Mol Breeding (2006) 18:157–169
DOI 10.1007/s11032-006-9024-3

An SSR-based linkage map of *Capsicum annuum*

Yasuhiro Minamiyama · Masato Tsuro · Masashi Hirai

Received: 9 November 2005 / Accepted: 22 May 2006 / Published online: 29 August 2006

Abstract There are five cultivated species of pepper, of which *Capsicum annuum* is the most widely cultivated as a vegetable or spice and the main experimental material of most pepper breeding programs. However, the number of simple sequence-repeat (SSR) markers known for *C. annuum* is limited. To develop SSR markers for *Capsicum* species, we constructed four SSR-enriched libraries from the genomic DNA of *C. annuum*, sequenced 1873 clones, and isolated 626 unique SSR clones. A higher percentage of these SSR markers were taken from dinucleotide motif libraries than from trinucleotide motif libraries. Primer pairs for the 626 SSR clones were synthesized and tested for polymorphisms; 594 amplified products were detected with the expected size. However, only 153 products were polymorphic between the parents of our mapping population. Using 106 highly reproducible pairs from the primer pairs, we constructed a linkage map of *C. annuum* in an intraspecific doubled haploid population (n=117) that contains nine previously reported SSRs as well as AFLP, CAPS, and RAPD markers and the trait of fruit pungency. The map contains 374 markers, including 106 new SSR markers distributed across all 13 linkage groups, and covers 1042 cM. The polymorphism information content (PIC) of these new SSR markers was calculated using 14 lines of *Capsicum* species. The average number of alleles per locus was 2.9 and the average PIC value was 0.46, even within *C. annuum*. The SSR markers developed in this study will be useful for mapping and marker-assisted selection in pepper breeding, and the linkage map provides a reference genetic map for *Capsicum* species.

Keywords *Capsicum annuum* · Linkage map · Molecular marker · Simple sequence repeats

Y. Minamiyama (✉) · M. Tsuro · M. Hirai
Kyoto Prefectural Institute of Agricultural Biotechnology, 74, Oji, Kitainayazuma, Seika, Soraku, Kyoto 619-0244, Japan
e-mail: yminami@kab.seika.kyoto.jp

M. Hirai
Graduate School of Agriculture, Kyoto Prefectural University, 74, Oji, Kitainayazuma, Seika, Soraku, Kyoto 619-0244, Japan

Present Address:
M. Tsuro
Faculty of Agriculture, Meijo University, Shiogamaguchi, Tenpaku, Nagoya 468-8502, Japan

Introduction

Hot and sweet peppers are members of the Solanaceae family. They have been categorized into five species, of which *Capsicum annuum* is the most widely cultivated as a vegetable or spice and the main subject of breeding programs for

commercial cultivars. The other four species, *C. chinense*, *C. baccatum*, *C. frutescens*, and *C. pubescens*, are used to produce spice or used as genetic resources for disease resistance genes (Moury et al. 2000; Caranta et al. 2002). Major breeding efforts on peppers focus on fruit traits, productivity, disease resistance, and the regulation of capsaicin content. However, many of these traits are quantitative, and simple selection based on phenotype is not always effective. More recently, the selection of these traits using molecular linkage markers – marker-assisted selection (MAS) – has been recognized as a powerful tool for breeding. Information on linkage maps is indispensable for elucidating quantitative trait loci and obtaining linkage markers. To date, several genetic linkage maps have been published for *Capsicum* species. These linkage maps are primarily based on restriction fragment length polymorphism (RFLP) markers generated in tomato, as well as on amplified (A)FLP and random amplified polymorphic DNA (RAPD) markers (Lefebvre et al. 1995; Livingstone et al. 1999; Ben Chaim et al. 2001; Kang et al. 2001). Because AFLP and RAPD markers are difficult to use as anchor markers, RFLP markers have been used as in their place to integrate these genetic linkage maps (Lefebvre et al. 2002; Paran et al. 2004). However, RFLP analysis is a complicated and time-consuming procedure that requires a large amount of genomic DNA, and these techniques cannot be routinely used for MAS in breeding programs. To use the information from genetic maps more efficiently in pepper breeding, the development of a high-throughput system for genotyping DNA fragments is necessary.

Simple sequence repeats (SSRs) or microsatellite markers are PCR-based markers that have been developed in many plant species, including most major crops, such as rice, maize, sorghum, wheat, and barley, as well as vegetable crops (Mba et al. 2001; Suwabe et al. 2002; Chiba et al. 2003). SSR markers have the advantage of being multiallelic, highly polymorphic, and codominant, and have been developed and used for the genetic mapping of solanaceous vegetables, including tomato, potato, and eggplant (Broun and Tanksley 1996; Smulders et al. 1997; Milbourne et al. 1998; Nunome et al. 2003). Unfortunately, the SSR markers developed in other solanaceous species cannot be used for breeding *Capsicum* species, despite a close phylogenic relationship among these species. The recent construction of a linkage map with SSR markers in pepper has been reported using an interspecific cross between *C. annuum* and *C. chinense* (Lee et al. 2004). The map contains 46 SSR markers, which shows a relatively high level of polymorphism in this interspecific cross. However, the level of polymorphism may greatly decrease in crosses between lines of *C. annuum*. Crosses within *C. annuum* are more frequently used in pepper breeding. Whether SSR markers generate enough polymorphism and are useful in intraspecific crossing in *C. annuum* remains to be determined.

In this study, we have developed SSR markers that are useful in intraspecific crosses in *C. annuum*, constructed a linkage map using these SSR markers, and evaluated their characteristics.

Materials and methods

Plant materials, pungency determination, and DNA extraction

A pepper cultivar, Manganji (*C. annuum*), which is locally grown in Kyoto, Japan, and has an occasionally pungent and long conical-shaped fruit, was used as one parent. The other parent was cv. Tongari (*C. annuum*), bred by Nanto Seed, Nara, Japan. The latter bears nonpungent and long block-shaped fruit. A segregating doubled haploid (DH) population (n=117) was developed by the anther culture of an F_1 plant of the two parents and was used for the genetic analysis (TMDH population). The polymorphism detecting ability of the SSR markers developed in this study was examined using a set of seven cultivars and breeding lines of *C. annuum* and seven lines of related *Capsicum* species (Table 1). All plants were grown in a greenhouse.

The pungency of the mature green fruit of the TMDH population was determined as previously described (Minamiyama et al. 2005).

DNA was extracted from young leaves using a DNA extraction kit (Amersham Biosciences, Buckinghamshire, UK).

Table 1 *Capsicum annuum* lines and *Capsicum* relatives used in this study

Capsicum annuum		*Capsicum* relatives	
Species	Name	Species	Name
C. annuum	SCM334	*C. baccatum*	Aji
C. annuum	LS2341	*C. frutescens*	Wabit
C. annuum	Bird Pepper	*C. frutescens*	Tabasco
C. annuum	AC2258	*C. chinense*	PI159236
C. annuum	California Wonder	*C. chinense*	Habanero
C. annuum	Fushimiamanaga	*C. chacoense*	PI260429
C. annuum	Ryokugei	*C. pubescens*	Rocoto

Development of SSR markers and marker selection

Four SSR-enriched libraries (motifs GA, GT, AAG, and AAT) were constructed by Genetic Identification Services (GIS, Chatsworth, Calif.) from genomic DNA of *C. annuum* cv. Manganji. A total of 1873 clones, randomly picked from the enriched libraries, were sequenced by Takara Bio, Shiga, Japan. SSRs were identified from the sequence data using the SSR identification tool that screens for all possible dimeric, trimeric, and tetrameric repeats (http://www.gramene.org/). Unique sequences containing a minimum of five di-, tri-, or tetranucleotide motif repeats were used for primer design using PRIMER 3.0 software (http://www.frodo.wi.mit.edu/) in a setting of the major parameters as follows: primer size from 18 to 27 bp; melting temperatures between 57 and 63°C; PCR product size from 150 to 250 bp.

