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Volume 61, Issue 1
Pages 1–214

Previous Issue | Next Issue

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In this issue
Advanced > Saved Searches >

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Review Jump to...

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T. F. Döring, M. Pautasso, M. R. Finckh and M. S. Wolfe
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Identification of isolate-specific resistance QTLs to phytophthora root rot using an intraspecific recombinant inbred line population of pepper (*Capsicum annuum*)

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Quantitative trait loci (QTL) for resistance to phytophthora root rot caused by *Phytophthora capsici* were investigated using two Korean *P. capsici* isolates and 126 F_8 recombinant inbred lines derived from a cross of *Capsicum annuum* line YCM334 (resistant parent) and local cv. Tean (susceptible parent). The experimental design was a split plot with two replications. Highly significant effects of pathogen isolate, plant genotype, and genotype × isolate were detected. QTL mapping was performed using a genetic linkage map covering 1486·6 cM of the pepper genome, and consisted of 249 markers including 136 AFLPs (Amplified Fragment Length Polymorphisms), 112 SSRs (Simple Sequence Repeats) and one CAPS (Cleaved Amplified Polymorphic Sequence). Fifteen QTLs were detected on chromosomes 5 (P5), 10 (P10), 11 (P11), Pb and Pc using two data processing methods: percentage of wilted plants (PWP) and relative area under the disease progress curves (RAUDPC). The phenotypic variation explained by each QTL (R^2) ranged from 6·0% to 48·2%. Seven QTLs were common to resistance for the two isolates on chromosome 5 (P5); six were isolate-specific for isolate 09-051 on chromosomes 10 (P10) and Pc, and two for isolate 07-127 on chromosomes 11 (P11) and Pb. The QTLs in common with the major effect on the resistance for two isolates explained 20·0–48·2% of phenotypic variation. The isolate-specific QTLs explained 6·0–17·4% of phenotypic variation. The result confirms a gene-for-gene relationship between *C. annuum* and *P. capsici* for root rot resistance.

Keywords: common QTL, isolate, isolate-specificity, *Phytophthora capsici*, resistance genes

Introduction

Phytophthora root rot, caused by *Phytophthora capsici*, is a major disease that limits pepper production in the world. It is a soilborne pathogen that can survive on host residues in soil for months (Oelke *et al.*, 2003). Various methods to control phytophthora root rot have been reported (Flett *et al.*, 1991; Biles *et al.*, 1992; Polizzi *et al.*, 1994; Stieg *et al.*, 2006); however, most treatments increase production costs as well as environmental and health risks. The use of resistant cultivars is a simple and effective strategy. Several resistance sources to phytophthora root rot have been reported (Ortega *et al.*, 1991, 1992; Reifschneider *et al.*, 1992), but commercial cultivars with good stable resistance in different environments against diverse isolates of the pathogen across regions are still lacking (Jee *et al.*, 2000).

Broadly speaking, two types of resistance to phytophthora root rot are known. Specific resistance to phytophthora root rot is oligogenic, effective against particular pathogen isolates and follows a gene-for-gene relationship (Monroy-Barbosa & Bosland, 2008; Sy *et al.*, 2008). Quantitative resistance is polygenic (Lefebvre & Palloix, 1996; Thabuis *et al.*, 2003). Several quantitative trait loci (QTLs) related to phytophthora root rot resistance have been identified (Thabuis *et al.*, 2003, 2004; Ogundiwin *et al.*, 2005; Bonnet *et al.*, 2007). Therefore, knowing the number and location of the resistance genes in the genome of pepper, in addition to understanding the genetic interaction between the plant and the pathogen, is very important in order to breed resistant cultivars.

QTLs associated with phytophthora root rot resistance against different isolates of *P. capsici* have been identified (Thabuis *et al.*, 2003, 2004; Ogundiwin *et al.*, 2005; Quirin *et al.*, 2005). However, in previous studies, the use of F_2 or F_3 mapping populations has not been fully used

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for practical purposes by breeders or geneticists; and for QTL analysis, it does not allow extensive evaluation of environmental and isolate effects. The use of a recombinant inbred line (RIL) population has many advantages over other populations that are used for genetic mapping and QTL analysis. A RIL can serve as a permanent mapping resource that will permit replicated trials in either multiple environments or with different pathogen isolates. Using RILs is especially powerful for analysing quantitative traits because replicated trials can be analysed using identical genetic materials (Burr & Burr, 1991). Ogundiwin *et al.* (2005) used RIL populations for mapping QTL resistance to phytophthora root rot against different isolates from different countries; however, QTL isolate-specific resistance was not found. Breeding for the local isolates present in specific geographic regions will lead to a successful, durable resistance by pyramiding combinations of isolate-specific resistance genes into *Capsicum* cultivars for those regions. Incorporation of understanding of complex *Capsicum–Phytophthora* interactions and new breeding approaches must be employed for global and local success. The objective of the present study was to investigate isolate-specific QTLs controlling resistance to two Korean *P. capsici* isolates using RILs generated from the hybridization of cv. Yolo Wonder and line CM334 with *P. capsici*-susceptible local cv. Tean.

