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SNP Marker Integration and QTL Analysis of 12 Agronomic and Morphological Traits in F_8 RILs of Pepper (*Capsicum annuum* L.)

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Red pepper, *Capsicum annuum* L., has been attracting geneticists' and breeders' attention as one of the important agronomic crops. This study was to integrate 41 SNP markers newly developed from comparative transcriptomes into a previous linkage map, and map 12 agronomic and morphological traits into the integrated map. A total of 39 markers found precise position and were assigned to 13 linkage groups (LGs) as well as the unassigned LGe, leading to total 458 molecular markers present in this genetic map. Linkage mapping was supported by the physical mapping to tomato and potato genomes using BLAST retrieving, revealing at least two-thirds of the markers mapped to the corresponding LGs. A sum of 23 quantitative trait loci from 11 traits was detected using the composite interval mapping algorithm. A consistent interval between a035_1 and a170_1 on LG5 was detected as a main-effect locus among the resistance QTLs to *Phytophthora capsici* at high-, intermediate- and low-level tests, and interactions between the QTLs for high-level resistance test were found. Considering the epistatic effect, those QTLs could explain up to 98.25% of the phenotype variations of resistance. Moreover, 17 QTLs for another eight traits were found to locate on LG3, 4, and 12 mostly with varying phenotypic contribution. Furthermore, the locus for corolla color was mapped to LG10 as a marker. The integrated map and the QTLs identified would be helpful for current genetics research and crop breeding, especially in the *Solanaceae* family.

INTRODUCTION

As a member of *Solanaceae* family, a divergent family comprised of around 3,000-4,000 species (Knapp et al., 2004) ranging from trees to annual herbs, red pepper (*Capsicum annuum* L.) is an important crop with an economic significance. It is one of the worldwide favorite ingredients when cooking food since its domestication in South and Central America about 6,000 years ago (Perry et al., 2007). Pepper's consumption is increasing worldwide, and the vegetable is an important source of vitamins and essential nutrients (Lee et al., 2007). Moreover, medical usage of pepper is also developed to alleviate the pain and long-term inflammation (Ben Chaim et al., 2006). Therefore, it has been attracting attention of breeders and geneticists (Hwang et al., 2009; Kwon and Kim, 2009; Seo et al., 2012).

Saturated genetic maps consisting of molecular markers are critical for quantitative trait loci (QTLs) analysis. So far, many markers have been used to develop linkage maps, such as amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), cleaved amplified polymorphic sequence (CAPS), and single nucleotide polymorphisms (SNP) (Cho et al., 2010; Lee et al., 2009a; 2009b). Despite the unavailable whole genome sequence information, numerous ESTs have been isolated, and provided valuable information for molecular marker development (Yi et al., 2006). Especially due to the advanced sequencing technique, much more gene-based molecular markers could be exploited from transcriptome data from massively-parallel next-generation sequencing (NGS) (Lu et al., 2011; 2012; Metzker, 2010). Researchers have benefited from the availability of techniques for the rapid and cost-effective development of molecular marker-based linkage maps (Yang et al., 2011). Four genetic maps were provided on Solanaceae Genomics Network (http://solgenomics.net/), of which pepper-FAO3 contained the most 720 markers with an averaged 1.89 cM interval (Mueller et al., 2005). Another integrated genetic linkage map was reported to consisting of 2,262 markers covering 1,832 cM with an average of 0.81 cM (Paran et al., 2004).

Linkage maps have proved to be powerful for discovering, dissecting and manipulating the genes that determine simple and complex traits in crop plants (Tanksley et al., 1992). Many studies have been carried out to discover those QTLs related to

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Table 1. Descriptors and scoring criteria for phenotypic investigation of 126 *Capsicum annuum* recombinant inbred lines (RILs)

Descriptors	Scoring criteria
Phytophthora capsici resistance at high-level infection (PcH)	1. Strongly resistance; 2. Highly resistant; 3. Intermediate; 4. Highly susceptible; 5. Strongly susceptible
Phytophthora capsici resistance at mediate-level infection (PcM)	1. Strongly resistance; 2. Highly resistant; 3. Intermediate; 4. Highly susceptible; 5. Strongly susceptible
Phytophthora capsici resistance at low-level infection (PcL)	1. Strongly resistance; 2. Highly resistant; 3. Intermediate; 4. Highly susceptible; 5. Strongly susceptible
Fruit Cross-sectional Corrugation (FCC)	1. No to light; 2. Light; 3. Middle; 4. Lightly strong; 5. Strong
Corolla Color (CC)	1. White; 2. Purple
Stem Diameter (SD)	Measured in the middle part to first bifurcation
Leaf Length (LL)	Measured on the longest part of the developed leaf
Leaf Width (LW)	Measured on the widest part of the developed leaf
Fruit Length (FL)	Average fruit length of 10 ripe fruits
Fruit Width (FW)	Measured at the widest point. Average fruit width of 10 ripe fruits.
Fruit Wall Thickness (FWT)	Average of 10 ripe fruits, measured at point of maximum width to one decimal point
Mean Fruit Weight (MFW)	Average fresh fruit weight of 10 ripe fruits

agronomic and morphological traits. Two loci *fs3.1* and *fs10.1* were identified to control the fruit shape as major QTLs, interpreting most of the variation in pepper (Ben Chaim et al., 2003a; 2003b; Borovsky and Paran, 2011). A total of 58 QTLs were detected based on 248 BC_2 plant and 92 restriction fragment length polymorphism (RFLP) markers, most of which distributed in 11 clusters (Rao et al., 2003). Using a set of 297 recombinant inbred lines (RILs), Barchi et al. (2009) found 76 QTLs grouped into 28 chromosome regions for 13 fruit and plant traits. Comparative QTLs on resistance against *Phytophthora capsici*, the causal agent of stem, collar and root rot, provided evidence for conservation of resistance loci across in *Solanaceae* (Thabuis et al., 2003).