All primer pairs developed in this study, plus 42 primer pairs that were used in the SNU2 map (Lee et al. 2004), were examined to confirm that the amplification of fragments resulted in sizes expected from the sequence data. The SSR primer pairs were then used to screen the parental lines for polymorphisms. SSRs were amplified under the following ''touchdown'' PCR conditions: one cycle of 94°C for 3 min; 10 cycles of 94°C for 30 s, 60°C for 60 s, decreasing by 1°C per cycle, and 72°C for 60 s; 30 cycles of 94°C for 30 s, 55°C for 60 s, and 72°C for 60 s; a final extension for 5 min. Subsequently, PCR products were labeled with Cyanine 5.5 (Amersham Biosciences) using a modified post-PCR fluorescence-labeling method (Inazuka et al. 1996). The labeled products were precipitated, washed twice with ethanol, and a mixture of the labeled sample and a molecular marker was loaded onto a Beckman CEQ 2000XL sequencer equipped with a 33 cm capillary (Beckman Coulter, Fullerton, Calif.). The resulting electrophoretogram was analyzed using the CEQ 8000 genetic analysis system (Beckman Coulter).

Scoring of DNA polymorphisms in the TMDH population

AFLP analysis was carried out according to Vos et al. (1995) with some modifications. The total DNA of each plant was digested with the restriction enzymes *Eco*RI and *Mse*I, ligated to the two adapters for *Eco*RI and *Mse*I cutting sites, and then preamplified with a pair of preselective primers for *Eco*RI and *Mse*I. The selective amplifications were performed using six *Eco*RI primers and eight *Mse*I primers, each with three additional nucleotides at the 3′-ends. The 5′-end of the *Eco*RI primer was labeled with D2-, D3-, or D4-fluorescent dye (Proligo Japan KK, Kyoto, Japan). Electrophoresis of the PCR products and fragment analysis were carried out using the same method at that used in the post-labeling SSR analysis.

RAPD analysis was carried out in a 12-μl volume containing 0.5 U *Taq* DNA polymerase (Takara Bio), 1× buffer (Takara Bio), 200 μM each of dNTPs, 0.05% Triton X-100, 0.42 μM of an arbitrary primer, and 12 ng of genomic DNA. Thirty-three 10-mer arbitrary primers that had previously been used to screen the polymorphism between the parental lines (Minamiyama et al. 2005; Operon Technologies, Alameda, Calif.) were used. DNA amplification was performed with a Program Temp Control System PC-800 (Astec, Fukuoka, Japan) under the following conditions: one step of 94°C for 3 min; 40 cycles of 94°C for 1 min, 40°C for 2 min, and 72°C for 2 min; a final step of 72°C for 5 min. The PCR products were separated on 2% agarose gels and visualized on a UV transilluminator after staining with ethidium bromide.

SSR primer pairs that detected polymorphisms between the parents were then examined using dye-labeled primers. The 5′-end of forward

primers was labeled with D2-, D3- or D4-fluorescent dye, and then used to score polymorphisms in the TMDH population. The PCR products were analyzed as described above.

The CAPS (cleaved amplified polymorphic sequence) marker linked to the *C* gene was scored as described previously (Minamiyama et al. 2005).

Linkage map construction

Linkage analysis was performed using JOINMAP3.0 software with a population type code, DH1 (Van Ooijen and Voorrips 2001). As the LOD score was less than six, a large number of markers would be disregarded as insufficient linkage markers in calculating the map after grouping. Therefore, the linkage groups were separated using an LOD score of 6.5. Recombination values were converted to genetic distances using the Kosambi mapping function (Kosambi 1944).

Evaluation of SSR markers using *Capsicum* lines

The selected SSR primers were examined for the detection of polymorphisms using the set of *Capsicum* lines described above. We calculated the number of alleles and polymorphism information content (PIC) of each marker locus according to Nei's statistic (Nei 1973): PIC = $1-\sum (p_I)^2$, where p_I is the frequency of the Ith allele at the locus.

Results

Development of SSR markers

We sequenced 1873 clones from four SSR-enriched genomic libraries (Table 2). Of these, 957 clones (51%) contained SSR sequences with more than five repeats. We excluded redundancies and clones with flanking sequences that were too short, and finally selected 626 SSR clones for primer construction. Among the four SSR-enriched libraries the percentage of clones containing SSR sequences was higher in the GT motif library (71%) than in the GA, AAG, and AAT motif libraries (32–55%). However, the number of primers designed from the GT motif library was the same as that designed from the GA motif library because of the high incidence of duplication in the GT motif library. Thus, a higher percentage of the final primer pairs designed was taken from the dinucleotide motif libraries than from the trinucleotide motif libraries. These 626 primer pairs were tested for polymorphisms between the parents, Manganji and Tongari using the modified post-PCR fluorescence-labeling method, of which 594 primer pairs (95%) amplified products with the expected size. However, only 153 primer pairs (26%) were polymorphic between the parental lines.

Additionally, SSR markers from the SNU2 map (Lee et al. 2004) were examined for polymorphisms between the parental lines. Of the 42 primer pairs, 36 were suitable for use in the post-labeling techniques. Nine primer pairs (25%) among these 36 were polymorphic between the parents.

Construction of linkage map

Dye-labeled primers were synthesized for the selected 153 polymorphic primer pairs. Among these, 106 pairs demonstrated reproducible patterns in the TMDH population. The remaining primer pairs generated fragments; however, they could not be used for mapping because of faint amplifications or ''stutter'' bands. Among the 106

Table 2 Development of markers from four simple sequence repeat (SSR)-enriched genomic libraries in pepper

Repeat motif	Clones sequenced	Clones with SSRs (%)	Primer pairs designed (%)	PCR amplification	Polymorphisms between parental lines (%)
GT/CA	565	401 (71)	237 (42)	217	48 (22)
GA/CT	553	303 (55)	202 (37)	196	54 (28)
AAT/TTA	376	119 (32)	90 (24)	87	20 (23)
AAG/TTC	379	134 (35)	97 (26)	94	31 (33)
Total	1873	957 (51)	626 (33)	594	153 (26)

primer pairs, 98 pairs amplified a single locus and eight pairs detected more than two loci. The 106 primer pairs detected 114 loci, of which 95 loci were codominant. Fifteen loci did not appear to be target SSRs because the fragment size obtained differed from that expected from the sequence data. Post-fixed figures of more than two were donated to the primer name in these non-target loci (Table 3). We also mapped nine SSR markers from the SNU2 map.

Consequently, we analyzed 123 SSR loci, along with 228 AFLP markers, 60 RAPD markers, one CAPS marker, and the trait of fruit pungency, and constructed a linkage map. The total map length for the TMDH population was 1042.4 cM with 13 linkage groups (LGs) at LOD 6.5 (Fig. 1). The average distance between the markers in our map was 2.8 cM (Fig. 1). We mapped 114 of the 123 SSR markers scored, and the remaining nine were independent. Although clusters of SSR markers were found on some LGs, including LG1, LG2, LG3, and LG5, new SSR markers were distributed throughout all of the linkage groups, with an average distance of 5.7 cM. Of the 114 mapped SSR markers, 28 loci (24.6%) deviated significantly ($p < 0.05$) from the expected ratio in the DH population. The pungency trait locus *C* and its linkage marker were mapped in LG13 where the SSR marker, CAMS327, was also mapped (Minamiyama et al. 2005).

Characterization and polymorphisms of the SSR markers mapped for the TMDH population

The sequences of 99 primer pairs along with other relevant information are shown in Table 3. Of the targeted SSRs, 54 were simple repeats and 45 were compound repeats, comprising two or more motifs. Most of the SSRs from the GT-enriched library were GT-AT compound repeats, similar to SSRs reported in eggplant and tomato (Nunome et al. 2003). In contrast, SSRs from the other three libraries (GA, AAT, and AAG) were mainly simple repeats. The repeats were often interrupted by several nucleotides, and the length of continuous repeat motifs varied from 5 to 32 motifs, with an average of 12 motifs per clone. Therefore, the total length of repeats varied from 5 to 39 motifs, with an average of 17 motifs.

The SSR markers used in the TMDH map were then examined for the ability to detect polymorphisms using seven cultivars and breeding lines of *C. annuum* and seven lines of related *Capsicum* species (Table 3). The average number of alleles per locus was 2.9 in the *C. annuum* lines and 4.0 in the *Capsicum* relatives. The average PIC value was 0.46 in the *C. annuum* lines and was slightly higher at 0.63 in the *Capsicum* relatives. The relationship of the PIC values to the mapping position of the SSR markers is shown in Fig. 2. About one half (34 of 82) of the SSR markers had PIC values greater than 0.7 in the *Capsicum* relatives; these were considered to be very useful in the mapping study. These highly polymorphic SSRs were distributed throughout 12 LGs, with the exception of LG13, where only one SSR was mapped. Moreover, in the *C. annuum* lines, 43 of the 87 SSR markers that were polymorphic had PIC values greater than 0.5 and were distributed throughout 12 LGs, again with the exception of LG13.