Materials and methods

Plant materials and genomic DNA extraction

The source of resistance is YCM334, a *C. annuum* line collected from AVRDC-The World Vegetable Center, Taiwan. It is an F_6 recombinant inbred line derived from a cross between cv. Yolo Wonder and CM334, which was developed at INRA, Montfavet, France. It was crossed with a local cultivar, Tean, which is highly susceptible to phytophthora root rot, in 2000 at the National Institute of Horticultural and Herbal Science (NIHHS), Rural Development Administration (RDA), Korea. A mapping population of 200 F_8 RILs obtained by the single seed descent method was advanced in 2008. For convenience of sample loading, a collection of 126 of the 200 RILs was selected as a mapping population. Genomic DNA of the 126 individuals and parents was extracted from young leaves of greenhouse-grown plants following the protocol described by Raz & Ecker (1997).

Evaluation of resistance to phytophthora root rot

Phytophthora *isolates and plant inoculation*

Two *P. capsici* isolates, 09-051 and 07-127, from Korea, provided by the Horticultural and Herbal Crop Environment Division, NIHHS, RDA, Korea, were used. Isolate 09-051 was obtained from sweet pepper in Chonnam province, located in the south of South Korea, and isolate 07-127 was isolated from hot pepper in Chungbuk province, located in the centre of South Korea. Based on preliminary tests, which only used resistant and susceptible parents as materials, isolate 09-051 was more aggressive than isolate 07-127 (data not shown). The inoculation test was performed as described by Kimble & Grogan (1960). Briefly, mycelial plugs of stored *P. capsici* isolates were cultured on sterile potato dextrose agar (PDA) medium and incubated at 27°C for 7 days. The core of mycelium grown on the PDA plate was sliced into 5 mm squares. These squares were placed in the centre of new PDA plates and incubated at 27°C for 8–9 days or until the plates were uniformly covered with mycelia. The mycelia were harvested and ground using a blender. One full mycelial PDA plate was prepared for 50 mL mycelial suspension. When plants were at the six/seven-leaf stage, 5 mL mycelial suspension were poured on the soil surface of each seedling, which were growing in 50-cell trays in a greenhouse. Disease reactions were assessed at 10, 20 and 30 days after inoculation. Plants were individually scored and sorted into proportions of healthy plants (0) and wilted plants (1).

Experimental design and data analysis

The evaluation was laid out as a split-plot design with two replications and 10 plants per replication. The split-plot design was followed with 'isolate' as main plot and 'plant material' as subplot. Each replication was conducted successively separated by 10 days in the same screenhouse. Two methods of data processing were used for analyses:

1 Percentage of wilted plants (PWP): PWP = $(N_W/N_T) \times 100$, where N_T is total number of plants and N_W is number of wilted plants.
2 Relative area under disease progress curves (RAUDPC) (Fry, 1978). Firstly PWP was used to calculate the area under the disease progress curves (AUDPC), which expresses the dynamics of disease development according to Shaner & Finney (1977). AUDPC was calculated following the formula: AUDPC = $\Sigma_{i = 1 \text{ to } n-1}[(Y_{i+1} + Y_i)/2] \times [X_{i+1} - X_i]$, where Y_i is percentage of wilted plants at the ith observation ($i = 1$ being the first observation at time zero), X_i is time at the ith observation, and n is the total number of observations. The RAUDPC was calculated for each isolate by dividing AUDPC values by the duration of the epidemic and multiplying the outcome by 100. RAUDPC is expressed as a percentage.

Transformation with $\log_{10}(x + 1)$ was performed for RAUDPC, while arcsine square root was used for PWP to improve the normality of the data. These transformed data were used for analysis of variance (ANOVA). ANOVA was performed with the PROC MIXED in SAS (SAS 8·2, SAS Institute). The entry mean comparison and entry comparison were performed under each replication or isolate when the entry × replication or entry × isolate or rep × isolate was significant. These analyses were carried out in order to determine the effect of genotype and isolate as well as the interaction between genotype and isolate on disease development. Significant differences were determined at $P < 0{\cdot}05$ by LSD. The function FREQ of

Microsoft Excel was used to analyse the frequency distribution of RILs and their parents for resistance to each *P. capsici* isolate, using percentage of wilted plants at final rating. Correlation among processed data was conducted with the CORR procedure of SAS.