In QTL analysis, SNP marker has increasingly become an important tool, as single nucleotide changes are the most abundant and stable small-scale genetic variation in a specific population (Chutimanitsakun et al., 2011). Previously, we reported two transcriptomes from two red pepper (*C. annuum* L.) accessions, YCM334 and Taean, using 454 GS-FLX pyrosequencing, and discovered transcriptome-wide sequence variations including SNPs, SSRs and InDels by comparison of both transcriptomes (Lu et al., 2011). In this study, 41 SNPs newly developed and verified from pepper transcriptomes were used to integrate into a previous linkage map containing 420 markers developed by Truong et al. (2010) using F_8 RILs from a cross of YCM334 and Taean. Besides, 12 plant agronomic and morphological traits, including resistance evaluation against *P. capsici* at three different infection levels, were used for QTL mapping.

MATERIALS AND METHODS

Plant materials and DNA extraction

A *C. annuum* F_6 line, 'YCM334', resistant to *P. capsici*, were derived from a cross between CM334 and Yolo Wonder at the World Vegetable Center (AVRDC), while a local variety, 'Taean', that is susceptible to *P. capsici* was developed in 2000 at National Institute of Horticultural and Herbal Science (NIHHS), Rural Development Administration (RDA), Republic of Korea. A YCM334 × Taean F_8 population of 200 RILs was advanced in 2008, of which a collection of 126 RILs was selected as the mapping population. Young leaves were collected from greenhouse-grown plants and stored at -80°C. Genomic DNA was extracted using RNeasy Plant Mini Kit (Qiagen Korea, Korea) following the manufacturer's instruction, and then diluted into 20 ng/μL using a BioSpec-nano spectrophotometer (Shimadzu, Japan).

Phenotype investigation

The 126-RIL seeds were sown in the 50-cell polystyrene trays with an organic plant substrate on Mar 9th in 2010. The seedlings were transplanted into the NIHHS experiment field (Korea) with 5 plants for each line on May 17th until they had two pairs of definitive leaves, and then cultured under field conditions. Spacing was 50 cm between rows and 50 cm between plants. These lines were characterized and evaluated according to the 12 morphological and agronomic descriptors (Table 1) detailed in the Descriptors for *Capsicum* by Korean Seed and Variety Service (KSVS; http://www.seed.go.kr). For evaluation of resistance against *P. capsici*, an isolate named 07-127 (Truong et al., 2012), provided by Horticultural and Herbal Crop Environment Division (HHVED) was used. Culture and inoculation of the *P. capsici* were performed following the method described by Andrés Ares et al. (2005) with three different concentrations of 10^6, 10^5, and 10^4 zoospores/ml for high, intermediate, and low-level resistance tests, respectively. Disease severity was recorded based on the scores described by Kim and Hwang (1992).

SNP verification

A total of 58 contigs from two sets of transcriptome (Appendix A) were chosen for SNP verification using classical PCR-cloning-sequencing strategy. From each contig, only one SNP locus was selected for primer design and SNP verification. Forward and reverse primers spanning the putative SNP were designed using Primer3 (Rozen and Skaletsky, 1999). Primer redesign was necessarily performed with those amplicons proved to contain more than one SNP by sequencing results. All PCR primers were synthesized at Bioneer Corporation (Korea).

Table 2. Gaussian test and Pearson correlation coefficients among 12 traits

	PcH	PcM	PcL	SD	LL	LW	FL	FW	FWT	FCC	MFW	CC
Min	1	1	1	0.53	5.17	2	3.5	1.43	1.53	1	4.31	0
Max	5	5	5	1.7	18.83	8	14.43	4.53	4.8	5	40.92	2
Skewness	-	-	-	-0.526	0.594	0.605	0.292	0.654	0.339	0.195	0.247	-
Kurtosis	-	-	-	0.653	-0.165	-0.254	0.516	1.597	-0.04	-0.069	-0.484	-
PcH												
PcM	0.821**											
PcL	0.730**	0.875**										
SD	0.179	0.162	0.182									
LL	0.149	0.166	0.13	0.545**								
LW	0.12	0.15	0.08	0.506**	0.886**							
FL	-0.024	0.013	0.059	0.006	0.064	0.053						
FW	-0.042	-0.038	-0.03	0.244*	0.245*	0.2	0.198					
FWT	0.055	0.009	-0.021	0.219*	0.066	0.009	0.289**	0.590**				
FCC	-0.177	-0.194	-0.227*	-0.043	0.054	0.075	0.121	0.343**	0.075			
MFW	0.009	0.002	0.045	0.200	0.196	0.166	0.700**	0.749**	0.703**	0.243*		
CC	0.152	0.125	0.032	0.194	-0.078	-0.100	-0.027	-0.141	0.098	-0.083	-0.056	

* Significant at $p < 0.05$; **Significant at $p < 0.01$

Each amplicon was purified using MG™ PCR Product Purification SV (Macrogen, Korea) and then cloned into a pTOP TA vector (Macrogen) according to the manufacturer's instructions. Finally, recombinant plasmids from five clones for each PCR product were sent for sequencing (Macrogen).