Discussion

SSR markers are useful in the genetic mapping of low polymorphic crops

Low levels of DNA polymorphism in crop species is an obstacle to applying molecular marker technology in breeding programs. Self-incompatible *Brassica* crops tend to show high levels of polymorphism among breeding lines and cultivars, whereas other crops, such as eggplant, soybean, and cucumber, tend to show low levels of polymorphic loci within each species (Maughan et al. 1996; Bradeen et al. 2001; Nunome et al. 2001). Therefore, interspecific crosses are sometimes used for mapping populations in these low polymorphic species. Breeding lines of sweet and hot *C. annuum* also show low levels of polymorphism. Overcoming this low polymorphism is a key to marker technology. AFLP is a powerful technique with which to score a number of polymorphic loci in a single experiment (Vos et al. 1995). In this study, 48 *Eco*RI/*Mse*I primer

Table 3 Polymorphism information content (PIC) of simple sequence repeat (SSR) loci of pepper located on the genetic map

SSR marker	Repeat motif	Total repeat	Maximum repeats	*Capsicum* relatives		*Capsicum annuum*		Forward primer	Reverse primer	Linkage group	Expected product size (bp)
				Number of alleles	PIC	Number of alleles	PIC				
CAMS-015	(ac)7at(ac)8a(ta)7	22	8	4	0.72	4	0.61	tcatgttgattatgcttttgttca	ccatgtattgtatgatacctgagaaa	2	112
CAMS-020	(tg)9...(tg)7	16	9	4	0.69	2	0.50	cagcagtaacagaggcaggtc	cacaagtgagtttattcatatcacca	5	171
CAMS-024	(tg)14tt(tg)3	17	14	5	0.73	2	0.24	tgttgaggcttgggaaaaac	caagataatgggtagaaaggcaac	8	219
CAMS-040	(ta)4tg(ta)4...(tg)10	18	10	–	–	–	–	tatagcctgtgggtgccttc	tggggtgaacaatagcatgt	3	248
CAMS-051	(gt)3a(ta)4(tg)11	18	11	4	0.69	3	0.57	acccagttccctttcttggt	gaaggttagcggaatgaacg	5	151
CAMS-056	(ta)6(tg)13...(tg) 3ta(tg)5ta(tg)5a(gt)3	35	13	6	0.82	2	0.24	cattgtccaggctgatgttt	aactcaggtacacgggataaaa	1	247
CAMS-063-2	(tc)6...(ac)4...(tc) 4(ac)6...(tc)3ct(tc)3	26	6	–	–	–	–	ccactctccaaaagcaaacc	tgtttgccactgtatgtgtctg	2	199
CAMS-065	(ac)12	12	12	4	0.72	3	0.61	ccagtctcatccagcagaca	catatgctgctcctgcattc	2	213
CAMS-070	(gt)3...(tct)3...(ac)16...(ac)3	25	16	4	0.69	3	0.57	ccctgaacttgtcctccaaa	gggtatggggtgtaggtgtg	6	248
CAMS-072	(ac)13	13	13	5	0.78	3	0.61	cccgcgaaatcaaggtaat	aaagctattgctactgggttcg	5	153
CAMS-075	(tg)10	10	10	2	0.49	1	0.00	actaattacacattctgcattttctc	aggctcgagtaccacgaaga	5	190
CAMS-081	(ta)7(tg)12	19	12	5	0.78	2	0.24	gttgggggagagttgggtat	tggggtgaacactagcatgt	7	238
CAMS-089	(tc)19	19	19	5	0.73	2	0.41	aacagcgctgatcctttacc	caacatcacagtggcagaaga	11	221
CAMS-090	(ca)3a(ac)15	18	15	5	0.73	2	0.24	tcgctcaaagcacatcaaag	cttgattgttcttccactgctg	8	243
CAMS-095	(ca)3...(ca)9...(ac) 5at(ac)9(at)6g(ta)3	35	9	3	0.57	2	0.41	cgctagcatgacactcaagg	aaacggcaaggctacacatc	5	228
CAMS-101	(tg)4ta(tg)10	14	10	6	0.82	4	0.69	tcagcaattaacatgccaaaa	tggattgggagaagatcgac	6	217
CAMS-117	(tg)21(ta)3	24	21	4	0.69	7	0.86	ttgtggaggaaacaagcaaa	cctcagcccaggagacataa	11	223
CAMS-122	(ta)3t(ac)8...(ac)3	14	8	3	0.57	3	0.57	catccacgtgcatagtcagg	ttctaagtgttgaaattatgggattt	5	178
CAMS-142	(ta)3...(ac)7...(ac)12a(ta)8	30	12	3	0.56	5	0.78	gagcgcttaagtggtcatagg	ctacaacgccccaaaacaat	7	241
CAMS-153	(ta)7(tg)14cg(tg)6	27	14	5	0.78	5	0.73	tgcacaaatatgaatcccaaga	aagtcagcaaacacatctgacaa	6	243
CAMS-156	(ac)14a(ta)6	20	14	4	0.72	4	0.71	ccctatgctttcacaactcct	gacgtggttatgacgataggc	10	181
CAMS-156-2	–	–	–	3	0.57	2	0.24	–	–	5	–
CAMS-162	(ta)7(tg)7ta(tg)6	20	7	2	0.50	4	0.69	ggaccgttcaggaggttaca	gccatcattcaaaaccgaat	1	210
CAMS-163	(at)7(gt)14	21	14	9	0.86	2	0.24	tccatatagcccgtgtgtga	gcgtgggaatacaatgctaga	5	250
CAMS-173	(cata)7...(ac)4	11	7	5	0.77	2	0.41	caaccgccagtagacaggtt	gtgcgtgtgcgtgtgtgtat	4	169
CAMS-173-2	–	–	–	–	–	–	–	–	–	3	–
CAMS-177	(ta)4tg(ta)4(tg)11	19	11	4	0.67	2	0.24	attctctacccctgcctgtg	ctcaggagatgtcccacgat	2	229
CAMS-190	(ta)8(tg)11...(ata)3	22	11	5	0.77	2	0.41	tttctgcagtgttaccaatatttca	cccatgggtcctacctcag	5	212
CAMS-191	(ac)10a(ta)4	14	10	4	0.69	2	0.24	cccgaatccaagtcattgag	taaatccggttccctttcct	3	224
CAMS-194	(ta)7...(tg)11aa(tg)3	21	11	5	0.78	3	0.45	tcatggaaaattaacaacgcata	gggggttggagaagaaagtt	1	245
CAMS-199	(ta)3(tg)9...(ta)3	15	9	4	0.69	3	0.65	ccctacctcggcatgtgata	tgcattgcatggggatataa	5	188
CAMS-201	(ta)7(tg)15ta(tg)4...(ta)3	29	15	1	0.00	2	0.24	ggttattggtttggttcattttt	ttggactaaatgttgatacgagaaa	9	201
CAMS-207-2	(ac)4at(ac)8(at)3	15	8	3	0.45	1	0.00	ctcacgagccacttgaacac	gccttgtttcctatcccaac	5	243
CAMS-215	(ta)7(tg)10tc(tg)5t(tg)5	27	10	1	0.00	3	0.52	cgtgggtggtctaggatgat	gctggcaagtcactctggat	7	220