Scoring of DNA polymorphism

An amplified fragment length polymorphism (AFLP) assay was performed as described by Vos *et al.* (1995). Selective amplification was performed using 17 *Eco*RI primers and nine *Mse*I primers, each with two to four additional selective nucleotides. The amplification products were analysed in parallel in a 5% denaturing polyacrylamide gel (19:1 acrylamide-bisacrylamide, 7·5 M urea) in 0·5× TBE buffer (25 mM Tris, 25 M boric acid, 0·5 mM EDTA, pH 8·0) using a S3S T-Rex™ Aluminum Backed Sequencer and visualized by silver staining. Silver staining and developing were performed according to Promega's DNA Silver Staining System.

Both 1667 Expressed Sequence Tag-Simple Sequence Repeat (EST-SSR) primer pairs and 197 consensus SSR primer pairs: 67 from the map Pepper-FAO3 (Mueller *et al.*, 2005) and 130 from the SNU3 map (Yi *et al.*, 2006) were tested for polymorphism between the parents YCM334 and Tean. Electrophoresis of the PCR products and fragment analysis were carried out using the same method as that for the AFLP analysis.

Polymorphic markers were visually scored. Band presence or absence associated with the YCM334 allele was coded as 1; band presence or absence associated with the Tean allele was coded as 2, and those with both parent bands were coded as 3 for heterozygote. Ambiguous bands were considered as missing data for map construction purposes. Each AFLP marker was assigned a name consisting of one letter such as 'a' and followed by a primer combination code and the number of polymorphic bands generated by its primer combination. For example: marker 'a015_4' located at 35·3 cM on chromosome 1 (P1) was an AFLP marker, '015' was the primer combination code, and '4' was the number of polymorphic bands generated by this primer combination. EST-SSR markers were named consisting of two letters such as 'ca' or 'cs' and serial numbers following the letters. For example: EST-SSR markers 'ca07096' and 'cs170141' were located at 0·0 and 32·8 cM, respectively, on chromosome 1 (P1), and '07096' and '170141' are serial numbers. SSR markers selected from the Sol Genomics Network (SGN) or the Pepper-FAO3 map (Mueller *et al.*, 2005) and the ESTs from the SNU3 map (Yi *et al.*, 2006) were named with their original names.

Map construction

Linkage analysis was performed with MAPMAKER/EXP 3·0 (Lander *et al.*, 1987). The 'triple error detection' feature was used to recognize the circumstance when an event was more probably the result of error than recombination. This feature avoids map expansion (Cervera *et al.*, 2001). Grouping was tested with different LOD (Logarithm of Odds) scores and recombination fractions to group anchor markers of each chromosome into one group using the 'group' command. This would determine which linkage group belongs on which chromosome. Linkage groups were established at a LOD score of 7·0 and a recombination fraction of 0·30. The best marker order of the linkage group having eight or fewer markers was identified using the 'compare' command, whereas the order of the groups with more than eight markers was identified using the 'order' and 'try' commands. The marker order of each linkage group was verified using the 'ripple' command. The 'delete' command would remove the markers causing unstable order in the map. The complete set of markers was then remapped using the 'try', 'compare' and 'ripple' commands. The Kosambi mapping function (Kosambi, 1944) was used to convert the recombination fractions into additive genetic distance (centiMorgans or cM). Linkage groups were drawn with the MAPCHART 2·2 program (Voorrips, 2002).

QTL analysis

Quantitative trait loci (QTLs) detection was performed using composite interval mapping analysis (Zeng, 1994) with QTL Cartographer (Basten *et al.*, 2005). A 1000-permutation test was performed with QTL Cartographer to estimate the appropriate significance threshold for analysis. A LOD threshold of 3·5 corresponding to a genome-wide significance level of 0·05 was chosen. MAPCHART was then used to draw QTLs on the linkage groups. QTLs were assigned a name consisting of two letters, such as '*Ph*', followed by three numbers indicating isolate code, number of linkage group, and number of QTLs obtained from linkage groups.

Results

Resistance to *P. capsici* isolates in the RIL population

There were no significant differences observed among replications, while highly significant effects of isolate, genotype, and genotype × isolate were detected with the two data processing methods (Table 1). Thus, differences in symptom development of the two isolates suggest variation in their virulence. The inconsistent genotype rank between isolates and the difference of magnitude indicates the significant effect of isolate × genotype. Such a significant effect of genotype × isolate could complicate the further mapping analysis.

