Construction of 2 intraspecific linkage maps and identification of resistance QTLs for *Phytophthora capsici* root-rot and foliar-blight diseases of pepper (*Capsicum annuum* L.)

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Abstract: Two linkage maps of pepper were constructed and used to identify quantitative trait loci (QTLs) conferring resistance to *Phytophthora capsici*. Inoculations were done with 7 isolates: 3 from Taiwan, 3 from California, and 1 from New Mexico. The first map was constructed from a set of recombinant inbred lines (RILs) of the PSP-11 (susceptible) × PI201234 (resistant) cross; and the second map was from a set of F_2 lines of the Joe E. Parker' (susceptible) × 'Criollo de Morelos 334' (resistant) cross. The RIL map covered 1466.1 cM of the pepper genome, and it consisted of 144 markers — 91 amplified fragment length polymorphisms (AFLPs), 34 random amplified polymorphic DNA (RAPDs), 15 simple sequence repeats (SSRs), 1 sequence characterized amplified region (SCAR), and 3 morphological markers — distributed over 17 linkage groups. The morphological markers mapped on this population were erect fruit habit (*up*), elongated fruit shape (fs_e), and fasciculate fruit clusters (*fa*). The F_2 map consisted of 113 markers (51 AFLPs, 45 RAPDs, 14 SSRs, and 3 SCARs) distributed in 16 linkage groups, covering a total of 1089.2 cM of the pepper genome. Resistance to both root rot and foliar blight were evaluated in the RIL population using the 3 Taiwan isolates; the remaining isolates were used for the root-rot test only. Sixteen chromosomal regions of the RIL map contained single QTLs or clusters of resistance QTLs that had an effect on root rot and (or) foliar blight, revealing a complex set of genetics involved in resistance to *P. capsici*. Five QTLs were detected in the F_2 map that had an effect on resistance to root rot.

Key words: Phytophthora capsici, QTLs, fasciculate, resistance, isolates, pepper, fruit shape.

Résumé : Deux cartes génétiques du poivron ont été produites et employées pour identifier des QTL conférant la résistance au *Phytophthora capsici*. Des inoculations ont été pratiquées avec 7 isolats dont 3 de Taiwan, 3 de Californie et 1 du Nouveau-Mexique. La première carte a été produite à partir d'une collection de lignées recombinantes (RIL) issues du croisement entre PSP-11 (sensible) et PI201234 (résistant). La seconde carte a été produite à l'aide d'une population F_2 issue du croisement entre Joe E. Parker (sensible) et Criollo de Morelos 334 (résistant). La carte RIL couvre 1466,1 cM du génome du poivron et compte 144 marqueurs (91 AFLPs, 34 RAPDs, 15 SSRs, 1 SCAR et 3 marqueurs morphologiques) distribués sur 17 groupes de liaison. Les marqueurs morphologiques examinés au sein de cette population étaient le port érigé du fruit (*up*), la forme allongée du fruit (*fse*) et les grappes fasciculées (*fa*). La carte F_2 comprenait 113 marqueurs (51 AFLPs, 45 RAPDs, 14 SSRs et 3 SCARs) totalisant 1089,2 cM du génome. La résistance aux pourritures racinaire et foliaire a été évaluée chez la population de RIL à l'aide des 3 isolats de Taiwan, tandis que les autres isolats ont été employés pour mesurer la résistance à la pourriture racinaire seulement. Seize régions chromosomiques sur la carte RIL portaient des QTL uniques ou des amas de QTL pour la résistance ayant des effets sur les pourritures racinaire ou foliaire, ce qui illustre la complexité génétique de la résistance au *P. capsici*. Cinq QTLs ayant un effet sur la résistance à la pourriture racinaire ont été détectés au sein de la population F_2.

Mots clés : Phytophthora capsici, QTL, *fasciculate*, résistance, isolats, poivron, forme des fruits.