Table 3 continued

SSR marker	Repeat motif	Total repeat	Maximum repeats	*Capsicum* relatives		*Capsicum annuum*		Forward primer	Reverse primer	Linkage group	Expected product size (bp)
				Number of alleles	PIC	Number of alleles	PIC				
CAMS-224-2	(ta)5(tg)12	17	12	–	–	–	–	tctctgctccaaaatggtga	gtgcgaacccactttgaaat	1	201
CAMS-227	(tgg)3...(tg)8	11	8	5	0.73	2	0.44	tttgtcctttaattcacctttga	gcatcaaaataaggataaagttatgg	5	296
CAMS-234	(ta)5(tg)6cg(tg)7	18	7	3	0.50	5	0.78	tatagcccatgggtgccttt	aaaacccaatattaaccatatgcaa	6	157
CAMS-236	(ac)14a(ta)10	24	14	4	0.60	5	0.70	ttgtagtttgcgtaccatttga	atgaatccagggttccacaa	2	191
CAMS-237	(ta)6(tg)13	19	13	4	0.61	1	0.00	ccttcccttgaaattgatattttac	tcccctatcactcacatctgc	2	221
CAMS-301	(tg)6tt(ag)12	18	12	2	0.50	2	0.41	ctgtccatgcttgtgatgct	tgatttgtgcctcgtttgag	8	180
CAMS-309	(ga)12	12	12	–	–	–	–	gaaaatcgacccgttttgaa	tcaattcggacaaaattagcaa	9	235
CAMS-311	(ga)3g(ga)4g(ga)7g(ga)3	17	7	2	0.48	3	0.66	ggtgcgctagagatggagag	tttgagtgttcgggactggt	6	234
CAMS-313	(ag)10...(ac)3...(ta)3	16	10	3	0.57	2	0.24	cagcctgcttggctagaact	tcgtcatgcatggctaatct	11	223
CAMS-321	(tc)5	5	5	2	0.49	2	0.24	ctgcaggtggttctctccat	tggcactcgaaccagtatga	3	235
CAMS-324	(tc)4...(tc)9...(tc)3	16	9	5	0.78	2	0.24	aacttgatccaacggctgag	tcgaaggagagaaacggttg	7	172
CAMS-327	(tc)7	7	7	4	0.69	2	0.41	gcatctaagtctacgcccttg	aaagcctttggcaatgaaca	13	243
CAMS-330	(ct)3...(tc)12	15	12	–	–	2	0.41	ggctaccgccttctgactta	ttcgtatctggggtgtcaaa	4	208
CAMS-336	(tc)16	16	16	6	0.82	4	0.61	ggtggaaacttgcttggaga	cccagaaccatccacctact	3	157
CAMS-340	(ta)3...(ag)13	16	13	6	0.82	3	0.61	tttatgcccattcacaaaataa	ggacgaattcaccgagtgc	10	250
CAMS-348	(ag)11	11	11	5	0.80	2	0.24	ctgaagtcggctagatgccta	tcaaagctatggaggaaaagga	3	218
CAMS-351	(tg)3...(ag)26	29	26	5	0.78	4	0.69	cgcatgaagcaaatgtacca	acctgcagtttgttgttgga	4	206
CAMS-352-2	(ag)8...(ag)3	11	8	2	0.44	2	0.49	gctccttatgtggtggagga	caccttctgactttggctga	4	240
CAMS-358	(tg)7	7	7	–	–	2	0.28	gacccttctccccttttttctt	cacatggacggatccttttt	6	218
CAMS-360	(ta)8t(ag)13	21	13	–	–	–	–	acggtcgattcctgtattgc	gcatgctaaacccaattttctc	1	209
CAMS-361	(ga)7	7	7	3	0.57	2	0.41	ttggtgtggttaggggagag	ggcgttcgaacttgtgaaat	4	207
CAMS-373	(ag)8	8	8	2	0.48	2	0.50	ggttgatggtccatgttcaa	cctcctaccctatccccaag	12	230
CAMS-376	(tc)6	6	6	2	0.41	2	0.32	ggtgctggcatagatgaaca	tatgtctggcttggtgctga	3	230
CAMS-378	(tc)6...(tc)4...(ct)3	13	6	4	0.61	2	0.24	gaaatcgacgcgtttctagc	tgtggggagagagaggaaga	1	168
CAMS-398	(ag)22	22	22	–	–	5	0.73	atggtccatggtcagcagat	gggcagaacagtggatgatt	7	165
CAMS-398-2	–	–	–	–	–	–	–	–	–	1	–
CAMS-405	(tc)18	18	18	5	0.78	7	0.83	ttcttgggtcccacactttc	aggttgaaaggagggcaata	11	241
CAMS-417	(tc)6ta(tc)3	9	6	2	0.49	2	0.41	cccaaagacgatttgctga	attgcctgtgagtgcaacaa	10	240
CAMS-420	(tc)5ca(tc)4	9	5	3	0.63	1	0.00	cagcgttctatcgtctcaaatg	ttgacaaaccagaaattgatcg	5	200
CAMS-424	(ag)16	16	16	4	0.69	3	0.65	tccacagcccacagtgtcta	gcttgtggttccgtgatttt	6	167
CAMS-450	(tct)3...(tct)4a(tct)9	16	9	–	–	–	–	ccttcttctttgccaccttc	tagcagcagctgatggagaa	5	220
CAMS-451	(tc)21t(ac)3	24	21	4	0.61	3	0.54	tgcattggtgggctaacata	gctcttgacacaaccccaat	11	233
CAMS-451-2	–	–	–	–	–	–	–	–	–	3	–
CAMS-454	(ct)3...(tc)4c(ct)3...(tc)5...(tc)5cc(tc)4	24	5	3	0.57	3	0.57	gagcctcttaatgtatctgaaaaca	aattttggtgaatcgcacct	9	243
CAMS-456	(tc)10	10	10	4	0.69	2	0.44	atggagctggggctaaaaat	gctcagcaaattgaggagaag	1	153
CAMS-460	(tc)20	20	20	6	0.82	3	0.65	cctttcacttcagcccacat	accatccgctaagacgagaa	7	215