The frequency distribution of RILs and their parents for resistance to both isolates showed continuous patterns, suggesting the resistance is controlled by a polygenic system (Fig. 1a,b). Severity appeared differently between the two isolates in the F_8 population: a greater

Table 1 Combined analyses of variance of the effects of *Phytophthora capsici* isolate (I: 09-051 and 07-127), *Capsicum annuum* genotype (G: 126 RILs and two parents), replication (R: two replications), G × I, and G × R on percentage of wilted plants (PWP) and relative area under disease progress curves (RAUDPC)

Source	d.f.	MS PWP	RAUDPC
Replication (R)	1	2·32^{ns}	0·15^{ns}
Isolate (I)	1	12·29*	2·26*
R × I	1	0·05^{ns}	0·003^{ns}
Genotype (G)	125	0·81**	0·23**
G × I	106	0·19**	0·06**
G × R	107	0·08^{ns}	0·02^{ns}

ns: not significant; MS: mean square; d.f.: degrees of freedom.
*, **Significant at $P < 0·05$ and 0·01, respectively.

number of F_8 families were closer to the susceptible parent when inoculated with isolate 07-127, whereas a large number of F_8 RILs were closer to the resistant parent when inoculated with isolate 09-051. Distribution of PWP was skewed toward the susceptible side (Fig. 1a), while distribution of RAUDPC followed an almost normal distribution pattern (Fig. 1b) when the population was inoculated with isolate 07-127. With isolate 09-051, distributions of PWP and RAUDPC of the F_8 RIL population were skewed toward resistance (Fig. 1a,b). Because of the different distributions observed with the two data processing methods, different QTLs could be detected. Thus, the two data processing methods can be used in QTL mapping, although common QTLs identified for the two methods could be more important. There were consistently high degrees of correlation between PWP and RAUDPC when inoculation was with isolates 09-051 and 07-125 (Table 2).

Table 2 Correlation between percentage of wilted plants (PWP) and relative area under disease progress curves (RAUDPC) following inoculation of *Capsicum annuum* with *Phytophthora capsici* isolates 09-051 and 07-127

	PWP-09-051	RAUDPC-09-051	PWP-07-127
RAUDPC-09-051	0·95**	–	–
PWP-07-127	0·60**	0·58**	–
RAUDPC-07-127	0·60**	0·60**	0·93**

**Significant at $P < 0·01$.

Genetic linkage map

A total of 249 markers (136 AFLPs, 112 SSRs and one CAPS) was used for QTL analysis. These markers were distributed over 18 linkage groups. The number of markers per linkage group ranged from two to 27, and the map covered 1486·6 cM of the pepper genome, with an average distance of 6·0 cM. Based on distribution of consensus SSR markers, 14 linkage groups were assigned into 12 chromosomes of pepper (Fig. 2a,b).

QTL detection

Composite interval mapping identified 15 QTLs associated with phytophthora root rot resistance against two Korean *P. capsici* isolates that explained 6·0–48·2% of phenotypic variation (Table 3, Fig. 2). Of these, eight QTLs for RAUDPC were detected on chromosomes 5, 10, 11 and Pc. Most of the QTLs inherited resistance alleles from the resistant parent, except for QTLs *Ph051-10·3* and *Ph127-11·1*. Seven QTLs on chromosomes 5, 10, Pb and Pc associated with PWP. Of these, QTLs *Ph051-5·1*, *Ph051-5·2*, *Ph051-10·1* and *Ph051-c·1* also associated with RAUDPC (Fig. 2b). This is in agreement with the high correlation between phenotypic values of

