), 51 out of 74 (69%) the BAC end sequences were found to be homologous to sequences in the database (data not shown), and 40 out of 74 end sequences had significant similarity (E value ≤ E-10) to pepper ESTs (http://plant.pdrc.re.kr/sol). A total of 21 of 40 BAC end sequences with significant similarity were matched to genes of known function and 9 of the 74 BAC end sequences contained microsatellite repeats. In a blastX search against the NCBI database, end sequences of BAC insertions obtained from screening showed greater homology with functional sequences than end sequences from a random selection of clones. STS from BAC-end sequences from clones screened with the mRNA probe were thus possibly more informative.

The end sequences from 74 BAC clones were sequenced to develop STS markers such as CAPS or SCAR. Seventy-one BAC clones were successfully amplified by PCR and 24 (9 SCAR and 15 CAPS markers) were polymorphic between the parents (*C. annuum* 'CM334' and *C. annuum* 'Chilsungcho') of an intraspecific population. Also, 46 amplified bands with BAC end-derived primers were polymorphic (17 SCAR and 29 CAPS) between the parents (*C. annuum* 'TF68' and *C. chinense* 'Habanero') of an interspecific population. Twelve STS markers from BAC-end sequences were developed in both populations together, and a total of 57 STS from BAC-end

Table 2. Number of common used markers on the four maps

Population	No. of F_2 individuals	Marker type	No. of common marker			
			1	2	3	4
SNU3	107	SSR	134	73	23	6
		RFLP	263	51	4	0
		BAC[a]	4	6	3	0
SNU4	100	SSR	4	11	11	6
		RFLP	151	52	4	0
		BAC	4	0	0	0
		STS from RFLP	1	8	0	0
		WRKY	19	12	0	0
SNU5	100	SSR	33	12	14	6
		RFLP	6	3	4	0
		BAC	8	10	2	0
		STS from RFLP	5	8	0	0
		WRKY	29	12	0	0
SNU6	93	SSR	9	58	3	6
		RFLP	0	0	0	0
		BAC	18	3	2	0
		STS from RFLP	0	0	0	0
		WRKY	143	0	0	0

[a] BAC is STS from BAC-end sequences

sequences were developed. Seven of the 57 developed markers were distorted in the interspecific cross; no distorted markers were found in the intraspecific cross.

SSR markers and RFLP-derived STS markers

A total of 207 SSRs from the SNU maps (Lee et al., 2004; Yi et al., 2006) and 109 SSRs from other maps (Minamiyama et al., 2006; 2007) were used as common markers. Out of the 109 SSRs, 39 SSRs were detected in the interspecific population and 28 in the intraspecific population. Among the 39 and 28 SSRs, 15 and 3 were distorted in the interspecific and intraspecific crosses, respectively. Three severely distorted markers (CAMS 081, 892-2, and 865) were found in the interspecific cross ($P < 0.01$), while 15 markers were weakly skewed with chi-square values below 5 ($P < 0.05$). A total of 62 out of 316 SSRs were newly developed on the SNU maps.

A total of 146 PST clones previously used as RFLP probes (Kang et al., 2001; Kim et al., 2008a) were sequenced, and only 15 out of 146 clones were confirmed as single copy type by Southern blot. Using sequences from 13 of the 15 PST clone, STS markers including seven SCAR markers (1 codominant and 6 dominant markers) and six dominant CAPS markers were developed in the intraspecific population (*C. annuum* 'CM334' × 'Chilsungcho'). Among these developed markers, two were excluded from further map construction due to distortion. Seven of the 13 STS sequences had significant similarity (E value ≤ E-10) to pepper ESTs in the pepper database (http://plant.pdrc.re.kr/sol).

RFLP, WRKY and rRAMP markers

Five hundred thirty-eight RFLP markers were from previously developed maps (Kang et al., 2001; Kim et al., 2008a). Among these RFLPs, 57 were used as common markers for further study of an integrated map. Two genes of known function (CT128 and CT204) and 11 RFLP markers (TG152, 74, 130, 62, 498, 281, 161, 83, 619, 379, and 46) were positioned on our linkage map and new common markers were developed in our map: TG44 and TG281 on chromosome 1; TG392, TG191, and TG48 on chromosome 2; TG130, TG421, TG74, CT179, and CT220 on chromosome 3; TG62 and TG587 on chromosome 4; TG232 on chromosome 6; CT135 on chromosome 7; CT143 and CT211 on chromosome 9; TG420 on chromosome 10; TG379 and TG619 on chromosome 11; and TG350 on chromosome 12. Out of the 538 RFLP markers, 138 were distorted. In addition, 215 WRKY markers were developed, 12 of which came from the intraspecific cross and was used as common markers. A total of 375 rRAMP markers were developed. There were no distorted markers among these developed WRKY and rRAMP markers.

Construction of the integrated map

We integrated four maps (SNU3, SNU4, SNU5 and SNU6) into a new integrated map using common markers, a subset of which was newly developed. A total of 808 markers from the interspecific maps and 747 markers from the intraspecific maps were integrated (Fig. 1). The interspecific map distance was 1858 cM with a marker density of 2.2 cM and the intraspecific map was 1892 cM with a marker density of 2.5 cM. Furthermore, this merged map contained 217 skewed markers (61, 18, 32, 7, 5, 8, 24, 3, 28, 2, 22, 7 on chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 respectively). During merging of the four different maps, the skewed and insufficient markers were excluded from the maps by the heterogeneity test in the Joinmap program. Because of many insufficient markers, 29, 24, 22, and 36 fewer markers were located on chromosomes 5, 7, 10, and 12, respectively. In the final analysis, 23 out of 57 STS from BAC-end sequences, 6 out of 13 STS from RFLP, and 168 out of 254 SSR markers were mapped on the integrated map (Table 3).

Due to the pseudolinkage of chromosome 1 and chromosome 8 caused by an interspecific cross, the integrated map has two different chromosomes 1 and 8 for interspecific and intraspecific populations. In the interspecific map, 109 markers were mapped on chromosome 1 containing chromosome 8, while in the intraspecific map, 24 markers were mapped on chromosome 1 and 25 markers on chromosome 8. A total of 70, 122, 69, 28, 84, 24, 149, 22, 92, and 36 markers were located on chromosomes 2, 3, 4, 5, 6, 7, 9, 10, 11, and 12, respectively. This integrated map contained a variety of markers such as AFLP, BAC, RFLP, rRAMP, SSR, STS, and WRKY markers. The marker information on this integrated map is shown in Table 3.

DISCUSSION

Pepper linkage maps and their common markers

This integrated map contains not only many PCR-based markers (SSR, STS from BAC-end sequences, and STS from RFLP) but also RFLP markers. These RFLP markers can be used as anchor markers to identify pepper chromosomes in several maps from different populations and to compare linkage groups containing TG or CT markers (Kang et al., 2001; Lee et al., 2004; Livingstone et al., 1999; Rao et al., 2003).

Most published pepper linkage maps consist of AFLP and RAPD markers. Multi-copy type markers such as AFLPs and RAPDs are difficult for pepper researchers and breeders to use in new map construction as the markers are not easily identified as being shared among different maps.