Table 3 continued

SSR marker	Repeat motif	Total repeat	Maximum repeats	*Capsicum* relatives		*Capsicum annuum*		Forward primer	Reverse primer	Linkage group	Expected product size (bp)
				Number of alleles	PIC	Number of alleles	PIC				
CAMS-478	(ag)12	12	12	5	0.72	1	0.00	gagtgccatgctgattaagga	cacgactgtcttgcctgaac	3	248
CAMS-489	(ag)4ca(ag)6	10	6	1	0.00	1	0.00	taagtttgtgtcgggcatgt	atggagcttgaactgggatg	1	240
CAMS-492	(tc)6...(ac)4(tc)4	14	6	4	0.75	2	0.41	gttcaaacacttccccctca	tgtcatcgttggtcgttacc	12	250
CAMS-494	(ag)7...(ag)3	10	7	2	0.50	1	0.00	ggtaggtgaggacccacaga	aactatacccccgctgctct	4	247
CAMS-606	(gt)3...(tc)14	17	14	4	0.61	5	0.78	gactagtccccgttcaacca	tttgcgagaagatgcttcag	7	208
CAMS-610	(taa)5...(taa)11	16	11	1	0.00	2	0.44	ttgggacatgacaattctgc	aaacgtacattaggtagatccggta	1	207
CAMS-619	(tct)16...(tct)4	20	16	4	0.67	2	0.41	tcctgctctcctcagaaagtaaa	gaaacaggaggaagaggaagaa	9	154
CAMS-630-2	(tct)5...(ctt)3	8	5	–	–	–	–	ccctcattatggagcaacca	ttgagggagggacataagaca	1	172
CAMS-635	(aat)3ga(taa)13...(att)3	19	13	–	–	–	–	gagattggtcggatggaaat	aaaatgtcaaagtcgatcaaaca	2	154
CAMS-644	(tg)3...(ag)26	29	26	5	0.78	3	0.61	cgcatgaagcaaatgtacca	acctgcagtttgttgttgga	4	206
CAMS-647	(tat)6tg(tta)3...(tat)21	30	21	10	0.95	3	0.65	cggattcggttgagtcgata	gtgctttggttcggtctttc	3	221
CAMS-655-2	(taa)12	12	12	–	–	–	–	ccttgctgctacctattgaaga	tccaatcgtcgatatgtttgtt	4	224
CAMS-668-2	(tc)17	17	17	–	–	–	–	atcgtccatttgtgggaaaa	tgtggtcggaaataggaagaa	10	158
CAMS-679	(tat)16	16	16	6	0.82	3	0.45	tttgcatgttttacccattcc	atgtgaaacacataggtagcactga	1	200
CAMS-684	(tat)4(taa)7...(taa)10	21	10	5	0.78	4	0.61	cagggtgagccaaaccatag	tcaagactactaggtcgtggaatg	6	232
CAMS-684-2	–	–	–	1	0.00	2	0.48	–	–	4	–
CAMS-806	(aga)19	19	19	6	0.74	5	0.79	tgtcacaagtgtcaaggtaggag	ccccaaaaattttccctcat	10	227
CAMS-806-2	–	–	–	3	0.63	3	0.50	–	–	5	–
CAMS-808	(ct)3...(tct)11	14	11	–	–	–	–	aatggcttgcctaacacagg	cggaattgatccctgacaaa	2	229
CAMS-811	(aag)3...(gaa)3...(gaa)7	13	7	–	–	6	0.82	gaagaaacgaaggatgaacaaaa	cctgtttcctcttcctcagc	9	260
CAMS-826	(gaa)6ga(gga)9...(gaa)3ga(gaa)3	21	9	4	0.61	3	0.50	cttgatctcaagaaccagctacaa	tgtacattgaagacacggaagaa	8	244
CAMS-838	(aga)19	19	19	3	0.56	2	0.50	ccaggatggtgttaagggttt	gtcgcatcaatgagcatagg	6	229
CAMS-842	(aga)17...(aga)8...(aga)5(agca)4...(aga)5	39	17	–	–	–	–	tggaaaagtagcagcagcag	cgcctcctcgttcatctg	1	210
CAMS-844	(gaa)6	6	6	4	0.72	4	0.69	gcaaagaaaaagaaaagcctga	ctgcaactgctgcttcattc	1	223
CAMS-846	(tct)10	10	10	–	–	–	–	tctttttcctcgccatcttc	cactgttatggcaaaagaggaa	3	189
CAMS-855	(agt)14a(gaa)9	23	14	6	0.83	4	0.69	aagtgtcaaggaaggggaca	cctaaccaccccccaaaagtt	8	243
CAMS-861	(aga)11	11	11	–	–	–	–	gcatgcaagcttagccaac	tgagattgaagctagaattttgga	2	183
CAMS-864	(aga)32	32	32	5	0.78	7	0.86	ctgttgtggaagaagaggaca	gcttctttttcaacctcctcct	7	222
CAMS-865	(gaa)7	7	7	4	0.69	1	0.00	agaaatcgtggttgggtgag	cactttggcacattttgctg	11	179
CAMS-876	(cct)3tc(tct)4...(tct)7	14	7	5	0.78	2	0.49	tgtgtctgaagcggaacaaa	aagcagtgagcgacgaaga	9	243
CAMS-880	(gaa)12	12	12	–	–	2	0.50	gagccaagaaaaaggtggaa	caactcatcgttcaacaacaca	6	237
CAMS-885	(gaa)28	28	28	3	0.64	4	0.61	aacgaaaaacaaacccaatca	ttgaaattgctgaaactctgaa	2	248
CAMS-888	(gaa)12	12	12	–	–	–	–	cctcggagtggtttgttgat	gcttgttcacgccaccttat	2	211
CAMS-891	(gaa)8	8	8	3	0.63	1	0.00	ctccgagaaggatgtcagga	actgagcgactgatgcctct	6	204
CAMS-892-2	(tct)12	12	12	–	–	–	–	cgtcgattgaccattgagtg	tccttctaggaggcatgttttt	2	184
CAMS-892-3	–	–	–	3	0.63	6	0.74	–	–	3	–

combinations generated 228 AFLP markers. However, the application of AFLP markers to other populations is difficult because of the use of nonspecific primers. Single nucleotide polymorphisms (SNPs) and SSRs are marker candidates for effective mapping in these low polymorphic crops and can specifically detect a single locus, even in different populations. In general, there are only two alleles at a single SNP locus; however, SNPs may be found in large numbers throughout a genome. In contrast, SSR loci may be far more rare than SNP loci, although enough can be found for ordinary linkage analysis and each SSR locus has many alleles (Goldstein and Schloetterer 1999). In model plants, such as rice, tomato, or *Arabidopsis*, information about SNPs is accumulating, and it is likely that SNPs will be more commonly used in the genetic mapping of these plants in the near future (Torjek et al. 2003; Shen et al. 2004; Yang et al. 2004). However, information on SNPs in most other crops is limited. As a result, SSR is currently a more useful marker in these crops, including *Capsicum*. SSRs developed for other members of the Solanaceae family are not useful in potato and eggplant (Nunome et al. 2003; Ghislain et al. 2004). Therefore, in this study, we developed SSR markers specifically for *C. annuum* and, because the sequence data for *Capsicum* are limited, we screened enriched library systems to develop these SSRs.

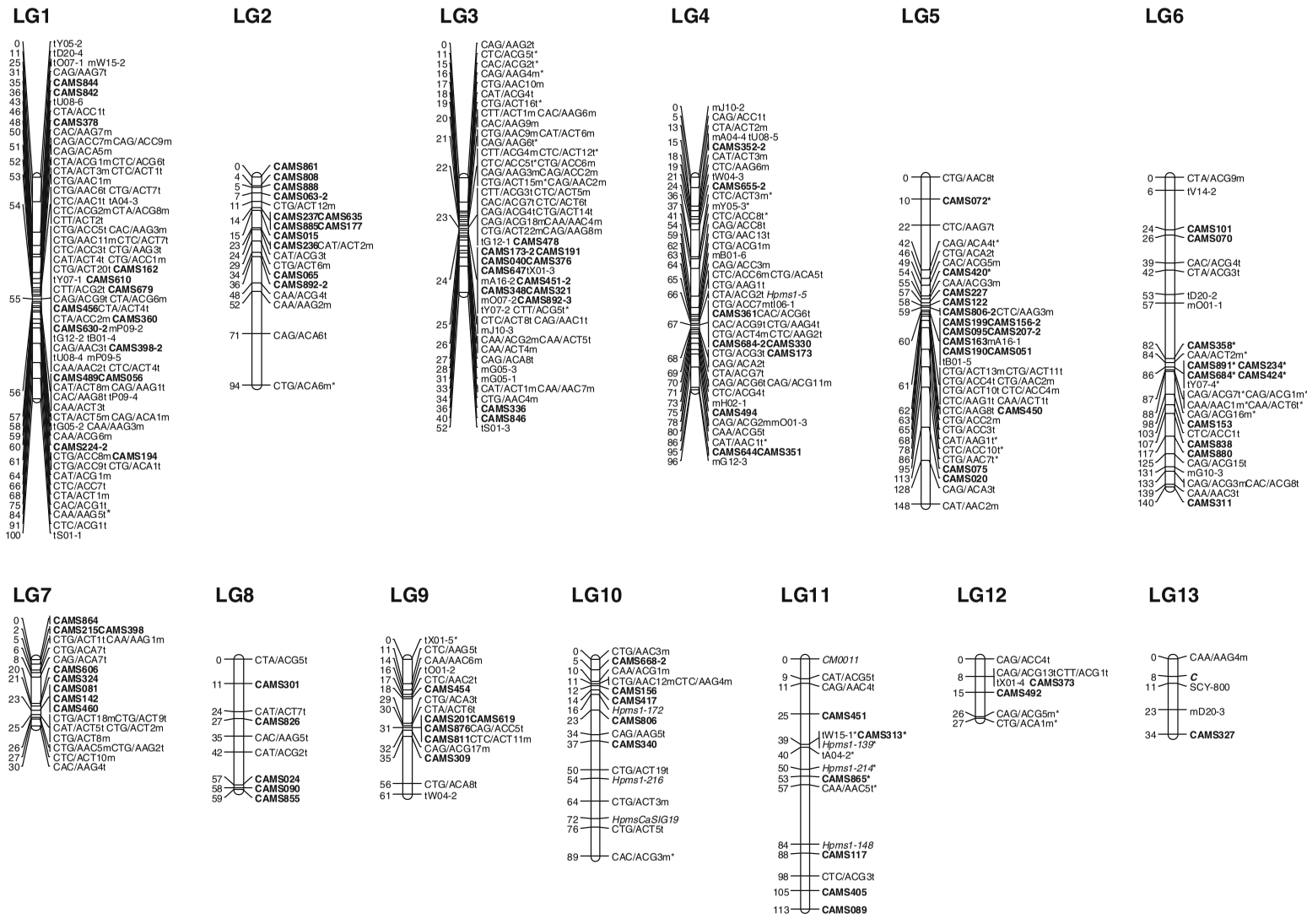
Development and characterization of SSR markers in *Capsicum*

We found that 51% of the 1873 clones in the enriched libraries contained SSRs, and we were able to design primers from 65% of these SSR-containing clones after excluding redundancies and clones with flanking sequences that were too short. The efficiency of the development of SSR markers for *Capsicum* using enriched library technology is similar to the results obtained in ryegrass (Jones et al. 2001) and timothy grass (Cai et al. 2003). There were 132 clones with flanking sequences that were too short, and the level of redundancy was 24%, which was lower than the 41% redundancy level found in 4000 clones of timothy grass using SSR-enriched libraries produced by GIS. However, the level of redundancy may have been higher in pepper if more clones had been sequenced.