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Introduction

Phytophthora capsici Leonian is a plant pathogen of global importance. It is responsible for different disease syndromes in pepper (*Capsicum annuum* L.), leading to significant economic loss in this vegetable and spice crop. The incidence of this pathogen has increased in recent years in the United States and worldwide (Hwang and Kim 1995; Ristaino and Johnston 1999). *P. capsici* can infect all parts of the pepper plant (Parra and Ristaino 2001), resulting in different disease syndromes, such as damping off, root rot, crown rot, fruit rot, and foliar blight. The foliar-blight phase is the biggest problem in regions with heavy rainfall or excessive sprinkler irrigation; the root- and crown-rot phases are significant problems in semiarid areas, such as California's Central Valley, where rain is infrequent during the growing season and furrow or drip irrigation is used (Café-Filho and Duniway 1996). *Phytophthora capsici* has also been reported to attack eggplant, cucumber, honeydew melon, pumpkin, squash, tomato, and watermelon (Polach and Webster 1972; Ristaino 1990).

Methods to control this disease include cultural practices that ensure well-drained soils, and the use of chemicals and resistant cultivars, but these are only partly effective. In addition, resistance of P. capsici to metalaxyl has been reported both in the laboratory and in field trials (Parra and Ristaino 2001; Lamour and Hausbeck 2001).

The use of genetically resistant cultivars promises to be the most effective and environmentally friendly method to control *P. capsici*. Development of resistant pepper cultivars will not only increase pepper yield but will also reduce the use of fungicides that are potentially detrimental to the environment. It has been reported that some accessions of pepper are resistant to *P. capsici* (Barksdale et al. 1984; Reifschneider et al. 1992; Ortega et al. 1992). It has been reported that the pepper land race 'Criollo de Morelos 334' ('CM334'), from the Mexican state of Morelos, and USDA PI201234, an accession from Central America, are highly resistant to *P. capsici* (Ortega et al. 1991; Walker and Bosland 1999; Oelke et al. 2003). Breeders are using these 2 resistant pepper lines in their breeding programs. Despite the availability of resistant pepper lines, the information in the literature on the genetics of resistance is conflicting, and there is no report of a commercial pepper cultivar with universal resistance to *P. capsici*. Several classic studies on the inheritance of *P. capsici* resistance in pepper have been conducted (Smith et al. 1967; Bartual et al. 1991, 1994; Ortega et al. 1991, 1992; Kim et al. 1989; Palloix et al. 1988, 1990; Walker and Bosland 1999). These studies do not agree on the number of genes conditioning resistance; the number of resistance genes reported varied from 1 to many, and the possibility of epistatic effects was raised. There is a strong indication, however, that resistance to *P. capsici* is polygenic (Lefebvre and Palloix 1996; Thabuis et al. 2003). The disparity in the results of these inheritance studies could be because of variations in the inoculation tests (isolates used, environmental conditions, plant physiological stage, disease rating system) and the resistant lines studied (Lefebvre and Palloix 1996). Oelke et al. (2003) and Glosier, B., and Prince, J.P. (unpublished results) reported the existence of 10 and 14, respectively, different physiological races of *P. capsici*. Walker and Bosland (1999) reported that different genes control resistance to root-rot and foliar-blight diseases of pepper caused by *P. capsici*. It is important to know the number and location of these resistance genes in the genome of pepper.

Several linkage maps of pepper have been reported (Prince et al. 1993; Livingstone et al. 1999; Kim et al. 1998; Lefebvre et al. 1995, 2002; Lee et al. 2004; Paran et al. 2004). Lefebvre and Palloix (1996) used the linkage map of Lefebvre et al. (1995) to detect, with a single isolate, up to 13 quantitative trait loci (QTLs) that control inheritance of *P. capsici* resistance. Thabuis et al. (2003), using the improved map of Lefebvre et al. (2002) and 2 strains of the pathogen, studied the genetics of *P. capsici* resistance in 3 different intraspecific populations of pepper; they detected several QTLs, some common to all, and some specific to each population.