A previously reported integrated pepper map (Paran et al., 2004) contains 400 RFLPs but among them, only few markers in our map are common: TG158 on chromosome 1, TG48 on

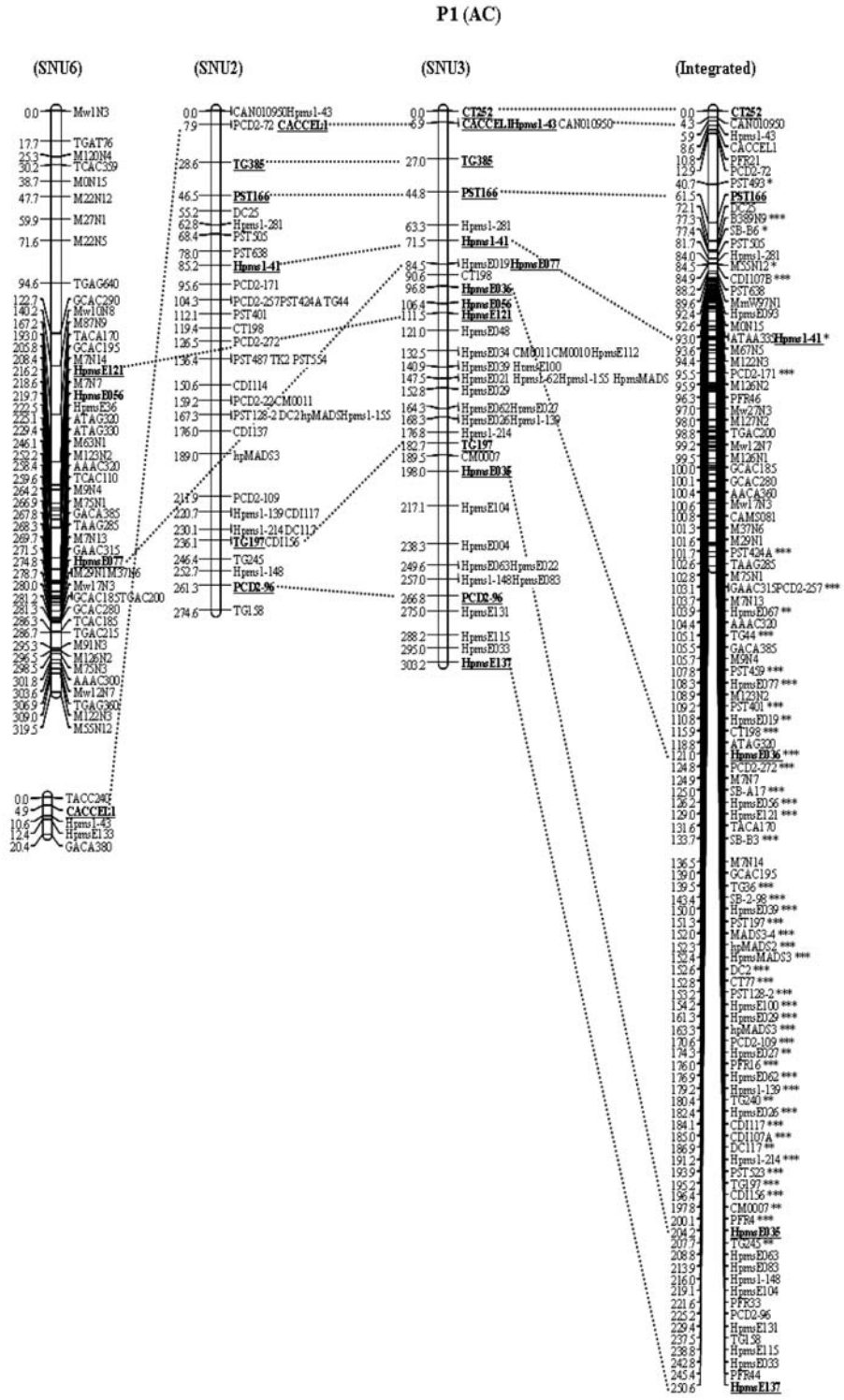
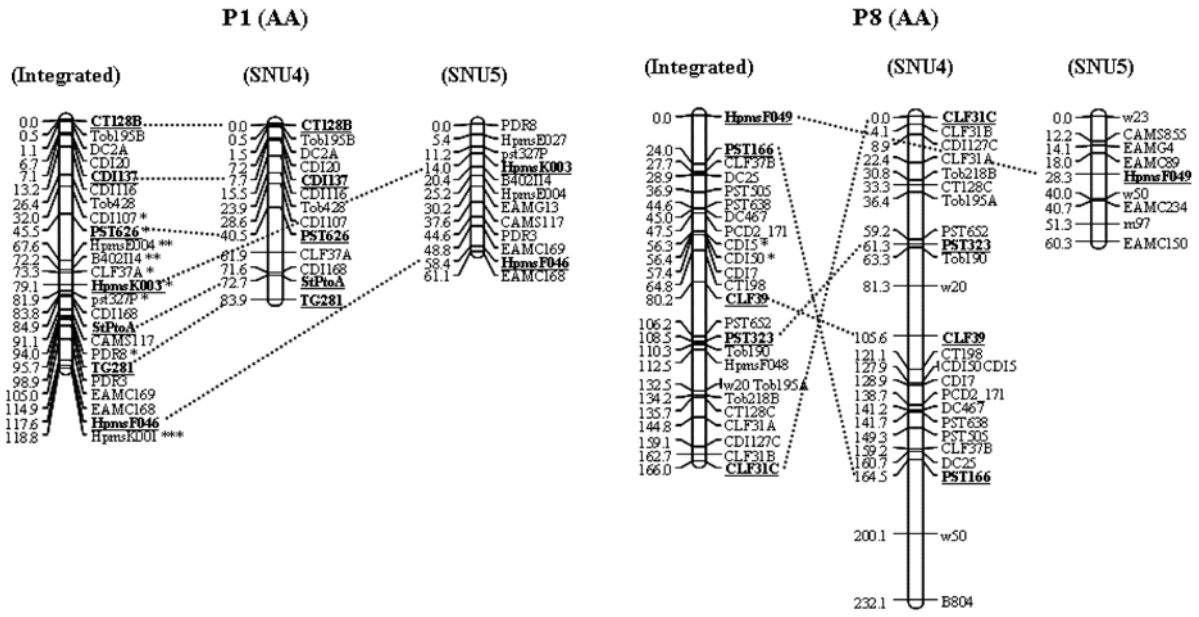


Fig. 1. An integrated pepper map (SNU7) consisted of 169 SSR, 354 RFLP, 23 BAC, 6 STS, 152 AFLP, 51 WRKY, and 99 rRAMP markers using the Joinmap 3.0 program with the Haldane mapping function. This integrated map was aligned with SNU2, SNU3, SNU4, SNU5 and SNU6 maps using two interspecific crosses of *C. annuum* 'TF68' and *C. chinense* 'Habanero' and two intraspecific crosses of *C. annuum* 'CM334' and *C. annuum* 'Chilsungcho'. Map distance (cM) is on the right side of the central bar and marker name is on the left side. P1 (AC) is pepper chromosome number 1 for an interspecific cross. P1 (AA) and P8 (AA) is pepper chromosome number 1 and 8 for an intraspecific cross, respectively. Marker types and designations are as follows: pepper SSRs (Hpms, CAMS, CM, and GenBank accession number); pepper RFLPs (PST, PCD, PDR, DC, CDI, PSY, GPS, LCY, CCS, CRTHYD, PFTF, PAL, PCS, pAMT, CAN, CFR, CLF, hpMADS, MADSP10, CaPR, N32, and RDNA); tomato RFLPs (TG, CT, CD); tobacco RFLP (M29869); pepper BAC (B); STS from PSTs (Pst); AFLPs (EAMC, EAMG, ECMC, ECMG, ANNN, CANN, GNNN, TANN, TCNN, TGNN); rRAMPs (m, M); WRKYs (w, Mw). * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$) indicate distorted markers with the significance on the right side of marker name.