SNP genotyping

A total of 94 RILs and two parents, YCM334 and Taean, were used for genotyping by high resolution melting (HRM). The HRM assay was conducted on a CFX96 Real-Time PCR Detection System (Bio-Rad, Korea) using Ssofast™ EvaGreen® Supermix (Bio-Rad) according to the manufacturer's protocol. The PCR reaction procedure and melting analysis were performed as follows: a 2-min initial denaturation followed by 40 cycles of denaturation at 98°C for 5 s, annealing at 60°C for 10 s, and a plate read. The amplification cycles were immediately followed by the following HRM steps: 98°C for 1 min, cooling to 40°C for 1 min, and a temperature increase to 60°C for 1 min. The temperature was subsequently increased to 95°C at intervals of 0.2°C for 10 s followed by a plate read. SNP genotyping was completed with Bio-Rad Precision Melt Analysis v1.0.534.0511 software (Bio-Rad).

Linkage mapping and physical mapping

Information of the target genetic map and genotypes was obtained as described previously (Truong et al., 2010). SNP markers were integrated into the previous linkage map using Mapmaker/EXP v3.0 program (Lander et al., 1987). The commands "near" and "assign" were used to attach the marker(s) to the corresponding linkage group (LG), while the "try" command was employed for localizing the precise position. Genetic distance between markers was calculated from recombination fractions using the Kosambi mapping function (Kosambi, 1943), and verified by the "map" and "ripple" commands. Linkage map was drawn using MapChart v2.1 (Voorrips, 2002). To support the linkage mapping information, homologous searches of 41 contigs were performed against the tomato genome (*Solanum lycopersicum*) database (version 2.4; http://solgenomics.net/organism/Solanum_lycopersicum/genome) and potato (*S. tuberosum* L.) pseudomolecules (The Potato Genome Sequencing Consortium, 2011) using BLAST v2.2.25 with an expected arbitrary value of 1e-5 (Altschul et al., 1990).

QTL mapping

Composite interval mapping (CIM) was performed to identify additive QTLs and to increase the resolution of QTL locations using WinQTL cartographer v2.5.10 (Wang et al., 2011) with a window size of 10 cM and walk speed at 0.5 cM. Significance thresholds for CIM were determined using 1,000 permutations for each trait (Doerge and Churchill, 1996; Searle et al., 1992). The proportion of observed phenotypic variation attributable to a particular QTL was estimated by the coefficient of determination (Rsq). The total phenotypic variance was estimated by fitting a model including all putative QTLs for the respective traits. Epistatic interactions between detected QTLs were accessed using QTLnetwork v2.0 (Yang et al., 2008). The running parameters were set up as follows: testing window at 10 cM, walk speed at 0.5 cM, filtration window at 10 cM and QTL effect at 0.05. Significance testing was conducted based on the F-tests using Henderson method III, and 10,000 permutations were used to calculate the critical F-value to control the genome-wise type I error (Doerge and Churchill, 1996; Searle et al., 1992)

RESULTS

Phenotype data evaluation

Among the 12 traits investigated, corolla color is suitable for Mapmaker analysis as a marker, since only two scores for the white and purple color were assigned. Its data distribution among 126 RILs followed 1.12:1, mostly abiding by the Mendel's Law of Segregation. The three resistance data at high-,

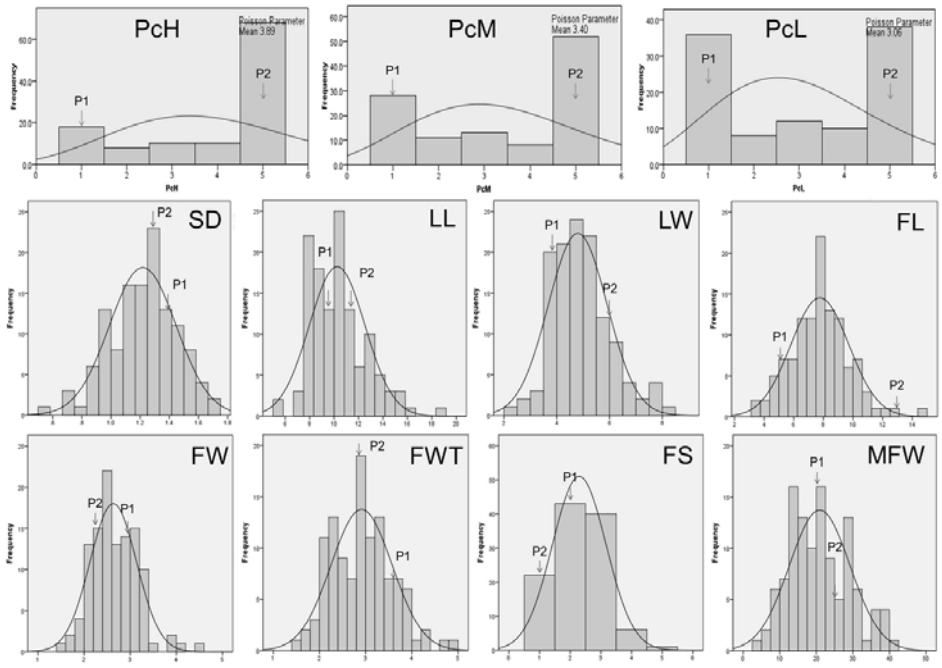

Fig. 1. Data distributions of 11 traits suitable for QTL analysis. Trait names refer to Table 1; P1, YCM334; P2, Taean.

intermediate- and low-level infections were highly correlated with each other (Table 2), which then were proved to confirm Poisson distribution. Furthermore, the other traits' phenotype data confirmed the Gaussian distribution (Fig. 1). It suggested that all traits were suitable to perform QTL analysis. Leaf length (LL) was significantly correlated with leaf width (LW), while mean fruit weight (MFW) was highly correlated with fruit length (FL), fruit width (FW), and fruit wall thickness (FWT). Other significant correlations were observed between stem diameter (SD) and LL/LW, whereas no correlation existed for corolla color (CC).