Fig. 1 Linkage map of pepper (*Capsicum annuum*) based on a doubled haploid population of a cross between cvs. Manganji and Tongari using 228 AFLP, one CAPS, 60 RAPD, and 123 SSR markers and the trait of fruit pungency. On the *left of the vertical double lanes* are the map distances in centiMorgans (cM), calculated by the Kosambi function; on the *right* are DNA markers with identification numbers and names. The SSR markers we developed are indicated in *bold* and the SSR markers from the SNU2 map (Lee et al. 2004) are indicated in *italics*. For the SSR markers we developed, nontarget loci were given a *hyphenated number following the primer name*. The markers with *asterisks* are those showing significant segregation distortion ($p < 0.01$)

Previous studies have shown that the majority of di- and trinucleotide motifs in plants are likely to be AT/TA and ATT/TAA (Lagercrantz et al. 1993). However, because of the presence of a palindromic sequence, the AT/TA motif is not used in SSR-enrichment procedures. In *Brassica rapa*, the GA/CT motif is nearly fivefold more abundant than the GT/CA motif (Suwabe et al. 2002). In contrast, we found that the GT/CA motif was slightly more frequent in *Capsicum* than the GA/CT motif. Moreover, most of the GT-containing clones had mainly GT-AT compound repeats. Similar results have been reported for other Solanaceae species, such as tomato and potato (Lagercrantz et al. 1993; Broun and Tanksley 1996; Milbourne et al. 1998; Areshchenkova and Ganal 1999), suggesting that these SSR features are common to Solanaceae species.

In this study, we obtained 594 primer pairs that amplified fragments of the expected size. Most of these primer pairs amplified SSR sequences and would be potentially useful in mapping studies of *Capsicum* species. However, we used only 106 of these primer pairs for the present mapping study. The low percentage of primer pairs used reflects the low polymorphism of the *C. annuum* population used in this study. Similarly, only 24% of the SSR markers were selected as polymorphic from the SSRs used in mapping based on an interspecific cross (Lee et al. 2004). A similar figure was obtained in other mapping populations within *C. annuum* (data not shown). The PIC


LG1
0 tY05-2
11 tD20-4
25 tO07-1 mW15-2
31 CAG/AAG7t
35 CAMS844
36 CAMS842
43 tU08-6
46 CTA/ACC1t
48 CAMS378
50 CAC/AAG7m
51 CAG/ACC7m CAG/ACC9m
CAG/ACA5m
52 CTA/ACG1m CTC/ACG6t
53 CTA/ACT3m CTC/ACT1t
CTG/AAC1m
CTG/AAC6t CTG/ACT7t
54 CTC/AAC1t tA04-3
CTC/ACG2m CTA/ACG8m
CTT/ACT2t
CTG/ACC5t CAC/AAG3m
CTG/AAC11m CTC/ACT7t
CTC/ACC3t CTG/AAG3t
CAT/ACT4t CTG/ACC1m
CTG/ACT20t CAMS162
tY07-1 CAMS610
CTT/ACG2t CAMS679
55 CAG/ACG9t CTA/ACG6m
CAMS456CTA/ACT4t
CTA/AAC2m CAMS360
CAMS630-2mP09-2
tG12-2 tB01-4
CAG/AAC3t CAMS398-2
tU08-4 mP09-5
CAA/AAC2t CTC/ACT4t
CAMS489CAMS056
56 CAT/ACT8m CAG/AAG1t
CAC/AAG8t tP09-4
CAA/ACT3t
57 CTA/ACT5m CAG/ACA1m
58 tG05-2 CAA/AAG3m
59 CAA/ACG6m
60 CAMS224-2
61 CTG/ACC8m CAMS194
CTG/ACC9t CTG/ACA1t
64 CAT/ACG1m
66 CTC/ACC7t
68 CTA/ACT1m
75 CAC/ACG1t
84 CAA/AAG5t*
91 CTC/ACG1t
100 tS01-1
LG2
0 CAMS861
4 CAMS808
5 CAMS888
7 CAMS063-2
11 CTG/ACT12m
14 CAMS237CAMS635
CAMS885CAMS177
15 CAMS015
23 CAMS236CAT/ACT2m
24 CAT/ACG3t
29 CTG/ACT6m
34 CAMS065
36 CAMS892-2
48 CAA/ACG4t
52 CAA/AAG2m
71 CAG/ACA6t
94 CTG/ACA6m*
LG3
0 CAG/AAG2t
11 CTC/ACG5t*
15 CAC/ACG2t*
16 CAG/AAG4m*
17 CTG/AAC10m
18 CAT/ACG4t
19 CTG/ACT16t*
20 CTT/ACT1m CAC/AAG6m
CAC/AAG9m
21 CTG/AAC9m CAT/ACT6m
CAG/AAG6t*
CTT/ACG4m CTC/ACT12t*
22 CTC/ACC5t*CTG/ACC6m
CAG/AAG3mCAG/ACC2m
CTG/ACT15m*CAG/AAC2m
CTT/ACG3t CTC/ACT5m
CAC/ACG7t CTC/ACT6t
CAG/ACG4t CTG/ACT1-4t
23 CAG/ACG18mCAA/AAC4m
CTG/ACT22mCAG/AAG8m
tG12-1 CAMS478
CAMS173-2CAMS191
CAMS040CAMS376
CAMS647tX01-3
24 mA16-2CAMS451-2
CAMS348CAMS321
mO07-2CAMS892-3
25 tY07-2 CTT/ACG5t*
CTC/ACT8t CAG/AAC1t
mJ10-3
26 CAA/ACG2mCAA/ACT5t
CAA/ACT4m
27 CAG/ACA8t
28 mG05-3
31 mG05-1
33 CAT/ACT1m CAA/AAC7m
34 CTG/AAC4m
36 CAMS336
40 CAMS846
52 tS01-3
LG4
0 mJ10-2
5 CAG/ACC1t
13 CTA/ACT2m
mA04-4 tU08-5
15 CAMS352-2
18 CAT/ACT3m
19 CTC/AAG6m
21 tW04-3
24 CAMS655-2
36 CTC/ACT3m*
37 mY05-3*
41 CTC/ACC8t*
54 CAG/ACC8t
59 CTG/AAC13t
62 CTG/ACG1m
63 mB01-6
64 CAG/ACC3m
CTC/ACC6m CTG/ACA5t
65 CTG/AAG11t
66 CTA/ACG2t Hpms1-5
CTG/ACC7mtI06-1
CAMS361CAC/ACG6t
67 CAC/ACG9t CTG/AAG4t
CTG/ACT4m CTC/AAG2t
CAMS684-2CAMS330
68 CTG/ACG3t CAMS173
CAG/ACA2t
69 CTA/ACG7t
70 CAG/ACG6t CAG/ACG11m
71 CTC/ACG4t
73 mH02-1
75 CAMS494
78 CAG/ACG2mmO01-3
80 CAA/ACG5t
86 CAT/AAC1t*
95 CAMS644CAMS351
96 mG12-3
LG5
0 CTG/AAC8t
10 CAMS072*
22 CTC/AAG7t
42 CAG/ACA4t*
46 CTG/ACA2t
49 CAC/ACG5m
54 CAMS420*
55 CAA/ACG3m
57 CAMS227
58 CAMS122
59 CAMS806-2CTC/AAG3m
CAMS199CAMS156-2
CAMS095CAMS207-2
60 CAMS163mA16-1
CAMS190CAMS051
tB01-5
CTG/ACT13m CTG/ACT11t
CTG/ACC4t CTG/AAC2m
61 CTG/ACT10t CTC/ACC4m
CTC/AAG1t CAA/ACT1t
62 CTC/AAG8t CAMS450
63 CTG/ACC2m
65 CTG/ACC3t
68 CAT/AAG1t*
78 CTC/ACC10t*
86 CTG/AAC7t*
95 CAMS075
113 CAMS020
128 CAG/ACA3t
148 CAT/AAC2m
LG6
0 CTA/ACG9m
6 tV14-2
24 CAMS101
26 CAMS070
39 CAC/ACG4t
42 CTA/ACG3t
53 tD20-2
57 mO01-1
82 CAMS358*
84 CAA/ACT2m*
CAMS891* CAMS234*
86 CAMS684* CAMS424*
tY07-4*
87 CAG/ACG7t*CAG/ACG1m*
CAA/AAC1m*CAA/ACT6t*
88 CAG/ACG16m*
98 CAMS153
103 CTC/ACC1t
107 CAMS838
117 CAMS880
125 CAG/ACG15t
131 mG10-3
133 CAG/ACG3mCAC/ACG8t
139 CAA/AAC3t
140 CAMS311
LG7
0 CAMS864
2 CAMS215CAMS398
5 CTG/ACT1tCAA/AAG1m
6 CTG/ACA7t
8 CAG/ACA7t
20 CAMS606
21 CAMS324
CAMS081
23 CAMS142
CAMS460
CTG/ACT18mCTG/ACT9t
25 CAT/ACT5t CTG/ACT2m
CTG/ACT8m
26 CTG/AAC5mCTG/AAG2t
27 CTC/ACT10m
30 CAC/AAG4t
LG8
0 CTA/ACG5t
11 CAMS301
24 CAT/ACT7t
27 CAMS826
35 CAC/AAG5t
42 CAT/ACG2t
57 CAMS024
58 CAMS090
59 CAMS855
LG9
0 tX01-5*
11 CTC/AAG5t
14 CAA/AAC6m
16 tO01-2
17 CTC/AAC2t
18 CAMS454
29 CTG/ACA3t
30 CTA/ACT6t
CAMS201CAMS619
31 CAMS876CAG/ACC5t
CAMS811CTC/ACT11m
32 CAG/ACG17m
35 CAMS309
56 CTG/ACA8t
61 tW04-2
LG10
0 CTG/AAC3m
5 CAMS668-2
10 CAA/ACG1m
11 CTG/AAC12mCTC/AAG4m
12 CAMS156
14 CAMS417
16 Hpms1-172
23 CAMS806
34 CAG/AAG5t
37 CAMS340
50 CTG/ACT19t
54 Hpms1-216
64 CTG/ACT3m
72 HpmsCaSIG19
76 CTG/ACT5t
89 CAC/ACG3m*
LG11
0 CM0011
9 CAT/ACG5t
11 CAG/AAC4t
25 CAMS451
39 tW15-1*CAMS313*
Hpms1-139*
40 tA04-2*
50 Hpms1-214*
53 CAMS865*
57 CAA/AAC5t*
84 Hpms1-148
88 CAMS117
98 CTC/ACG3t
105 CAMS405
113 CAMS089
LG12
0 CAG/ACC4t
8 CAG/ACG13tCTT/ACG1t
tX01-4 CAMS373
15 CAMS492
26 CAG/ACG5m*
27 CTG/ACA1m*
LG13
0 CAA/AAG4m
8 C
11 SCY-800
23 mD20-3
34 CAMS327