chromosome 2, CT220 and TG244 on chromosome 3, TG62 and TG587 on chromosome 4, TG123 on chromosome 5, TG232 on chromosome 6, CT143 on chromosome 9, TG420 on chromosome 10, TG619 and TG379 on chromosome 11, and TG508 on chromosome 12. Although the integrated map (Paran et al., 2004) did not contain enough useful common markers to compare with a map containing SSRs (Lee et al., 2004; Yi et al., 2006), according to these RFLP-based chromosome numbers, the chromosome number was assigned in this integrated map. Because of insufficient common markers, the marker order on chromosomes in the two integrated maps could not be directly compared. In this study, the marker order was in concordance with that of previous pepper maps (Ben Chaim et al., 2001; Lee et al., 2004; Livingstone et al., 1999;



(Fig. 1, continued)

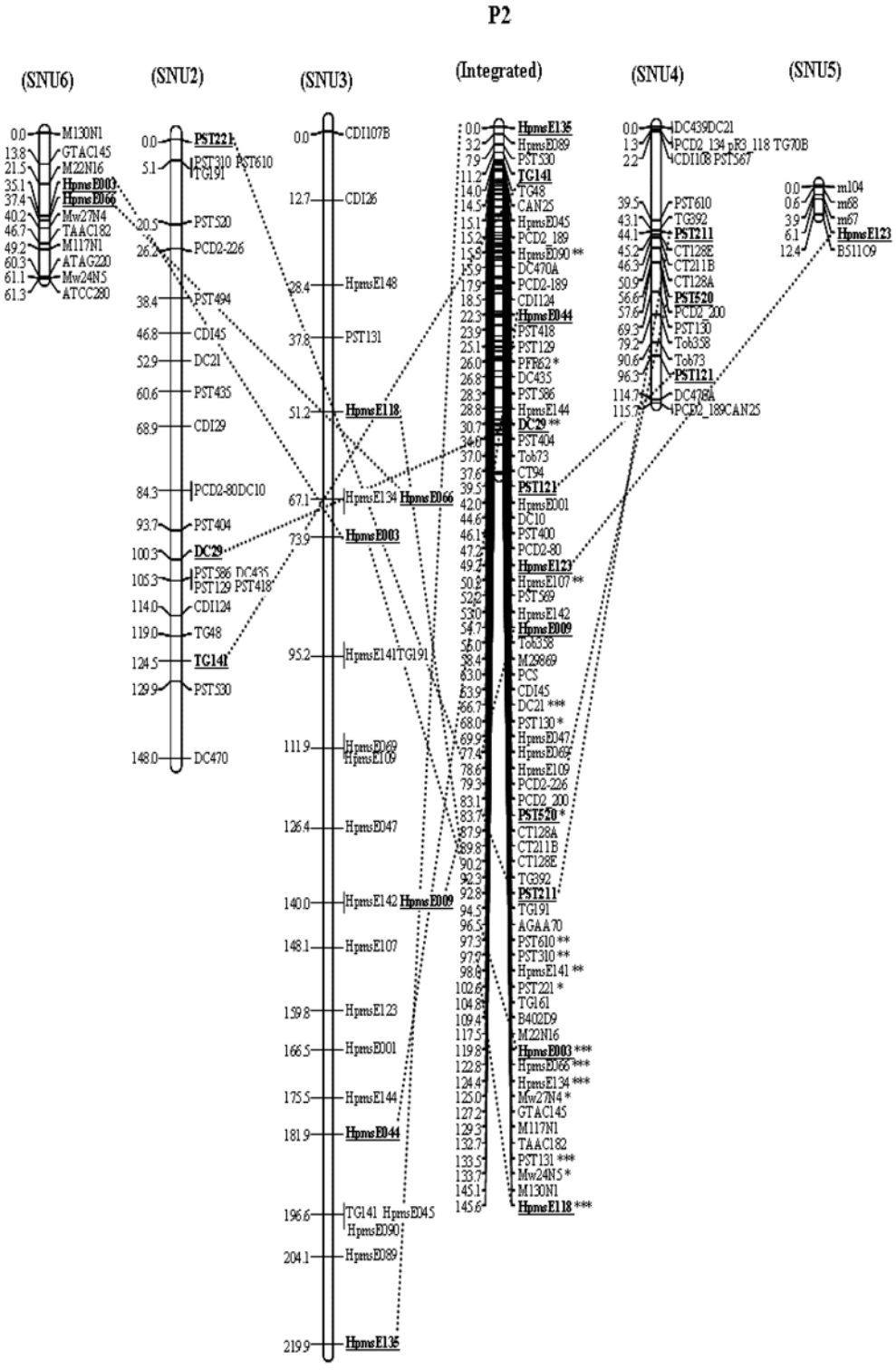
Table 3. The marker information mapped on the integrated map of SNU7

Chro[a]	No. of marker types								Map length (cM)	Average distance[d]
	AFLP	BAC[b]	RFLP	rRAMP	SSR	STS[c]	WRKY	Total		
P1 (AA)	2	1	15	-	5	1	-	24	119.0	4.9
P1 (AC)	12	1	45	15	32	-	4	109	251.0	2.3
P2	3	1	41	4	19	-	2	70	146.0	2.0
P3	15	1	67	8	21	-	10	122	237.0	1.9
P4	12	1	13	22	15	-	6	69	145.0	2.1
P5	-	1	20	-	7	-	-	28	164.0	5.8
P6	16	2	42	7	13	-	4	84	178.0	2.1
P7	-	-	19	-	5	-	-	24	73.0	3.0
P8 (AA)	-	-	22	-	2	-	1	25	166.0	6.6
P9	52	7	21	26	26	-	17	149	225.0	1.5
P10	3	1	8	1	5	2	2	22	120.0	5.4
P11	31	5	17	13	18	3	5	92	191.0	2.0
P12	6	2	24	3	1	-	-	36	128.0	3.5
Total	152	23	354	99	169	6	51	854	2143.0	2.5

[a]Chr is chromosome
[b]BAC is STS from BAC-end sequences
[c]STS is from RFLP
[d]Average distance between markers (cM)

Tanksley et al., 1992; www.sgn.cornell.edu). Only RFLP markers were useful as common markers when comparing our integrated map with previous linkage maps (Ben Chaim et al., 2001; Lefebvre et al., 2002; Rao et al., 2003).