SNP verification and genotyping

To further verify those SNP loci detected by comparative transcriptomes of red pepper YCM334 and Taean accessions (Lu et al., 2011), a sum of 58 contigs with different SNP number and variation frequencies was selected (Appendix B). The number of SNPs in each of the contigs selected varied from one to 15, whereas the variation frequency changed from 83% to 100%, with an average of 99.39%. Total depth of those SNP loci varied from three to 186, with an average of 13. Among the 58 amplicon alignments, 51 were found to contain 75 SNP loci, of which 43 alignments were responsible for single SNP loci, leaving another eight for multiple SNP loci (more than one SNP locus). Those SNP variation types, including C/T and G/A nucleotide transitions, accounted for two-thirds of 75 SNP loci discovered from 51 contigs (Fig. 2). In the 51 contigs, 49 SNP loci were proved to be consistent with the SNP calling results from 454 pyrosequencing. The other 26 SNP loci were additionally discovered in the exon and intron parts. A total of nine contigs were confirmed to contain false-positive SNPs, of which seven contigs contained no SNP variations. Furthermore, one polymorphic SSR locus with an AAG motif was found to be polymorphic in contig00487. Finally, based on the HRM requirements and performance assay, 29 primers were proved to have a good performance, while another 12 primers were necessarily redesigned with the amplicons' size less than 250 base pairs (bp). Therefore, these 41 SNPs were finally selected for marker integration (Table 3).

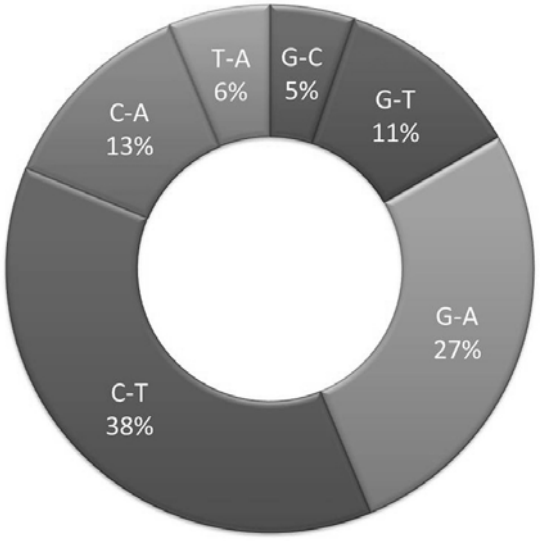

Fig. 2. Variation pattern types of all 75 SNPs discovered from 51 contigs.

SNP markers mapping

From the previous 420 markers mapped into 19 LGs, 419 were obtained based on the available genotyping data (Truong et al., 2010). Among those 41 SNP markers developed, 39 were finally mapped into 13 main LGs plus the unassigned LGe, with a relatively abundant distribution in LG1, 3, 4 and 9 (Fig. 3). Only two markers (CA00518 and CA00519) were found to have