We report here the development of 2 linkage maps of pepper, based on 2 intraspecific mapping populations generated from the hybridization of 'CM334' and PI201234 with 2 *P. capsici*-susceptible pepper lines, 'NuMex Joe E. Parker' ('JEP') and PSP-11, respectively. Resistance QTLs detected from these maps that affect resistance to foliar blight and root rot, involving 7 different *P. capsici* isolates, are also reported.

Materials and methods

Plant material, mapping populations, and DNA extraction

A set of 94 recombinant inbred lines (RIL: F_7) generated from the PSP-11 × PI201234 cross constituted the first mapping population (RIL). PSP-11 is a *P. capsici*-susceptible pepper accession, whereas PI201234 is resistant. The RIL population was developed at the Asian Vegetable Research and Development Center (AVRDC), in Taiwan, by single-seed descent. The second mapping population (F_2) was a set of 94 F_2 individuals obtained by crossing a susceptible commercial pepper line, 'JEP', with 'CM334'. DNA was isolated from leaf tissue of RIL and F_2 individuals, as well as from their parental lines, in accordance with the method described by Prince et al. (1997). The quality of DNA samples was checked on 1% (*w*/*v*) agarose gel, and quantification was done by means of spectrophotometry.

Molecular markers

The following molecular markers were used in this study: random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), and sequence characterized amplified regions (SCARs). These markers were analyzed using standard procedures. Microsatellite primers were either designed from SSR sequences (generated in S.L.'s laboratory), with the use of Primer3 software (Rozen and Skaletsky 2000), or obtained from previous pepper-linkage maps (Lee et al. 2004). A GenBank search was made for candidate resistance genes to be included on the map, and for the possible localization to *P. capsici*-resistance QTL. The sequence of the *Bs2* gene (*Capsicum chacoense* disease-resistance protein mRNA), cloned by Tai et al. (1999), was obtained from GenBank (accession No. AF202179). Primers were designed for this

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gene, and the amplified products were digested with the *Eco*RV restriction enzyme to reveal polymorphism between the parental DNA samples. Also obtained from GenBank was the sequence of a transcript obtained from *P. capsici*-challenged roots of *C. annuum* (acc. No. CA847364).

Attempts were made to convert RAPD markers that either associated with QTLs or were common to both maps to SCAR markers. The band of interest was excised from the agarose gel and the DNA was re-extracted from the gel using a DNA gel extraction kit (Millipore Corporation, Bedford, Mass.) in accordance with manufacturer's instructions. The isolated DNA was then cloned into the pCRII-TOPO vector (Invitrogen, Carlsbad, Calif.) or PGEM T-Easy vector (Promega Corp., Madison, Wis.). The clones were sequenced with the ABI310 DNA Analyzer (Applied Biosystems, Foster City, Calif.), and the sequences analyzed with Sequencher v. 4.1 computer software (Gene Codes Corp., Ann Arbor, Mich.). Primer information for SSR and SCAR markers and candidate gene homologs is given in Table 1.

Survey of polymorphism, marker scoring, and nomenclature

A survey of polymorphism for the 2 pairs of parental lines was conducted for each of the marker types. For RAPD analysis, a survey was done twice for each primer, and only repeatable polymorphic bands were followed up in the segregating populations. Bands that were too faint or ambiguous were excluded from RAPD data. RAPD markers generated from Operon decamers (Operon Technologies, Alameda, Calif.) were named using the letter that identifies the set, the primer number in the set, and the approximate size (in base pairs) of the marker as a subscript. Names of RAPD markers obtained from the University of British Columbia primers start with UB, and the primer number with approximate fragments size, in base pairs, follows as a subscript. AFLPs were scored as dominant markers, and each marker was named after the primer combination used for selective amplification (E+3M+3), with the approximate size of the fragment, in base pairs, as a subscript. SSR markers developed in our lab were simply assigned serial numbers (Table 1).