The pepper linkage map reported by Minamiyama et al. (2006) consisted of 106 SSRs and was compared to the SNU2 map using 46 SSR markers. In this study we tested all SSRs from the Minamiyama's study (2006) in our mapping populations which revealed a polymorphism of 24.5% (26/106 × 100) in a cross of *C. annum* 'CM334' and *C. annum* 'Chilsungcho' and of 34.9% (37/106 × 100) in a cross of *C. annuum* 'TF68' × C. *chinense* 'Habanero'. The greater level of polymorphism displayed in the interspecific population was consistent with previous studies (Barchi et al., 2007; Lefebvre et al., 2002). A clustering of SSR markers (Minamiyama et al., 2006) was similarly observed on chromosome 9a (CAMS456, 424, 360, 679, and 056 orderly) in this study. The discrepancy between the identification of the linkage group containing the CAMS424 marker in this study and a previous study could be a result of severe distortion ($P < 0.01$). The pepper linkage map published by Barchi et al. (2007) contained 40 SSR, 19 RFLP, and 507 AFLP markers in a RIL population, which has some advantages, such as decreased skewed segregation and accurate mapping. We would have liked to compare the Barchi map with our map to



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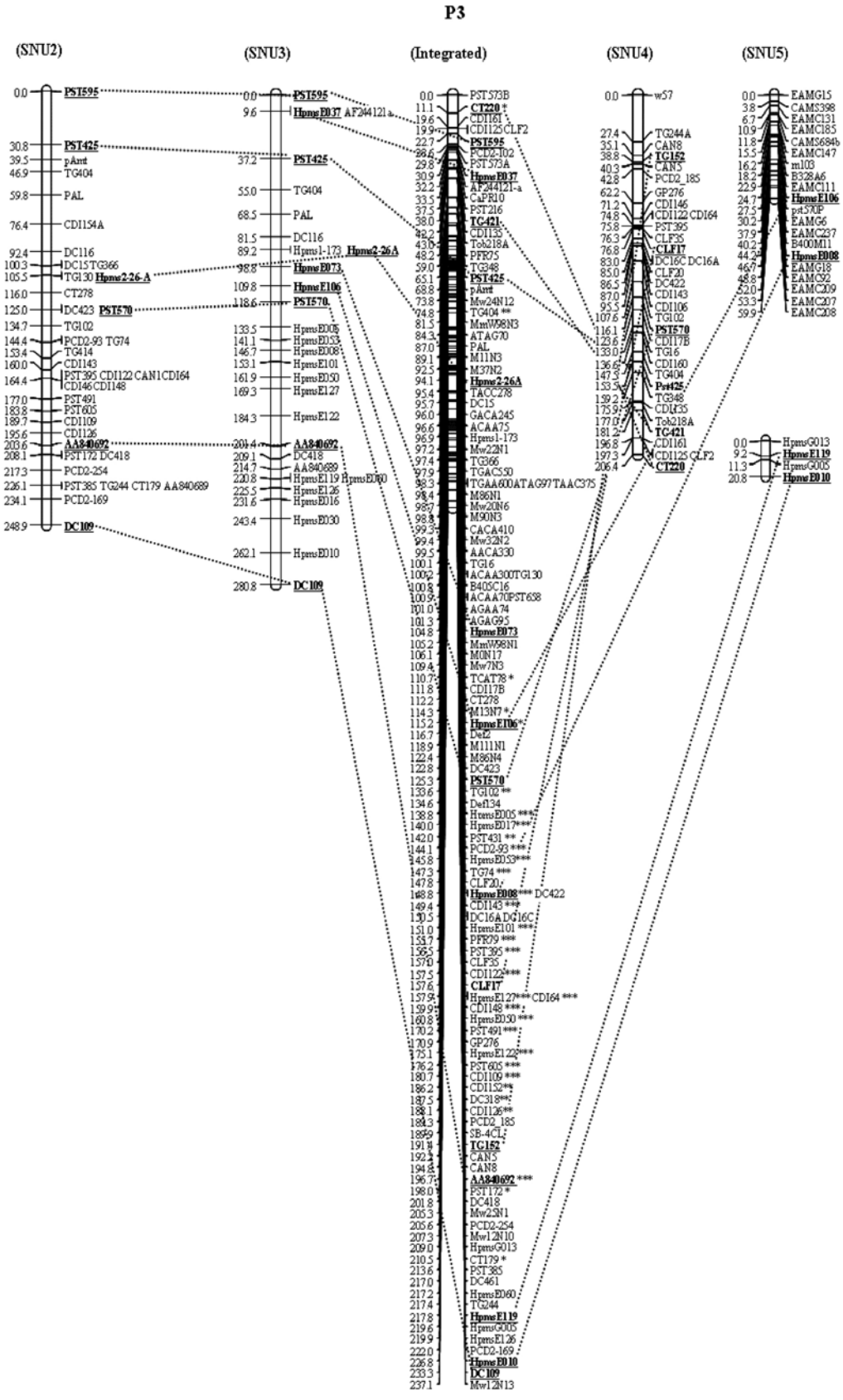
take advantage of the information in a RIL population. However, the limited number of common markers, namely seven SSRs (Hpms 1-1, 1-214, 1-5, 1-62, 2-13, 2-24, and 1-69) and two RFLPs (TG046 and TG281), between the maps prevented comparison between these two maps.

STS from BAC-end sequences, STS from RFLP, and SSR markers

The integrated map developed in this study contains more single copy type markers such as STS from BAC-end sequences, STS from RFLP, and SSR markers than other pepper maps. In



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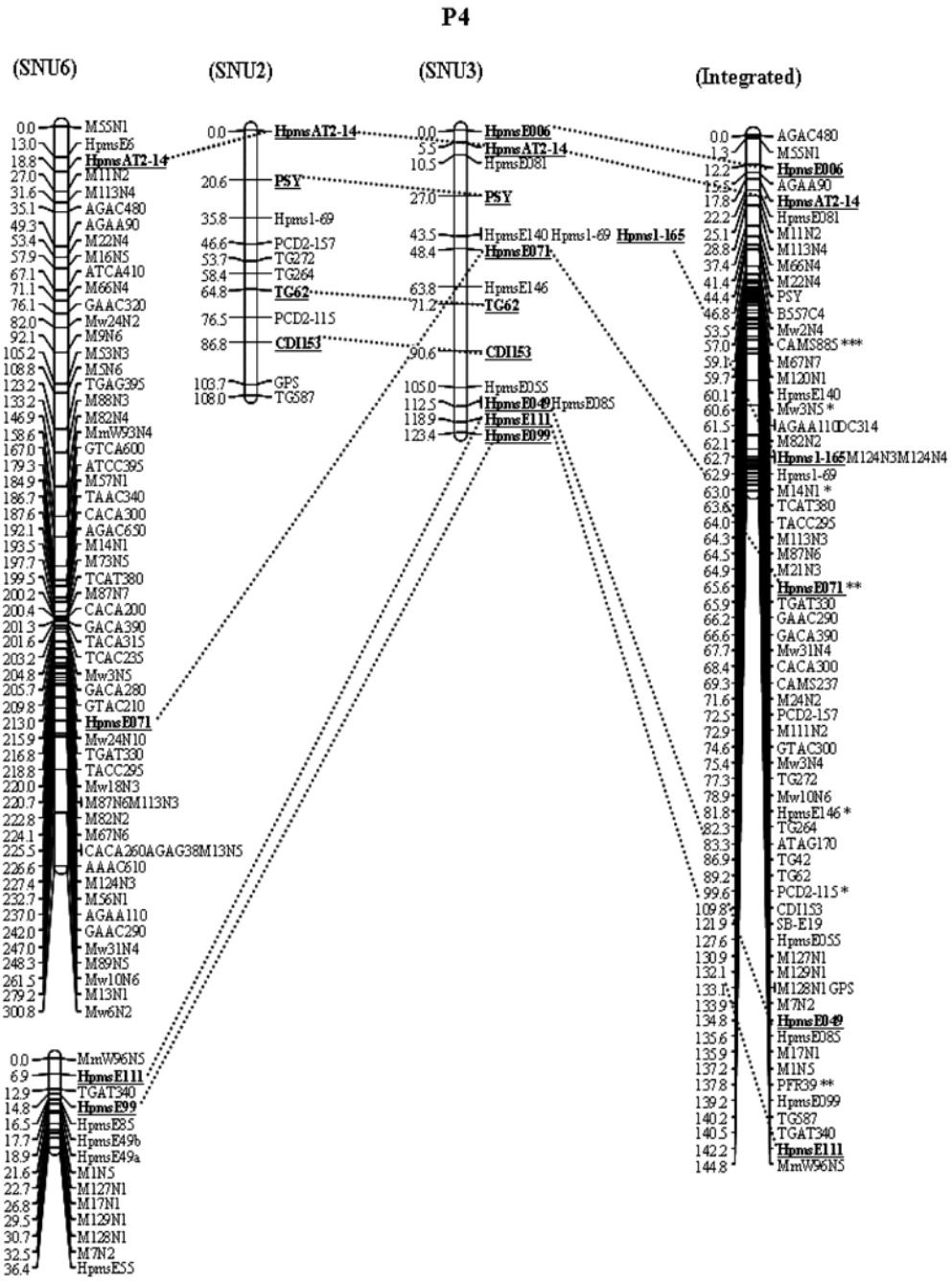