values shown in Table 3 also demonstrate the lower polymorphism among *C. annuum* lines compared with that in related species. Other problems may be related to technical aspects. For example, we used a less expensive post-PCR fluorescence-labeling method for the first survey of the primer pairs, dye-labeled primers were synthesized for the selected primer pairs only, some of the primer pairs showed different amplification patterns between the two techniques, the reason for which is not clear, and some of the SSR primer pairs amplified poor or nonreproducible bands, and others sometimes amplified more complex bands and were not suitable for scoring alleles, as described by Röder et al. (1998). The redesign of primer pairs may improve the amplification to some extent, and the remaining primer pairs have the potential to be used for mapping in future studies. Some of the primer sequences of the SSR clones will be disclosed elsewhere.

The degree of polymorphism increases with the total length of the repeat among cultivars in tomato (Smulders et al. 1997). This correlation was also observed among *C. annuum* in this study: SSRs with fewer than 16 repeats had an average PIC value of 0.37; however, those with more than 17 repeats had an average PIC of 0.54. In contrast, this correlation was not apparent when related species were included in the test of polymorphism because the PIC value of most SSRs shifted into the high range, as in the case of tomato (Smulders et al. 1997). Additionally, more than one half of the mapped SSR markers had interruptions in the repeat motif sequence, although this did not reflect the degree of polymorphism.

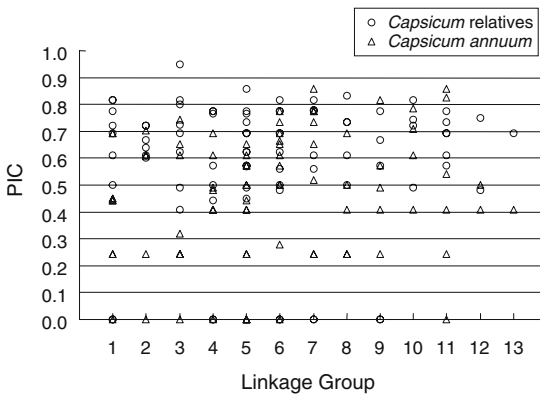


Fig. 2 The polymorphism information content (PIC) of SSR markers located in each linkage group for 14 genotypes in *Capsicum* spp

Map construction based on SSRs

Using the 106 highly reproducible SSR primer pairs, along with nine previously reported SSR, 228 AFLP, one CAPS, and 60 RAPD markers and the trait of fruit pungency, we constructed a linkage map of *C. annuum* in an intraspecific DH population (n=117). At total of 106 new SSR loci produced by 99 primer pairs were mapped in the present linkage map. The characteristics of these 99 SSRs are shown in Table 3. The map contained 374 markers, including 106 new SSR markers in 13 LGs, and covered 1042 cM. The total map length of the pepper is estimated to be between 1245 cM and 1762 cM according to previous reports (Livingstone et al. 1999; Ben Chaim et al. 2001; Kang et al. 2001; Lefebvre et al. 2002; Lee et al. 2004); however, the map we produced here was shorter than this. We consider that the map we constructed covers most of the *Capsicum* genome because there were only 38 unlinked markers after the linkage analysis. The 106 new SSR markers were distributed across all linkage groups; however, clusters of SSR markers were found on some of these. A similar clustering of SSR markers has been observed for markers developed from genomic libraries in several crops (Areshchenkova and Ganal 1999; Jones et al. 2002). Previous studies have also shown that gene-based SSRs generally do not cluster (Scott et al. 2000; Blair et al. 2003). To saturate a linkage map in pepper with SSR markers, it may be necessary to develop the markers from expressed sequence tag (EST) database entries or gene sequences.

A reference map for *Capsicum* crops

Although many mapping studies have been reported on *Capsicum* crops, and a number of agricultural traits have been mapped using different mapping populations (Ben Chaim et al. 2001; Thabuis et al. 2003), the relative map position of loci found in different mapping populations is difficult to determine without a reference

map filled with suitable anchor markers. RFLP markers have been used as anchor markers in previously published maps. However, an RFLP-based map is difficult to use as a reference map because RFLP technology is time-consuming and requires the transfer of probes between laboratories.

A reference map should contain enough polymorphic markers, such as SSRs and SNPs, that are useful in different mapping populations. Although the SNU2 map contains 46 SSRs, it is difficult to use as a reference map. This is due to the fact that maps based on the crossing of *C. annuum* and *C. chinense* have the problem of the adhesion of linkage groups due to chromosome interchange (Ben Chaim et al. 2001); in addition, some linkage groups did not contain any SSR markers in the SNU2 map. As well, only 24% of the SSRs are polymorphic in the present mapping population, and the majority of the SSRs in the SNU2 map may shown a low level of polymorphism in intraspecific crosses of *C. annuum*.