Map construction

Each marker was tested using χ^2 analysis for goodness-of-fit to the expected Mendelian segregation ratios (3:1 and 1:2:1 for the F_2 population; 1:1 for the RIL). Linkage analysis was performed with Mapmaker, version 2.0, for Macintosh (Lander et al. 1987). Linkage groups were identified using the "group" command, with minimum log of odds ratio (LOD) threshold between 3.0 and 4.5, and maximum recombination fraction of 35%. The ordering of markers in each linkage group was done by first performing pair-wise comparisons. A subset of the linkage group was ordered using the "compare" command at LOD of 3.0. Other markers in the group were placed on the ordered linkage group at LOD of 3.0, using the "try" command. Markers ordered at LOD ≥ 3 formed the framework map. Unlinked markers at LOD of 3.0 were placed on the map at LOD ≥ 2.0. The Kosambi mapping function (Kosambi 1944) was used to convert recombination fractions to map distances in centimorgans (cM).

Data analysis and QTL detection

The disease reactions of the RILs to foliar blight and root rot of the 3 Taiwan isolates, PC1E, PC33E, and PC17E, were compared using the pooled *t* test analysis function in Data Desk 4.2 (Velleman 1985). QTLs for the framework maps were detected using composite interval mapping (CIM) on Windows QTL Cartographer, version 2.0 (Basten et al. 1997; Wang et al. 2003). The 1-way analysis of variance (ANOVA) function in Data Desk 4.2 (Velleman 1985) was used to identify markers that were either unlinked at LOD ≥ 3.0 or placed on the maps at 2 ≥ LOD ≤ 3, but that had significant ($P \leq 0.01$) association with resistance. For the CIM, a QTL was declared at minimum LOD of 2.5. Estimates of epistatic interactions among the detected QTLs for each isolate/disease syndrome were obtained with the multiple interval mapping analysis of the Windows QTL Cartographer. The percentage of phenotypic variation explained by all the QTLs associated with resistance to a given isolate/disease syndrome was obtained using the multiple regression analysis of the flanking framework markers. Dominance ratio was calculated for each QTL detected in the F_2 map from the CIM analysis, as described by Thabuis et al. (2003): dominance ratio = 2|d/a|, where *a* and *d* are the additive and dominance estimates, respectively.

Phytophthora disease-resistance assays

Fungal isolates and experimental layout

Three *P. capsici* isolates from Taiwan, PC1E, PC33E, and PC17E (hereafter referred to as T1, T2, and T3, respectively), were used for foliar- and root-rot inoculations of the RIL population at the AVRDC. The RIL population was tested for root-rot disease reaction with 3 Californian isolates: GPS1-1 (hereafter referred to as Gps), PPc2, and PPc4 at California State University, Fresno, California (CSUF). The F_2 population (F_3 families) was inoculated with isolate M (from New Mexico) at New Mexico State University to evaluate for root-rot resistance. Seeds of 94 RILs were sown into a commercial peat moss mix in plastic flats with 72 cells (cell size: 4.2 cm × 4.2 cm × 5 cm) at AVRDC in Taiwan, or in sterilized soil (Supersoil, Rod McLellan Co., San Mateo, Calif.) in plastic flats with 32 cells (cell size: 4 cm × 6 cm × 7 cm) at CSUF in California. The seeds of 69 F_3 families obtained from the F_2 population were planted in 72-cell trays (cell size: 3.8 cm × 3.8 cm × 5.7 cm) filled with Sunshine peat-moss mix (McCalif, Ceres, Calif.). Experimental design was a randomized complete block, with 3 or 4 replicates, and 6 plants per line per replicate. The parental lines were included as susceptible and resistant checks.

Root-rot assay and disease-resistance score

The root-rot assay was a modification of that described by Bosland and Lindsey (1991). *P. capsici* isolates were grown in V8® juice agar medium at 25 °C for 7 days. About 15 mycelial plugs (10 mm diameter) were then cut out with a sterile core borer and placed in a sterile petri plate. Sterile water was added to cover the agar plugs and the plate was incubated at 25 °C for 48 h, after which the water was decanted and replaced with sterile soil–water extract. The plate was incubated at 25 °C for another 48 h,transferred to a 10 °C incubator for 90 min to initiate zoospore formation,

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Table 1. PCR primers used to generate some specific markers on the 2 pepper intraspecific maps. F, forward sequence of the primer; R, reverse sequence of the primer.