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particular, the STS from BAC-end sequences are the first to be developed in pepper and could allow a direct link between physical and genetic distances and marker orders without BAC library screening.

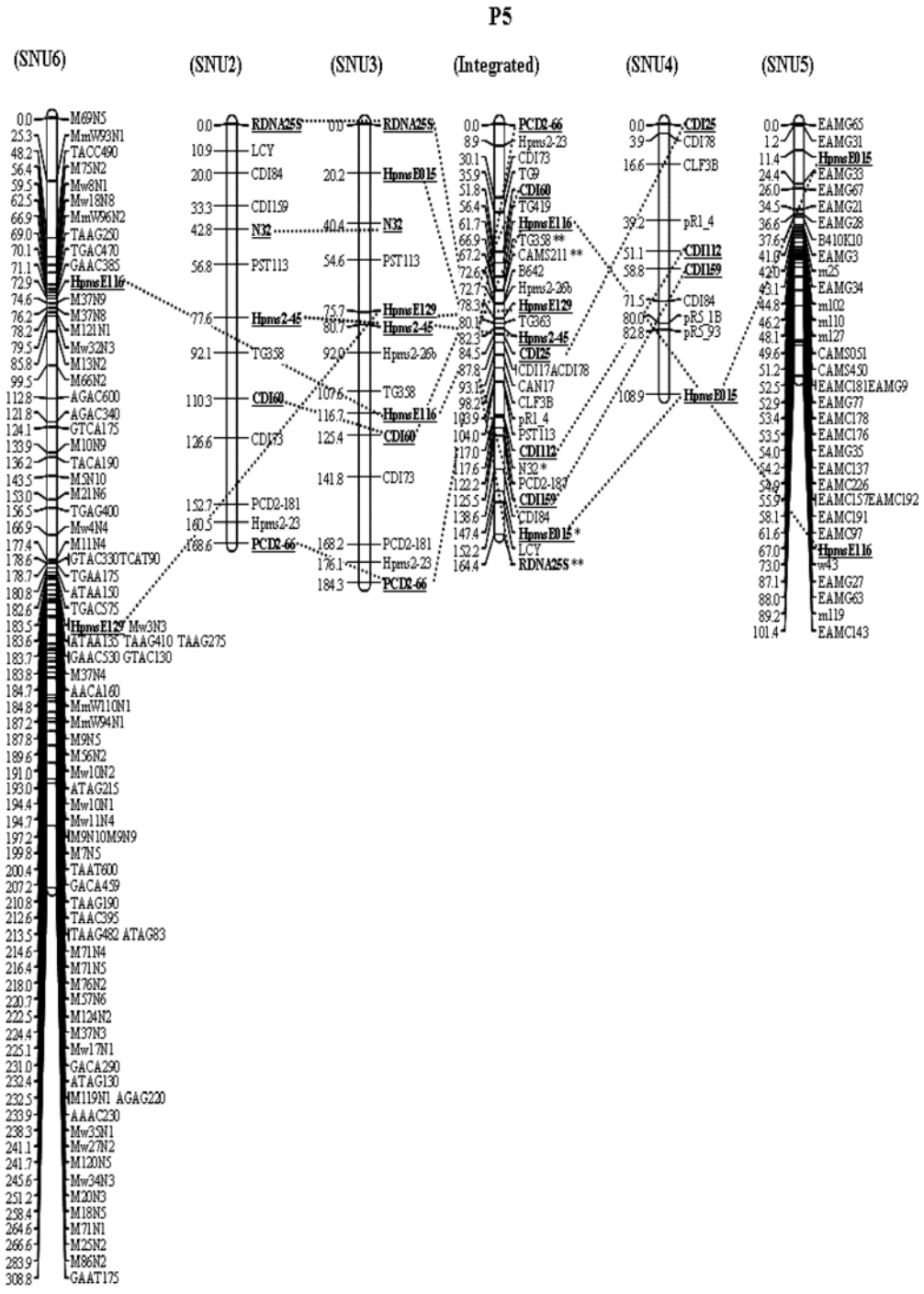
The STS markers were derived from PST clones which were used as probes for RFLP markers. Over 140 PST clones were sequenced and from these, 13 STS markers from RFLP were developed for our maps.

All SSR markers (Lee et al., 2004; Minamiyama et al., 2006; 2007; Yi et al., 2006) were applied to our maps, but additional ones were needed as common markers to have deep coverage of the genome in the integration of several maps.

The integrated map

This integrated pepper map was constructed using 57 STS from BAC-end sequences, 13 STS from RFLP, 254 SSR, and 477 RFLP markers. Single copy type markers such as STS from BAC-end sequences, STS from RFLP, and SSR are more useful for rapid construction of linkage groups as well as for the assignment of the groups to pepper chromosomes.