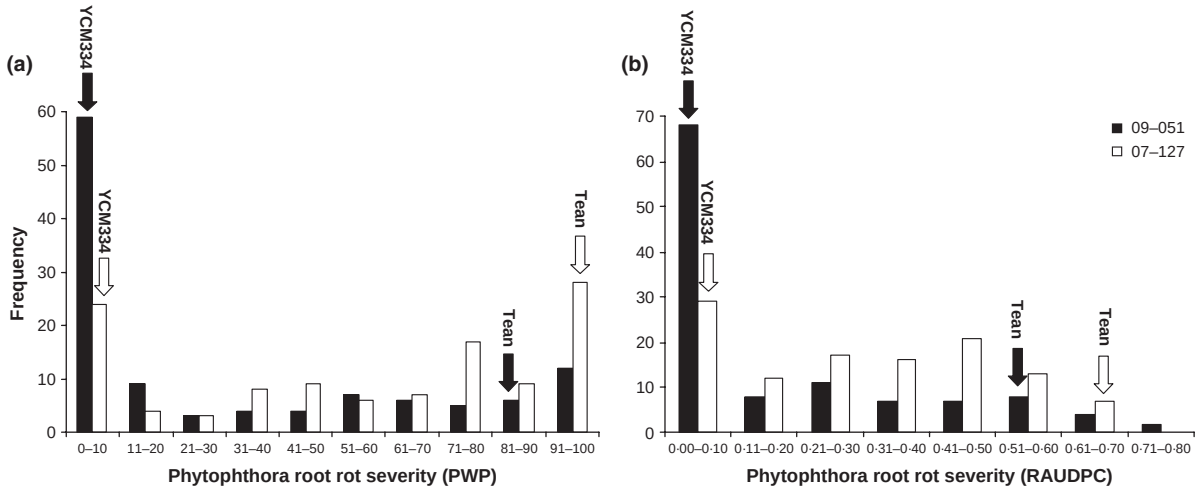

Figure 1 Frequency distribution of phytophthora root rot severity expressed as (a) percentage of wilted plants (PWP) and (b) relative area under the disease progress curves (RAUDPC) in *Capsicum annuum* F_8 population after inoculation with *Phytophthora capsici* isolates 09-051 and 07-127.

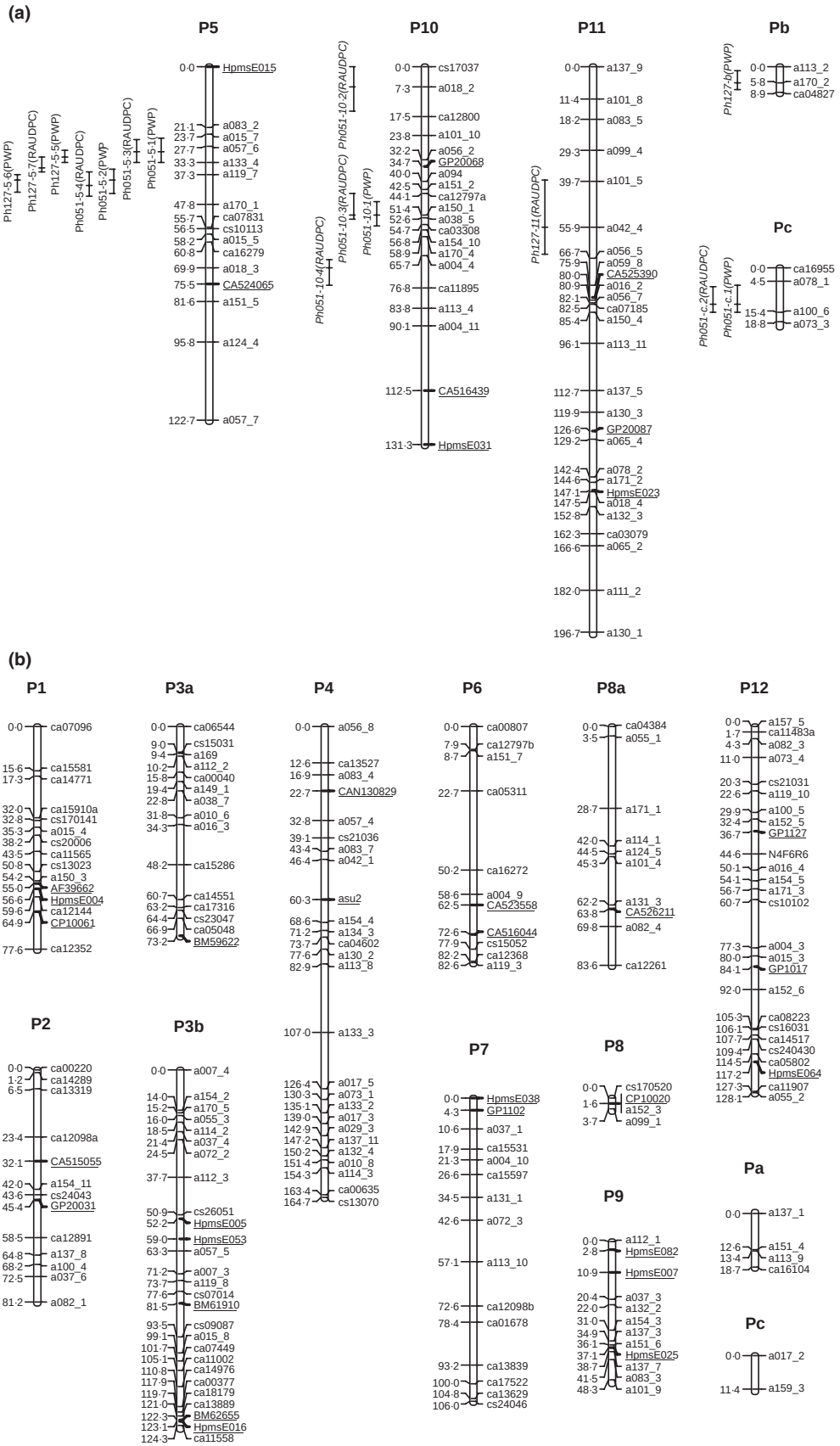
(a)
P5
P10
P11
Pb
Pc
(b)
P1
P2
P3a
P3b
P4
P6
P7
P8
P8a
P9
P12
Pa
Pc