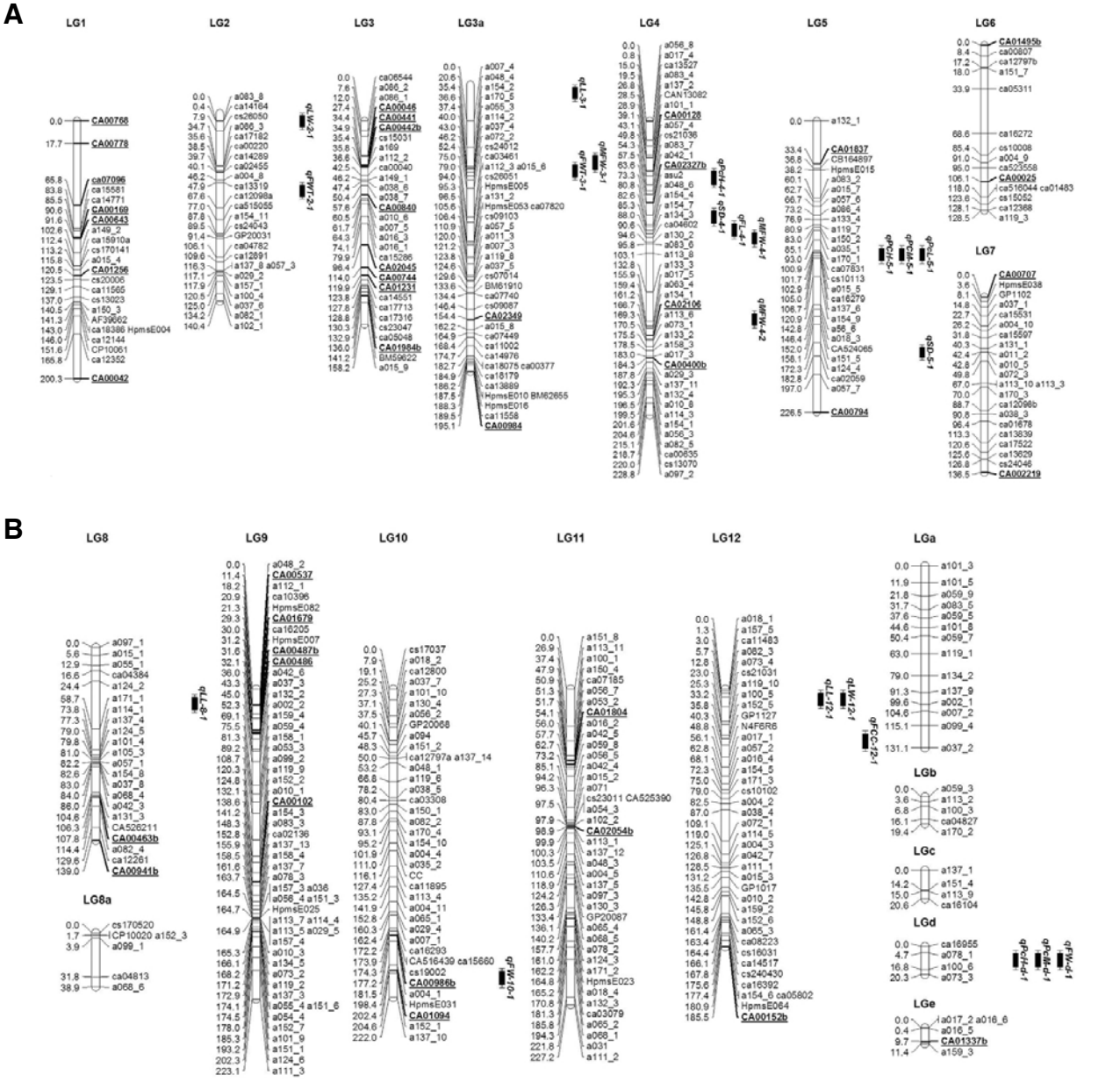

Fig. 3. Integrated linkage map of 458 markers by adding new 41 SNP markers. (A) Linkage groups for LG1-7; (B) Linkage groups for LG8-e. The underlined texts indicated the newly integrated markers.

no close linkage with any located markers. In addition, the CC trait should be treated as a marker, not a quantitative trait, since only two scores were allocated. The CC trait was fine mapped to the interval between a035_2 and ca1895 on LG10, with a genetic distance of 5.1cM and 11.3cM from the two neighbor markers, respectively. In all, those 458 markers were mapped into 19 LGs in present genetic map, with few rearrangements of marker position and orders. The genetic distance of the 14 main linkage groups ranged from 125.2 cM (LG6) and 226.6 cM (LG4) with a total of 2,599.1 cM, while the averaged genetic distance varied from 4.1 (LG9) to 9.3 cM (LG5) with an average of 5.7 cM for the whole map.

Physical mapping

To support the linkage mapping information, BLAST was executed to explore the homologous locations from two *Solanum* species, tomato and potato genome databases. All the 41 contigs were aligned with at least one hit except CA02054b showed not a match in potato genome but a target on chromosome 11 (STChr11) in tomato genome database (Table 3). Moreover, the top target chromosome ID from potato for each contig was consistent with those from tomato, indicating highly conserved regions exist between two species. Except the CA01337b in LGe and two non-mapped loci CA00518/CA00519, the target LGs of 25 loci were in accordance with the BLAST results. In the remaining 13 loci, seven mapped onto pepper

Table 3. Primer sequences and physical mapping of tomato and potato genome database