Over half of the 1839 markers were not mapped because 321 markers in an interspecific population were distorted, and markers that had insufficient linkage analysis were discarded. We could not map the skewed markers on the maps although the “fix order” function in the Joinmap 3.0 program supported



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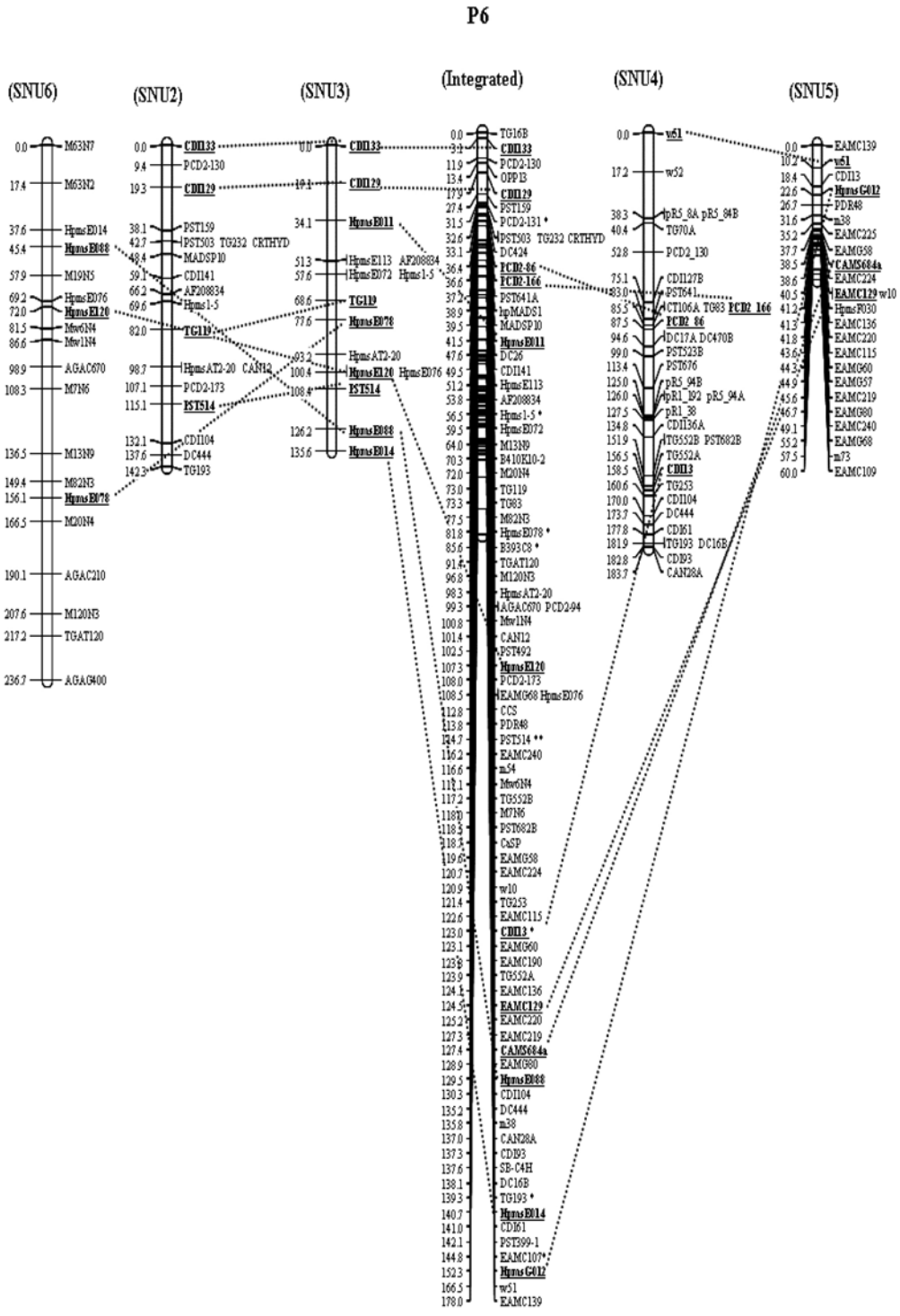
the localization of these skewed markers on each map. The distorted markers of segregation ratios were located fewer on this integrated map as we compared parental map such as SNU2, SNU3 and SNU6 of an interspecific population. This integrated map contained a total of 217 skewed markers which came mostly from an interspecific cross population as we expected to produce many skewed markers of an interspecific population in our previous result (unpublished data), which is concordance with previous studies (Barchi et al., 2007). Among 12 chromosomes, P1 (AC), P3, P7, P9, and P11 contained more skewed markers than other chromosomes, and it could be explained that the skewed markers on this linkage groups mostly came from the interspecific population.

We compared to reliability of the integrated map and parental maps previously reported. It showed that most of marker orders were identified with parental maps (SNU3, 4, 5 and 6), except some regions such as M67N7-M82N2 in chromosome 4, M7N6 in chromosome 6, HpmsE042 and CAMS424 in chromosome 9.



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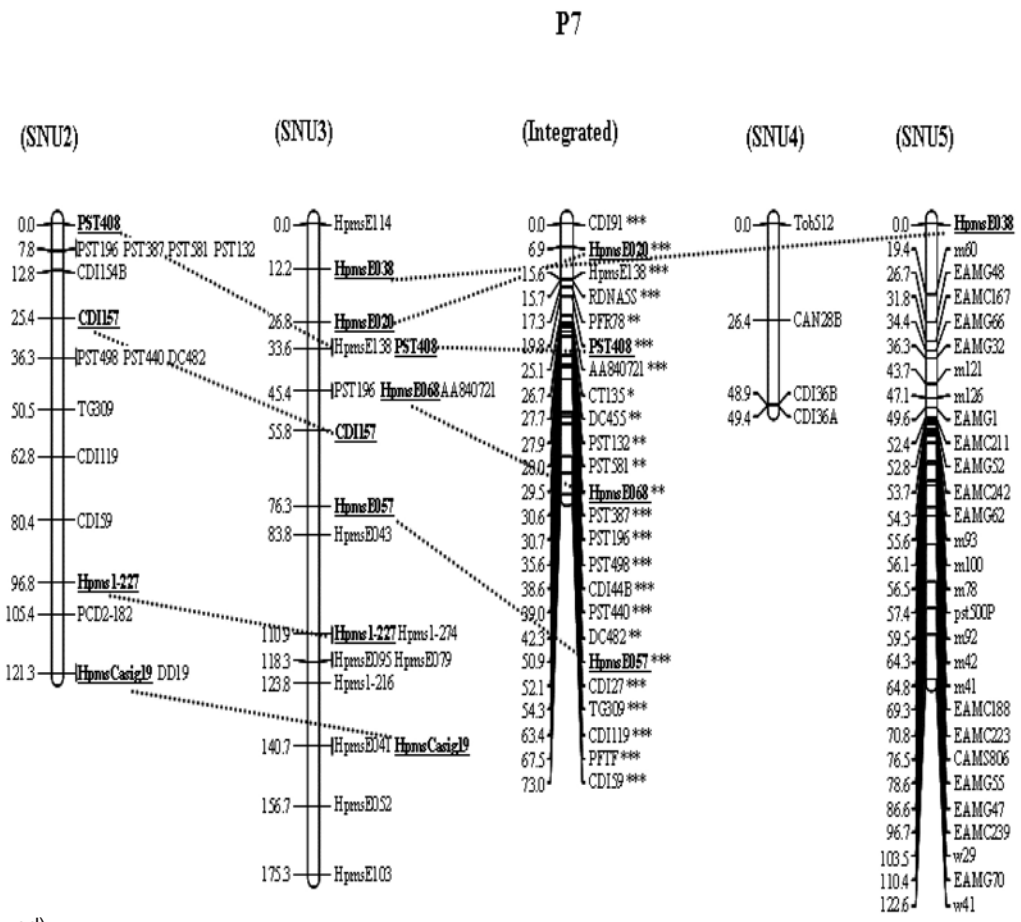
These discrepancies could be explained by lots of dominant markers containing fewer markers with high significance (more than LOD 5) than the markers in other SNU maps or by highly skewed marker. The length of merged map was similar to one of the parental maps (SNU3, 4, and 5). However, there was some gap of map length between this merged map and SNU6, because this merged map and SNU6 was constructed by Joinmap 3.0 and Carthagene, respectively and the map length using Carthagene is longer than one using Joinmap (Doligez et al., 2006). Moreover, most dominant markers of SNU6 map using Carthagene program could more increase the map length.