Table 3 Quantitative trait loci (QTLs) detected in association with phytophthora root rot resistance from composite interval mapping using percentage of wilted plants (PWP) and relative area under the disease progress curves (RAUPDC) following inoculation of *Capsicum annuum* with *Phytophthora capsici* isolates 09-051 and 07-127

Trait	QTL	Chromosome	Location (CI)[a]	LOD[b]	R^2 (%)[c]	A[d]
PWP-09-051	*Ph051-5·1*	5	29·7 (24·7–33·3)	7·1	20·0	17·0
PWP-09-051	*Ph051-5·2*	5	39·3 (35·9–44·3)	13·4	34·5	22·5
RAUDPC-09-051	*Ph051-5·3*	5	29·7 (25·4–33·5)	8·3	21·8	0·1
RAUDPC-09-051	*Ph051-5·4*	5	41·3 (36·6–44·9)	15·4	37·2	0·1
PWP-07-127	*Ph127-5·5*	5	31·7 (29·2–33·3)	16·6	39·6	23·1
PWP-07-127	*Ph127-5·6*	5	39·3 (37·3–43·5)	21·6	46·8	25·2
RAUDPC-07-127	*Ph127-5·7*	5	35·3 (31·7–36·4)	20·6	48·2	0·2
PWP-09-051	*Ph051-10·1*	10	51·4 (47·0–55·5)	5·8	11·8	−18·8
RAUDPC-09-051	*Ph051-10·2*	10	7·3 (0·0–15·6)	4·1	7·1	0·1
RAUDPC-09-051	*Ph051-10·3*	10	51·4 (44·1–52·9)	3·5	7·8	−0·1
RAUDPC-09-051	*Ph051-10·4*	10	69·7 (67·1–76·1)	3·2	9·8	0·1
RAUDPC-07-127	*Ph127-11·1*	11	55·9 (39·4–65·0)	3·7	6·0	−0·1
PWP-07-127	*Ph127-b*	Pb	5·8 (1·4–7·8)	7·5	11·6	12·7
PWP-09-051	*Ph051-c·1*	Pc	12·5 (5·9–15·4)	7·1	17·4	15·5
RAUDPC-09-051	*Ph051-c·2*	Pc	12·5 (6·5–15·4)	4·6	10·5	0·1

[a]The most likely location of the QTL is indicated in cM from the top of the linkage group, followed by the confidence interval (CI) of this location.
[b]LOD: maximum value of the log-likelihood in the marker interval.
[c]R^2 (%): partial coefficient of determination, i.e. percentage of phenotypic variation explained by the QTL calculated by QTL Cartographer.
[d]A: Additive effect; minus sign indicates alleles contributing resistance were carried in susceptible parent Tean; for values without a minus sign, alleles contributing resistance were carried in resistant parent YCM334.

the two disease traits (Table 2). Except for the QTL *Ph051-10·1* on chromosome 10, all QTLs inherited resistance alleles from the resistant parent.

Clusters of QTLs were detected on chromosome 5 in the region between two AFLP markers, a015_7 and a170_1, associated with resistance against both isolates 09-051 and 07-127 (Fig. 2b) that explained 20·0–48·2% of phenotypic variation. The large range of phenotypic variation could be explained by the fact that different isolates had different disease pressure. Four QTLs for RAUDPC, *Ph051-10·2*, *Ph051-10·3* and *Ph051-10·4* on chromosome 10 (P10) and *Ph051-c·2* on Pc, and two QTLs for PWP, *Ph051-10·1* on chromosome 10 (P10) and *Ph051-c·1* on Pc, were detected for resistance against isolate 09-051 but not 07-127, explaining 7·8–17·4% of phenotypic variation. In contrast, one QTL for RAUDPC, *Ph127-11*, located on chromosome 11, and one QTL for PWP, *Ph127-b*, on Pb, were detected for resistance against isolate 07-127 but not 09-051, explaining 6·0% and 11·6% of phenotypic variation, respectively. In addition, QTLs detected for PWP, *Ph051-10·1* and *Ph051-c·1* on chromosome 10 and Pc, respectively, also associated with QTLs for RAUDPC, *Ph051-10·3* and *Ph051-c·2*, respectively (Fig. 2b). These results demonstrate the presence of isolate-specific resistance QTLs in the F_8 RIL population. Three QTLs, namely *Ph051-10·2* and *Ph051-10·4* on chromosome 10 (P10) and *Ph127-11* on chromosome 11, associated with RAUDPC but not PWP. These QTLs explained 7·1%, 9·8% and 6·0% of the total phenotypic variation, respectively. In contrast, QTL *Ph127-b* on Pb associated with PWP but not RAUDPC and explained 11·6% of the total phenotypic variation. This is in agreement with different distributions of the two processed data (Fig. 1).