Primer ID	Forward primer	Reverse primer	V(T/Y)	Tm	Am	P_ch	T_ch	LG
CA00025	catgatacgaagaacttctccaac	atcaagagttgtgtcgtactccaa	A/G	53	169	6	6	6
CA00042	taacaatgggtattttcagggc	gagtgtcgaacatgcagatttagt	A/G	54	214	1	1	1
CA00046	agcgatggatcgtagtaagaattg	gagaaacaagtacgtcactcctcc	T/G	53	167	9	9	3
CA00102	aggccaacttatgagatcactttc	aattagtacactcgaaccccagag	T/C	54	139	9	9	9
CA00128	gtaaaaagttgctatccttccgtc	gatgttggttggtactgtatacgc	A/C	54	162	4	4	4
CA00169	gcgtaacagatgaggttgctaata	ccgattaatcttgatttgagcc	G/A	53	117	1	1	1
CA00441	ctgtcagtttctattcccctcagt	ggcaaatacgagaagaaggtgtat	G/A	54	190	9	9	3
CA00486	catactccggaggacgatataact	attcttcaacaactcccacaactc	A/G	54	257	9	9	9
CA00518	ggcgttgagaaacttatacaagga	aggaagagcaacaaggtaagagaa	G/A	54	214	6	6	-
CA00519	gaatcacactgtaagtccttgtgg	cagacaaatatgagaatctcgtgg	A/G	54	182	11	11	-
CA00537	aattgctaagctcttgaagctcac	actttgttcctatctaaacgcagg	G/A	54	195	9	9	9
CA00643	gtttgtaagttggaactttagggg	gtctacaagccgaaaccagaac	T/C	53	91	1	1	1
CA00707	aaccacccttacgatcatttg	cccggttttgatagtcgtataaag	A/G	54	197	7	7	7
CA00744	caagatcccctaaaccagaaagta	tgaatcagtaggggaactaaaagg	T/G	54	203	9	9	3
CA00768	gaggggtagataacagtagcaag	tatgtctgaatctgaactcgagga	T/C	54	147	1	1	1
CA00778	gatctcctaaagaatatggatgcg	tatgggttagtatggtgctttcct	A/G	54	230	1	1	1
CA00794	gagtatgtaaggagctaaagggga	gaacacttcctcaacattctcctc	A/G	53	206	5	5	5
CA00840	agaaaggaactgatacaacttggc	cttggcctcaatacatatcctctt	A/G	54	191	9	9	3
CA00984	atctcctgagtaccctgttgagtc	ttactctcaccaagtatgttccca	C/A	54	246	3	3	3
CA01094	aaattctcatccagtagaagctgg	ggaacctatattgtcctttgcatc	C/T	54	154	10	10	10
CA01231	tcgggtctaggagaagtaaagaag	acaaacaataagtcgccttacctc	T/C	54	187	9	9	3
CA01256	gtcataaaggcctcattctgagtt	gttgagttgagttacaacaatgcc	T/G	54	221	1	1	1
CA01679	catcagatgtgcccactaaactac	gtaccaaaagtgacacaatgagga	G/A	54	133	9	9	9
CA01804	ctctctaagaatctttctcgctgg	actgcagacctgccttaatagaac	C/T	54	127	11	11	11
CA01837	tgttgaaatacctaagagtcgaagc	ataacgcgatctctaacttcatcc	C/T	54	151	4	4	5
CA02045	tactacaacaacaatggcaactcc	gctagaaccagaatcaacgatgta	T/C	54	178	3	3	3
CA02106	acaaagaaagaagagttagcggtg	ccgccattagtagtatccctaaga	T/G	53	151	6	6	4
CA02219	catagcaggaaatggtttgtctac	gcataggcttttccattacctaac	G/A	53	237	7	7	7
CA02349	tatcgacaattgagagacttggtc	taatcttcagggaccagatacaca	A/G	54	157	3	3	3
CA00152b	actccctatgtgtctcaaccctac	gtctttaatttcactgcgtccac	T/C	54	112	12	12	12
CA00400b	aggagtggagaaagataccaagaa	taatcctccaattcctcaatgg	T/G	54	117	6	6	4
CA00442b	acgagaagaaggtgtatgtcttgc	ccttggtaagtttggtgagcttag	T/G	55	99	9	9	3
CA00463b	catttaatccagcacctttgtcag	atagtccgtttgcatctcctaaag	G/T	54	96	1	1	8
CA00487b	gatttttcacctctcgaatat	atcttcttcttcatcaattac	A/C	50	104	9	9	9
CA00941b	agaatgggtttctcggattggtgaa	gaacatcagtaacagcaaaggagagac	T/C	56	104	1	1	8
CA00986b	aacacatgatttcaagaaagcaccctg	ttggtacagcaaccatggcaact	A/G	58	62	10	10	10
CA01337b	acatgataagcgcaatcttcag	ggaaccaccattagataccgataa	G/A	53	97	8	8	e
CA01495b	ggtgacattcacaagaagaccatat	tgaaatcaatagcagcagcagtcct	G/C	55	122	8	8	6
CA01984b	ttcttggtcgtgtgagggctt	aaatcagttatgatcaacaag	C/A	53	106	9	9	3
CA02054b	gttctggagcctttattttaccac	ctgcaagaactgttccttaccat	G/C	54	98	-	11	11
CA02327b	gcagaatacctaaagaacaacctct	gagatcttctctggttcatacctatagct	G/A	53	100	4	4	4

V(T/Y), SNP variation type of Taean/YCM334; Am, Amplicon size; P_ch, Chromosome number in potato (*Solanum tuberosum* L.); T_ch, Chromosome number in tomato (*S. lycopersicum*); LG, linkage group of pepper (*Capsicum annuum*) in present study. Those 12 markers with a suffix "b" were redesigned based on the HRM requirements and performance.

LG3 but matched both SLChr09 and STChr9, whereas another locus CA02045 on pepper LG3 hit the Chr3 of tomato and potato. In addition, two loci CA00463b and CA00941b on pepper LG8 found their homologous region in Chr1 of tomato and potato, while another two loci CA00400b and CA02106 on pepper LG4 were homologous with some regions from Chr6 of tomato and potato.

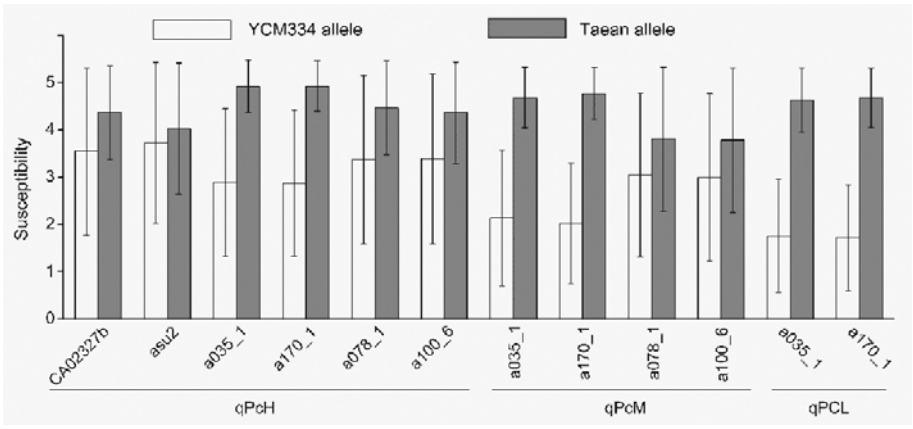

Fig. 4. Allelic effect of those markers covering resistance QTLs against *Phytophthora capsici*. The resistance scaling standard refers to Table 1.