Though this integrated map does not contain numerous markers, the many single copy type markers used in the four maps will provide good marker information for future mapping



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studies. Furthermore, the STS from BAC-end sequence markers can directly connect a genetic map with a physical map in pepper like they have in cotton, papaya, and Brassica (Chen et al., 2007; Frelichowski et al., 2006; Guo et al., 2008).

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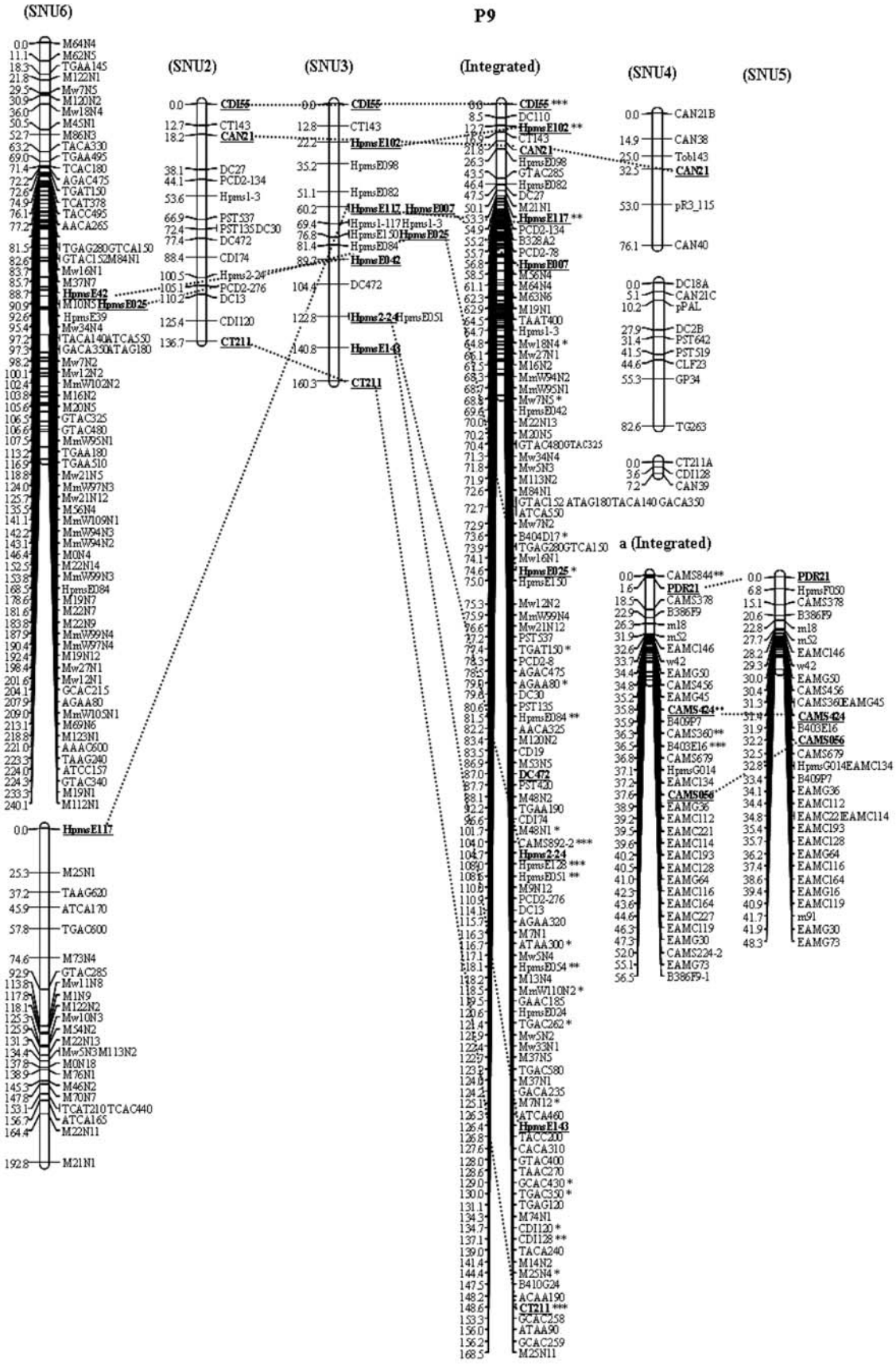

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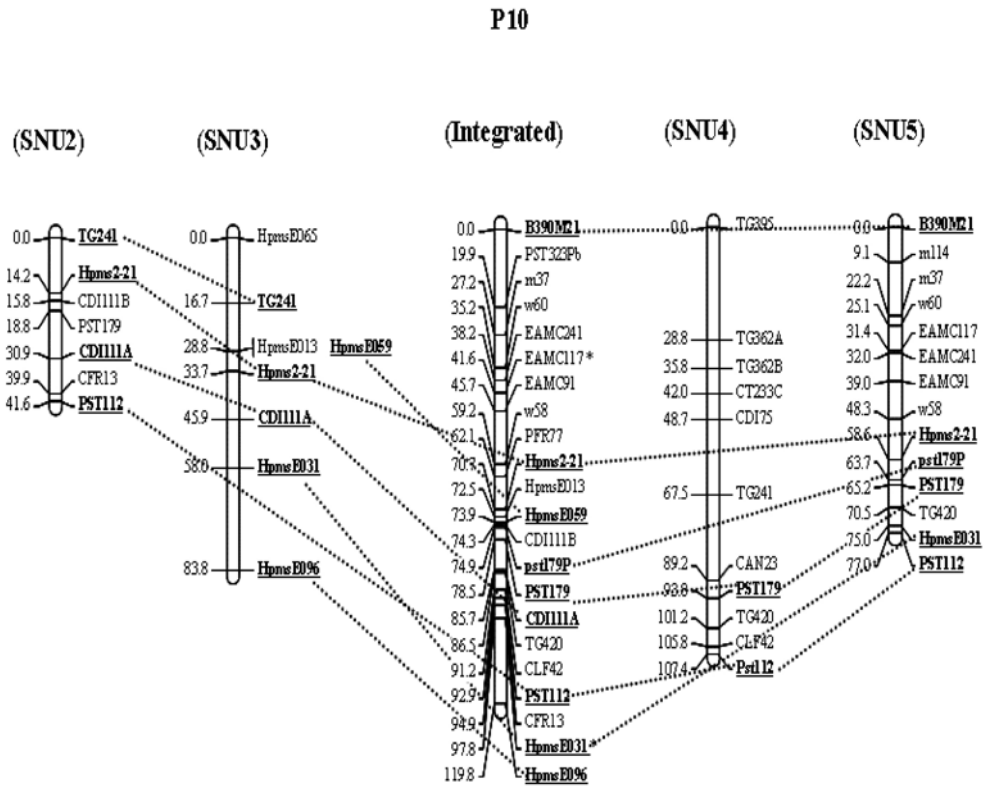