Discussion

This study mainly compared the present map with two published maps: SNU3 (Yi *et al.*, 2006) and Pepper-FAO3 (Mueller *et al.*, 2005). The map developed in this study consisted of 249 markers (136 AFLPs, 112 SSRs and one CAPS) distributed over 18 linkage groups. Comparison of the present intraspecific map with the interspecific maps developed by Yi *et al.* (2006) and Mueller *et al.* (2005) revealed high linkage conservation in at least three linkage groups. In the SNU3 map, 11 of 14 linkage groups were assigned into 11 chromosomes (Yi *et al.*, 2006); in the present map, 14 of 18 linkage groups were assigned to 12 chromosomes of pepper. However, the map distances differed. These differences could result from the lower genome homology between parents in an interspecific cross, reducing recombination and map size, or the dra-

Figure 2 *Capsicum annuum* F_8 RIL population linkage map. (a) Chromosomes carrying QTLs for phytophthora root rot resistance. (b) Chromosomes not carrying QTLs for phytophthora root rot resistance. QTL positions, together with their confidence intervals, are presented to the left of linkage groups and indicated by vertical lines. Isolate-specific QTLs are indicated by italics. Common QTLs are indicated by non-italics. Genetic markers are to the right of each linkage group; genetic distances (cM) between adjacent markers are to the left. Underlined markers are consensus SSR markers in common with previously published pepper linkage maps. Assignment of numbers to linkage groups corresponds to the basic chromosome numbers of pepper.

matic decrease in EST-SSRs and SSR markers and absence of RFLP markers in the present map. For example, in potatoes, the map size of an interspecific cross was found to be 65% smaller than that of an intraspecific one (Gebhardt *et al.*, 1991). Anchor markers BM59622, BM61910 and BM62655 belong to chromosome 3, and CA52611 and CP10020 belong to chromosome 8 (Mueller *et al.*, 2005), but in the present map, these markers belong to separate groups (Fig. 2a,b). Thus, P3a and P3b were assigned to chromosome 3, and P8a and P8b were assigned to chromosome 8. The four linkage groups Pa, Pb, Pc and Pd could be merged into chromosomes when more markers are added.

Distances between two anchor SSR markers on chromosomes 1 (10·9 cM), 2 (12·8 cM), 3 (39·4 cM), 4 (35·4 cM) and 6 (8·7 cM) in the Pepper-FAO3 map were similar to those on P1 (9·9 cM), P2 (13·3 cM), P3b (40·8 cM), P4 (37·6 cM) and P6 (10·1 cM), respectively (Fig. 2a,b). The distribution and order of reference markers of the SNU3 map were consistent with those in the present map except for some minor differences. This indicates those markers are highly conserved across *Capsicum* species. However, distances between anchor markers, such as GP20068 and CA516439 on P10, CA525390 and GP20087 on P11, and GP1127 and GP1017 on P12, were greater than those on chromosomes 10, 11 and 12, respectively. More comprehensive coordination among the *Capsicum* maps would be helpful for pepper genetics and breeding. The present map covered a total genetic distance of 1486·6 cM. Within the *C. annuum* genome, map coverage in the present study is similar to that of previous maps (Ogundiwin *et al.*, 2005; Barchi *et al.*, 2007; Kim *et al.*, 2008).

Expression of resistance to phytophthora root rot in pepper depends on genetic variability of the pathogen (Sy *et al.*, 2008). The RILs presented highly significant genetic variation for resistance to the two *P. capsici* isolates used in this study (Table 1) and the performance of the RIL population was highly correlated among the traits inoculated with different isolates (Table 2). This indicates there are common QTLs associated with resistance expression under various environments. The term 'environment' here includes factors possibly affecting resistance expression, such as pathogen isolate, temperature and relative humidity. QTLs commonly detected for more isolates would indicate the greater importance of those QTLs contributing to stable resistance. Based on the QTL analysis results, QTLs located in the region of 24–44 cM on chromosome 5 (P5) that associated with the resistance against both isolates fit this criterion. The importance of QTLs in this region was also supported by the large percentage contributing to the overall disease variation, ranging from 20·0% to 48·2%. The R^2 values of those QTLs in this region were the largest among QTLs detected from the evaluation using two data processing methods (Table 3). In previous studies, QTLs for phytophthora root rot resistance against different pathogen isolates from different geographic regions have been identified on chromosome 5; for example, a major QTL was identified for resistance against strains S101 and S197 (Thabuis *et al.*, 2004), or isolate Pc197 (Bonnet *et al.*, 2007). In addition, Kim *et al.* (2008) identified QTLs affecting phytophthora root rot resistance against a Korean isolate, Pa23, located between markers HpmsE015 and pR5-93, and CDI78 and CDI25 within intervals of 0–30 and 105–109 cM on chromosome 5, respectively. In the present map, reference marker HpmsE015 was in the same position as in the reference map of Kim *et al.* (2008). Thus, the QTL region associated with resistance against Korean *P. capsici* isolates detected by Kim *et al.* (2008) and in the present study overlapped by about 10 cM on chromosome 5. This indicates that QTLs in this region could be related to the stable resistance of YCM334. Markers such as those in the region from 23 to 40 cM on chromosome 5, which flank stable resistance QTLs, would be useful for marker-assisted foreground selection to improve resistance in commercial cultivars.