The present map included 114 SSR loci, including eight SSR loci reported by Lee et al. (2004). Each linkage group contained more than two SSR loci, with the exception of LG13, to which the *C* locus was mapped. Markers with a high PIC are evenly mapped in most of the linkage groups (Fig. 2). Therefore, the present map would be useful as a reference map for *Capsicum* crops. The map positions of the loci are easily found using some of the SSR primer pairs presented here. A comparison between the present map and the SNU2 map (Lee et al. 2004) with respect to some of the map positions is as follows: (1) the SSRs mapped in LG1 of the SNU2 map, Hpms1-139, Hpms1-148, Hpms1-214, and CM0011, were mapped on LG11 in the present study and showed consistency in their order; (2) Hpms1-172, Hpms1-216, and HpmsCaSIG19 were on LG10 in the present study, while Hpms1-172 was on LG11, and Hpms1-216 and HpmsCaSIG19 were on LG7 in the SNU2 map. The reason for this disagreement may be that Hpms1-172 amplified more than two loci. Therefore, the locus mapped by Hpms1-172 primers in the present study would be different from that mapped in the SNU2 population, and LG10 in the present study may correspond to LG7 in the SNU2 map. Further studies on mapping the SSRs detected in this study in the SNU2 map or in other pepper maps are needed to clarify these discrepancies.

Acknowledgements We thank Drs. T. Nunome and H. Fukuoka for their valuable suggestions. We are also grateful to Ms. T. Ohta and Ms. W. Okai for their technical assistance.

References

Areshchenkova T, Ganal MW (1999) Long tomato microsatellites are predominantly associated with centromeric regions. Genome 42:536–544

Ben Chaim A, Grube RC, Lapidot M, Jahn M (2001) Identification of quantitative trait loci associated with resistance to cucumber mosaic virus in *Capsicum annuum*. Theor Appl Genet 102:1213–1220

Blair MW, Pedraza F, Buendia HF, Gaitan-Solis E, Beebe SE, Gepts P, Tohme J (2003) Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 107:1362–1374

Bradeen JM, Staub JE, Wye C, Antonise R, Peleman J (2001) Towards an expanded and integrated linkage map of cucumber (*Cucumis sativus* L.). Genome 44:111–119

Broun P, Tanksley SD (1996) Characterization and genetic mapping of simple repeat sequences in the tomato genome. Mol Gen Genet 250:39–49

Cai HW, Yuyama N, Tamaki H, Yoshizawa A (2003) Isolation and characterization of simple sequence repeat markers in the hexaploid forage grass timothy (*Phleum pratense* L.). Theor Appl Genet 107:1337–1349

Caranta C, Pflieger S, Lefebvre V, Daubeze AM, Thabuis A, Palloix A (2002) QTLs involved in the restriction of cucumber mosaic virus (CMV) long-distance movement in pepper. Theor Appl Genet 104:586–591

Chiba N, Suwabe K, Nunome T, Hirai M (2003) Development of microsatellite markers in melon (*Cucumis melo* L.) and their application to major cucurbit crops. Breed Sci 53:21–27

Ghislain M, Spooner DM, Rodriguez F, Villamon F, Nunez J, Vasquez C, Waugh R, Bonierbale M (2004) Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. Theor Appl Genet 108:881–890

Goldstein DB, Schloetterer C (1999) Microsatellite evolution and applications. Oxford University Press, Oxford

Inazuka M, Tahira T, Hayashi K (1996) One-tube post-PCR fluorescent labeling of DNA fragments. Genome Res 6:551–557

Jones ES, Dupal MP, Kolliker R, Drayton MC, Forster JW (2001) Development and characterisation of simple sequence repeat (SSR) markers for perennial ryegrass (*Lolium perenne* L.). Theor Appl Genet 102:405–415

Jones ES, Dupal MP, Dumsday JL, Hughes LJ, Forster JW (2002) A SSR-based genetic linkage map for perennial ryegrass (*Lolium perenne* L.). Theor Appl Genet 105:577–584

Kang BC, Nahm SH, Hhu JH, Yoo HS, Yu UW, Lee MH, Kim BD (2001) An interspecific (*Capsicum annuum* × *C. chinense*) F2 linkage map in pepper using RFLP and AFLP markers. Theor Appl Genet 102:531–539

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lagercrantz U, Ellegren H, Andersson L (1993) The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res 21:1111–1115

Lee JM, Nahm SH, Kim YM, Kim BD (2004) Characterization and molecular genetic mapping of microsatellite loci in pepper. Theor Appl Genet 108:619–627

Lefebvre V, Palloix A, Caranta C, Pochard E (1995) Construction of an intraspecific integrated linkage map of pepper using molecular markers and doubled-haploid progenies. Genome 38:112–121

Lefebvre V, Pflieger S, Thabuis A, Caranta C, Blattes A, Chauvet JC, Daubeze AM, Palloix A (2002) Towards the saturation of the pepper linkage map by alignment of three intraspecific maps including known-function genes. Genome 45:839–854

Livingstone KD, Lackney VK, Blauth JR, van Wijk R, Jahn MK (1999) Genome mapping in *Capsicum* and the evolution of the genome structure in the Solanaceae. Genetics 152:1183–1202

Maughan PJ, Saghai Maroof MA, Buss GR, Huestis GM (1996) Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. Theor Appl Genet 93:392–401

Mba REC, Stephenson P, Edwards K, Melzer S, Nkumbira J, Gullberg U, Apel K, Gale M, Tohme J, Fregene M (2001) Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: towards an SSR-based molecular genetic map of cassava. Theor Appl Genet 102:21–31

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C, Waugh R (1998) Isolation, characterization and mapping of simple sequence repeat loci in potato. Mol Gen Genet 259:233–245

Minamiyama Y, Kinoshita S, Inaba K, Inoue M (2005) Development of a cleaved amplified polymorphic sequence (CAPS) marker linked to pungency in pepper. Plant Breed 124:288–291

Moury B, Pflieger S, Blattes A, Lefebvre V, Palloix A (2000) A CAPS marker to assist selection of tomato spotted wilt virus (TSWV) resistance in pepper. Genome 43:137–142

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323

Nunome T, Ishiguro K, Yoshida T, Hirai M (2001) Mapping of fruit shape and color development traits in eggplant (*Solanum melongena* L.) based on RAPD and AFLP markers. Breed Sci 51:19–26

Nunome T, Suwabe K, Iketani H, Hirai M (2003) Identification and characterization of microsatellites in eggplant. Plant Breed 122:256–262

Paran I, Rouppe van der Voort J, Lefebvre V, Jahn M, Landry L, van Schriek M, Tanyolac B, Caranta C, Ben Chaim A, Livingstone K, Palloix A, Peleman J (2004) An integrated genetic linkage map of pepper (*Capsicum* spp.). Mol Breed 13:251–261

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS, Henry RJ (2000) Analysis of SSRs derived from grape ESTs. Theor Appl Genet 100:723–726

Shen YJ, Jiang H, Jin JP, Zhang ZB, Xi B, He YY, Wang G, Wang C, Qian L, Li X (2004) Development of genome-wide DNA polymorphism database for map-based cloning of rice genes. Plant Physiol 135:1198–1205

Smulders MJM, Bredemeijer G, Rus-Kortekaas W, Arens P, Vosman B (1997) Use of short microsatellites from database sequences to generate polymorphism among *Lycopersicon* esculentum cultivars and accessions of other *Lycopersicon* species. Theor Appl Genet 97:264–272

Suwabe K, Iketani H, Nunome T, Kage T, Hirai M (2002) Isolation and characterization of microsatellites in *Brassica rapa* L. Theor Appl Genet 104:1092–1098

Thabuis A, Palloix A, Pflieger S, Daubeze AM, Caranta C, Lefebvre V (2003) Comparative mapping of *Phytophthora* resistance loci in pepper germplasm: evidence for conserved resistance loci across Solanaceae and for a large genetic diversity. Theor Appl Genet 106:1473–1485

Torjek O, Berger D, Meyer RC, Mussig C, Schmid KJ, Sorensen TR, Weisshaar B, Mitchell-Olds T, Altmann T (2003) Establishment of a high-efficiency SNP-based framework marker set for *Arabidopsis*. Plant J 36:122–140

Van Ooijen JW, Voorrips RE (2001) JOINMAP 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Vos P, Hogers R, Bleeker M, Reijians M, Lee T, van de Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Yang W, Bai X, Kabelka E, Eaton C, Kamoun S, van der Knaap E, Francis D (2004) Discovery of single nucleotide polymorphisms in *Lycopersicon esculentum* by computer aided analysis of expressed sequence tags. Mol Breed 14:21–34