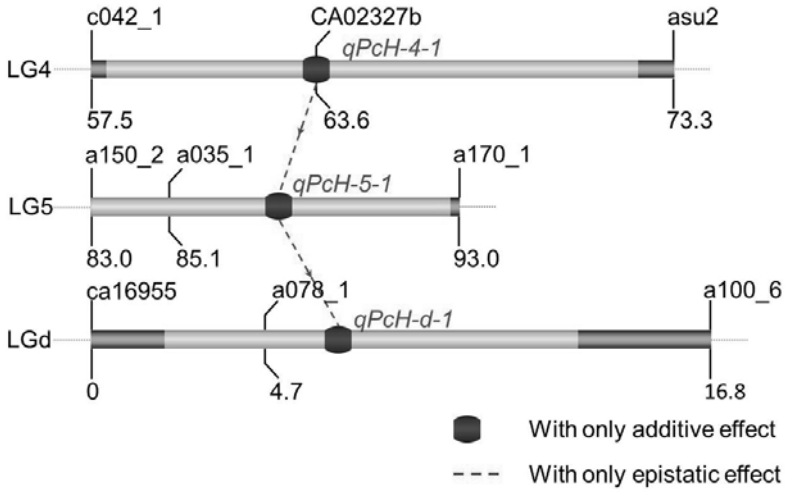

Fig. 5. Interaction QTLs detected between the QTLs for high-level resistance assay. Epistasis between *qPcH-4-1* and *qPcH-5-1*: heritability = 0.0489; epistasis between *qPcH-5-1* and *qPcH-d-1*: heritability = 0.0759.

QTL identification

A total of 23 QTLs were detected for the 11 traits' data (Table 4). In terms of resistance-related six QTLs, all seemed to be involved in three marker intervals, and QTL number for each trait decreased with the lowering infection level. The negative additive values indicated the susceptibility were inherited from Taean whereas the resistance from YCM334 (Fig. 4). In addition, a consistent main-effect QTL locating between a035_1 - a170_1 on pepper LG5 was found concerning the plant resistance feature against *P. capsici* at all three infection levels, interpreting 70.38, 70.38 and 66.08% of the phenotypic variations at high, intermediate and low resistance test levels, respectively. And one of the newly integrated markers, CA02327b, with 7.16% phenotype contribution was involved in the *qPcH-4-1* localization, and this QTL appeared to share the same map position with CA02327b in the LG4. Moreover, two sets of loci showed interactions between both *qPcH-4-1* and *qPcH-5-1*, and *qPcH-5-1* and *qPcH-d-1*, which were referred to epistatic QTLs (Fig. 5). Considering the contribution from additive effect and epistatic effect, the three QTLs totally explained 98.25% of high-level resistance-related variation. For intermediate-level resistance test, two QTLs were uncovered on LG5 and LGd with a total of 75.99% phenotype contribution.

With respect to the other eight morphological traits, CIM analysis detected 17 QTLs, and no epistatic QTLs were identified. Two QTLs, *qSD-4-1* and *qSD-5-1* were found in LG4 and LG5, respectively, and interpreting 19.86% of the heritability of additive effect in all. Six QTLs were revealed for LL and LW, interpreting 28.36 and 34.70% of the heritability, respectively. And one newly developed marker, CA00025, was closest to the locus *qLW-6-1*, and the *qLL-12-1* and *qLW-12-1* loci shared the same marker interval in LG12. Three QTLs for FL and FW on LG4, LG10 and LGd explained 10.98 and 21.44% of the heritability, respectively. And one newly integrated marker CA00986b localized the locus *qFW-10-1*. The additive effect indicated the responsible alleles for FL and FW were separately from Taean and YCM334. Two and one QTLs for FWT and FCC were separately found on LG2 and LG3a, and LG12, interpreting 19.14 and 9.08%, respectively, and the alleles from YCM334 contributed to the increased trait values. Three MFW QTLs on LG3a and LG4 explained 30.20% of phenotypic variations, and the *qMFW-4-1* effective allele was from Taean, whereas the other two from YCM334.

DISCUSSION

Genetic map is of great importance for its role in QTL mapping, gene localization and discovery (Kloosterman et al., 2010; Li et

Table 4. QTLs detected for 11 traits in 126 recombinant inbred lines using composite interval mapping algorithm