Specific resistance is oligogenic against particular pathogen isolates and follows a gene-for-gene relationship (Keen, 1990). Breeding for durable resistance to phytophthora root rot therefore requires knowledge of the distribution of isolate-specific resistance. The objective of the study was to identify such resistance in *C. annuum* that would be relevant to a pepper breeding programme in Korea. This study used two *Phytophthora* isolates collected from different locations in Korea and identified different disease reactions in the RIL population. Resistance alleles originated more frequently from the resistant parent, but they occasionally originated from the susceptible parent. Comparing the QTLs detected as associated with resistance to two *P. capsici* isolates, eight QTLs were detected with one or other of the isolates, but not both (Fig. 2b). Among these, the alleles *Ph051-10·1*, *Ph051-10·3* and *Ph127-11* from the susceptible parent were associated with resistance. Resistance alleles originating from the susceptible parent using the cv. Yolo Wonder × CM334 population have been reported previously (Thabuis *et al.*, 2003). Thus, resistance alleles originating from the susceptible parent in this study could be inherited from Yolo Wonder. The inheritance of a resistance allele from a susceptible parent is not uncommon and has been reported in many plant species (Young *et al.*, 1993; Pilet *et al.*, 1998; Wang *et al.*, 2000). QTLs for phytophthora root rot resistance in pepper were previously detected with resistance alleles inherited from susceptible parents (Lefebvre & Palloix, 1996; Ogundiwin *et al.*, 2005). These QTLs may be responsible for the occurrence of individuals with transgressive phenotypes (deVicente & Tanksley, 1993; Dirlewanger *et al.*, 1994; Darvishzadeh *et al.*, 2007). Trangressive segregation was previously reported by Palloix *et al.* (1988) for phytophthora root rot resistance of pepper with other isolates of *P. capsici*.

QTLs for phytophthora root rot resistance were previously identified on chromosome 11, but not 10, using populations with resistance alleles generated from CM334 (Thabuis *et al.*, 2003, 2004; Ogundiwin *et al.*, 2005). The present study found four QTLs for specific

resistance to isolate 09-051 on chromosome 10 (P10) and one QTL for specific resistance to isolate 07-127 on chromosome 11 (P11). The number of QTLs detected for RAUDPC was greater than that for PWP. This could be because RAUDPC was calculated using all data and does not obscure variation in rate of disease development; thus, the effect of minor differences in disease severity is included. This is in agreement with the frequency distribution of RAUDPC. QTLs associated with resistance against only isolate 09-051, namely *Ph051-10·1* on chromosome 10 (P10) and *Ph051-c·1* on Pc for PWP, also associated with RAUPDC for QTLs *Ph051-10·3* and *Ph051-c·2*, respectively. In addition, two QTLs for RAUDPC, *Ph051-10·2* and *Ph051-10·4* on chromosome 10, associated with resistance against isolate 09-051 but not 07-127. In contrast, QTLs *Ph127-11* for RAUDPC on chromosome 11 and *Ph127-b* on Pb for PWP associated with resistance against isolate 07-127 but not 09-051. Thus, markers in the region from 40 to 55 cM on chromosome 10 would be useful for marker-assisted foreground selection to improve root rot resistance in commercial cultivars. The results confirm the gene-for gene relationship between *C. annuum* and *P. capsici* for root rot resistance reported by Sy *et al.* (2008) and Monroy-Barbosa & Bosland (2008). Thus, the results indicate that at least a few specific gene functions are important components of root rot resistance to different *P. capsici* races/isolates in the YCM334 × Tean population. Identification of isolate-specific resistance QTLs in *P. capsici*–*C. annuum* interactions will help breeders in selecting appropriate resistant lines for future hybridization. Breeders may need to breed for resistance against a specific isolate from different regions, and then pyramid a number of specific genes to confer resistance into a cultivar. The approach for further studies following the results of the present study could be to develop near-isogenic lines carrying different combinations of QTLs and challenging the isogenic lines with different pathogen isolates.

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