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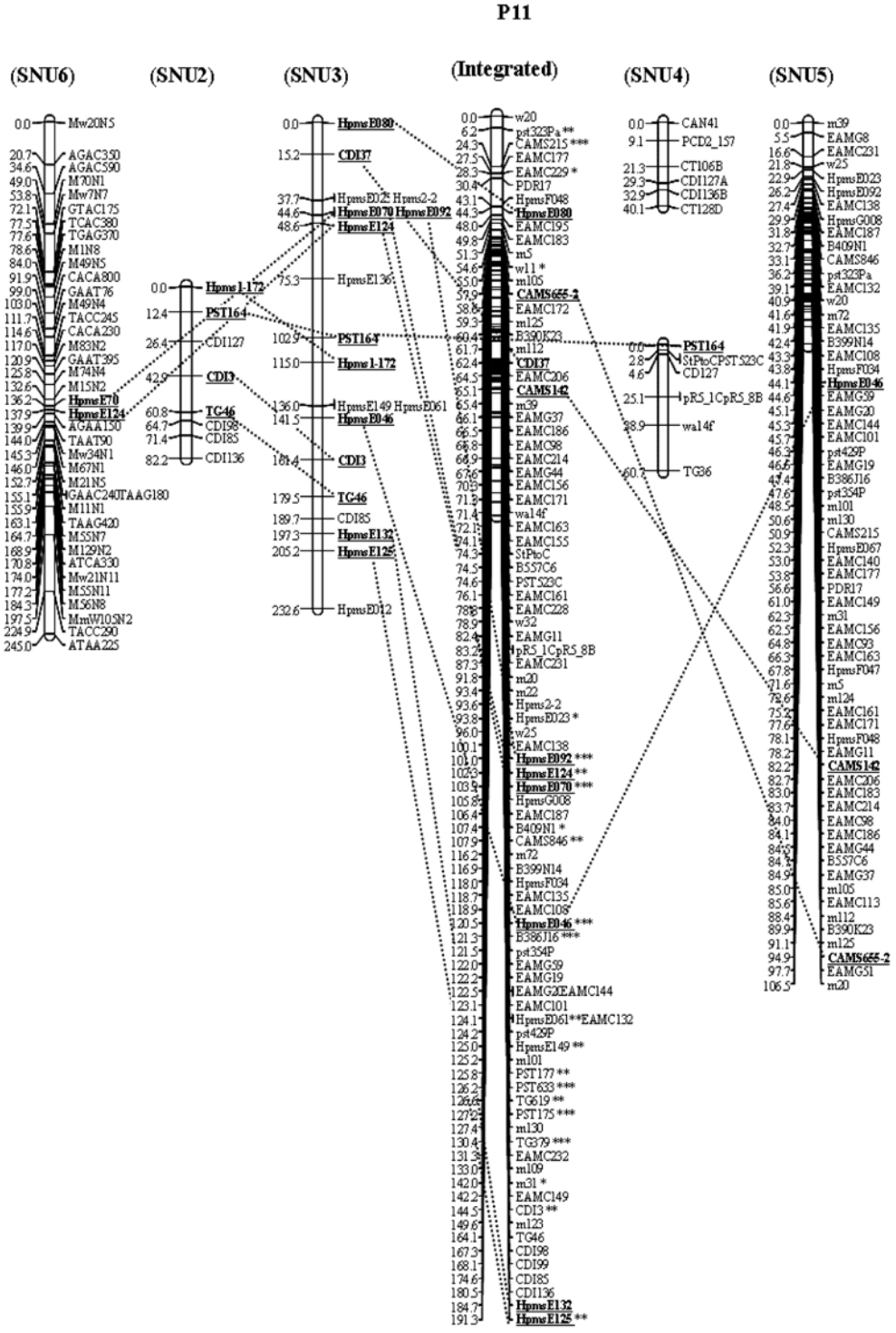

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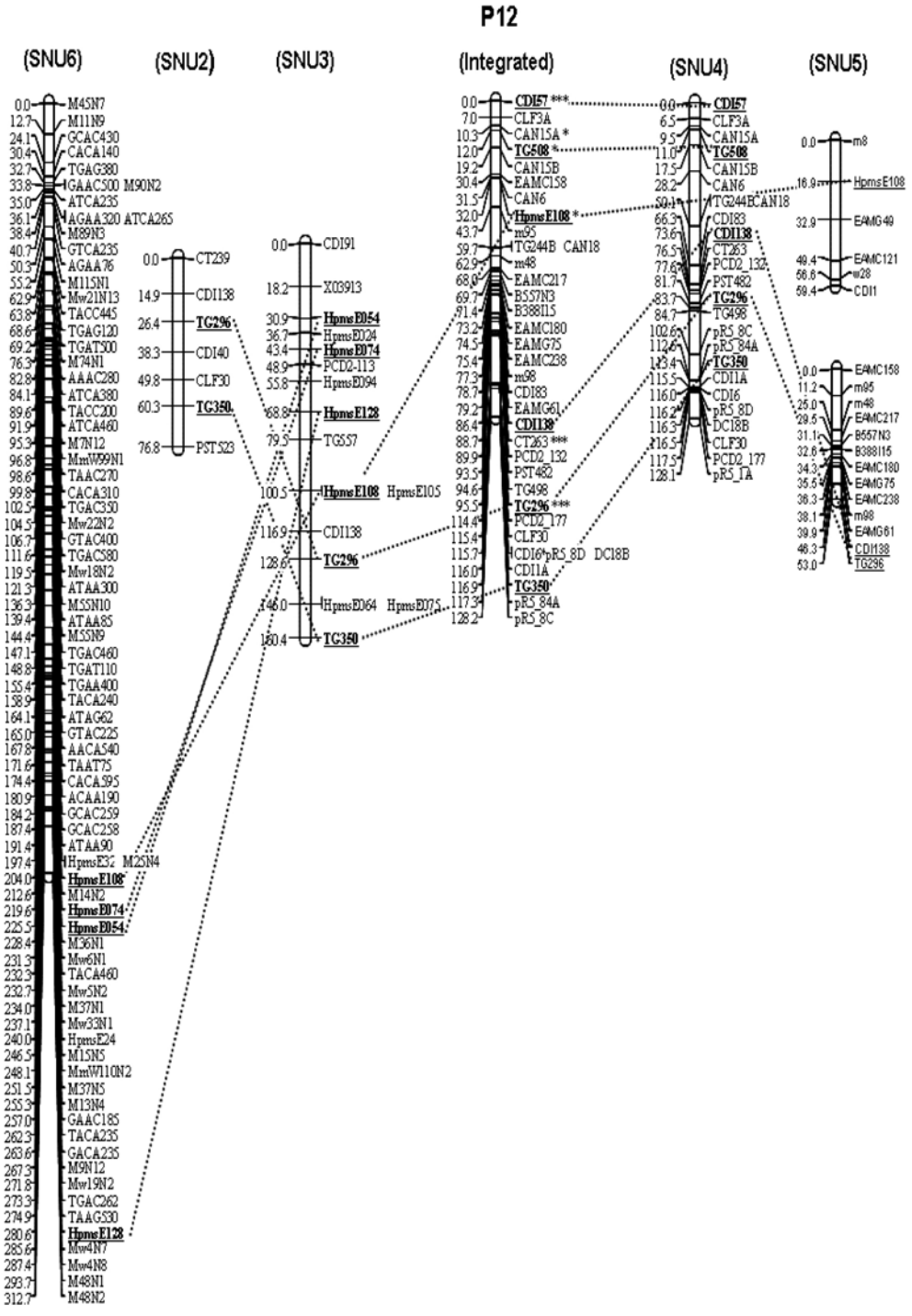

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