QTLs	LG	Interval	Range (cM)	Position (cM)	LOD	A	R2
qPcH-4-1	4	CA02327b-asu2	63.6-73.3	63.6	4.6318	-0.4669	0.0716
qPcH-5-1	5	a035_1-a170_1	85.1-93.0	88.1	31.4572	-1.4787	0.7038
qPcH-d-1	d	a078_1-a100_6	4.7-16.8	6.7	4.8461	-0.4744	0.0833
qPcM-5-1	5	a035_1-a170_1	85.1-93.0	88.1	31.4572	-1.4787	0.7038
qPcM-d-1	d	a078_1-a100_6	4.7-16.8	8.7	4.3947	-0.4038	0.0561
qPcL-5-1	5	a035_1-a170_1	85.1-93.0	86.6	31.9698	-1.4877	0.6608
qSD-4-1	4	a154_7-a134_3	85.3-88.0	86.8	3.5348	0.0731	0.0955
qSD-5-1	5	CA524065-a151_5	152.0-158.1	157.0	3.6104	-0.0809	0.1031
qLL-3-1	3a	a154_2-a170_5	35.4-36.6	35.4	4.1274	-0.7442	0.1014
qLL-8-1	8	a171_1-a114_1	58.7-73.8	71.2	3.4618	0.7309	0.1001
qLL-12-1	12	a100_5-a152_5	33.2-35.8	33.2	3.3898	0.6750	0.0821
qLW-2-1	2	cs26050-a086_3	7.9-34.7	8.9	4.3136	-0.3740	0.1064
qLW-6-1	6	ca523558-CA00025	95.0-106.1	102.5	4.0416	0.4208	0.1221
qLW-12-1	12	a100_5-a152_5	33.2-35.8	33.2	5.1596	0.3966	0.1185
qFL-4-1	4	ca04602-a130_2	90.6-94.6	90.6	4.1049	-0.6541	0.1098
qFW-10-1	10	cs19002-CA00986b	174.3-177.2	174.3	3.2406	0.1493	0.0852
qFW-d-1	d	a078_1-a100_6	4.7-16.8	7.2	4.1439	0.1847	0.1282
qFWT-2-1	2	ca13319-ca12098a	47.9-67.6	48.4	2.6845	0.1996	0.0870
qFWT-3-1	3a	a015_6-cs26051	79.0-94.0	85.5	2.8688	0.2164	0.1044
qFCC-12-1	12	a017_1-a057_2	56.1-62.8	57.1	2.8910	0.2674	0.0908
qMFW-3-1	3a	ca03461-a112_3	75.0-79.0	75.5	2.8755	2.4635	0.0834
qMFW-4-1	4	a130_2-a083_6	94.6-95.8	94.6	4.5180	-3.1103	0.1312
qMFW-4-2	4	a113_6-a073_1	169.3-170.5	169.3	3.0995	2.5019	0.0874

LG, Linkage group; A, Additive effect, positive values indicated that YCM334 carries the allele for an increase in the trait, while negative values indicate that Taean contributed the allele for an increase in the pathogen susceptibility; R2, Heritability of the additive QTLs.

al., 2011). This study represents an attempt to develop and verify a new set of 41 SNP markers, and integrate these markers into a previous genetic map, and an attempt to map the loci controlling 12 traits using a F_8 RIL population from a cross by pepper (*C. capsicum* L.) accessions YCM334 × Taean, which were proved to be efficiently resistant and susceptible pepper accessions, respectively. RILs have been demonstrated to show various advantages than other populations for fine genetic mapping and QTL analysis due to the almost fixed genotypes (Kumar et al., 2009; Meyer et al., 2010; Ordas et al., 2010). It could serve as a permanent mapping population with permissibility for replicated tests in multiple environments or evaluation of effect from different pathogen strains. Much more precise QTL location will be expected, and lots of genes were successfully localized with fine mapping using a RIL population (Wang et al., 2003).

As a kind of co-dominant markers stably distributed in the pepper genome with high frequency, SNP performs perfectly in the map-based research, for instance, linkage mapping, association mapping and the consecutive positional cloning (Chen et al., 2011; Setter et al., 2011; Shi et al., 2011). To further confirm the SNPs identified from transcriptome comparisons (Lu et al., 2011) and to provide more precise marker information to future research, one must realize that these SNPs have to be evaluated due to sequencing errors and SNP calling algorithms. In the present study, up to 87.93% (51) in those 58 contigs were verified to contain SNPs, and 84.48% (49) from 58 PCR amplicons were proved to include the expected SNPs using a classical PCR-cloning-sequencing strategy. The validation rate is similar with the report of 85% by Barbazuk et al. (2007), suggesting the necessities to validate the SNPs discovered from NGS data.

Among the newly developed SNP markers, most of them were mapped into the linkage map generated using the same pepper population as described by Truong et al. (2010). It further filled in more markers into the previous map, with slight genetic distance expansion. To the most important point, these SNP markers will provide more information than AFLP when localizing some gene(s) related to critical agronomic or morphological traits based on the transcriptome information provided (Lu et al., 2011). Moreover, several new SNP markers were quite close to some loci, for instance, CA02327b to *qPcH-10-1*, CA02106 to *qMFW-4-2*, and CA00986b to *qFW-10-1*, providing opportunities to localize the potential corresponding gene(s).

Many studies have been dedicated to characterize the major or minor QTLs, and identify the genes involved in the resistance against *P. capsici*, one of the most serious pathogens to pepper breeding (Bonnet et al., 2007; Minamiyama et al., 2007; Thabuis et al., 2004). This study presented three main-effect loci related to *P. capsici* resistance, and the locus on LG5 could independently explained over 60% of the heritability of additive effect at least, which was supposed to be a major-effect gene involved in the pathogen defense response. The QTL number difference at three infection tests may indicate more genes

would be mobilized with the increasing severity of pathogen invasion. Several reports previously have identified some QTLs on chromosome 5 (Bonnet et al., 2007; Kim et al., 2008; Pflieger et al., 2001; Thabuis et al., 2003; 2004). Truong et al. (2012) ever reported 15 QTLs on P5, P10, and P11, and we provided more detailed locus information on LG5, facilitating the main-effect gene discovery.

The QTLs controlling morphological traits were most likely concentrated on pepper chromosome P2, P3, P4, and P10 (Barchi et al., 2009; Ben Chaim et al., 2001; Rao et al., 2003; Zygier et al., 2005). Two main-effect QTLs (*fs3.1* and *fs10.1*) were detected on chromosome P3 and P10, respectively (Ben Chaim et al., 2003a; 2003b). In present study, the fruit-related QTLs, accounting for around 10% contribution to phenotype variance for each, were mainly centered on the LG3 and LG4, which showed consistency with previous studies. The QTLs detected in this study will help discover the resistance mechanism of plant defense against *P. capsici* in *Solanaceae* family for geneticists and breeders.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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