Primer		Sequence (5′ to 3′)	Description	Source
SSR6	F	TGGGAAGAGAAATTGTGAAAGC	(CAT)n repeats	Present study
	R	AGACCCAATGTGGTCCAATC		
SSR16	F	TAACACCTCTCTAACCGTCACC	(AAT)n repeats	Present study
	R	AAAAGCCCCAAACCAACC		
SSR24	F	GGAGTGGACTTTGGAACATTT	(CGT)n repeats	Present study
	R	CGCTTCACAATCATCACACT		
SSR19	F	AGGTGGATATGCGGTTCAAT	(GA)n repeats	Present study
	R	CTCAAAAGTGATGGTGGCAA		
D11Scar$_{650}$	F	AATCACACTGGGTTGTTGAC	SCAR primer[a]	Present study
	R	CTGGATAAGATGGAAGAGGA		
Bs2[b]	F	TGCCTGGGCTACCATATCTC	*Bs2* resistance gene of pepper	Tai et al. 1999
	R	ACAGATCCACTTGGGCAATC	—	—
CA847364	F	AGGCCGCATTGTCTCTCAT	cDNA of putative resistance protein	Richins et al. 2002[c]
—	R	CCGCGTAATCTTCAGTGTGT	—	—

Note: F, F_2 population; R, recombinant inbred line (RIL) population; SSR, simple sequence repeats.
[a]Sequence characterized amplified region (SCAR) developed from random amplified polymorphic DNA (RAPD) marker D11$_{842}$.
[b]PCR product was digested with *Eco*RV.
[c]Unpublished sequence obtained from GenBank. acc. No. CA847364.

and then transferred to a 25 °C incubator for 30 min for zoospore release. The zoospore suspension was decanted and the zoospores were counted with the aid of a hemacytometer. Zoospore concentration was estimated and the stock suspension was diluted to 10^5 zoospores/mL with chilled water immediately before inoculation. Inoculation was done by releasing 5 mL of the diluted inoculum (10^5/mL) at the base of each 28- to 35-day-old plant. Test plants in the flats with holes were transferred to holeless flats and flooded with water immediately before inoculation. The flooded flats were maintained for 2 days after inoculation, thendrained, after which the plants were transferred to flats with holes. The greenhouse temperature was maintained above 28 °C, and watering was done twice daily to maintain high soil moisture.

Inoculated plants were scored for disease reactions 7, 14, and 21 days after inoculation, on a scale of 0 to 5 (0 = no symptoms, no necrosis, white/tan roots, healthy shoot; 1 = leaf chlorosis but no necrosis; 2 = leaf chlorosis, slight necrotic crown; 3 = necrotic crown plus severe wilting; 4 = severe necrosis, almost dead; 5 = dead plant). Plants were scored individually. Plants with a score of 0 or 1 were considered to be resistant; plants with a score of 2 or higher were considered susceptible. Counts of susceptible and resistant plants were made for each line, based on the cutoff. The disease-resistance score (DRS) for each line was expressed as a percentage of resistant plants.

Foliar-blight assay and disease-resistance score

The 3 Taiwan isolates (T1, T2, and T3) were also used for the foliar-blight assay of the RIL population. Inoculum preparation was the same as for the root-rot assay, and inoculation was carried out on 35- to 38-day-old plants by atomizing the foliage, to the point of runoff, with the zoospore suspensions (10^5/mL). Inoculated plants were held for 24 h at 28 °C and 100% relative humidity in the dark to retain the leaf wetness. After 24 h, the foliage was allowed to dry, and thereafter the plants were maintained at 28 °C and 60% – 95% relative humidity, with 14 h of light (70 $\mu E \cdot m^{-2} \cdot s^{-1}$) per day.

The disease reaction was scored at 7 and 14 days after inoculation. Plants were scored individually. The DRS scale, with ratings of 0 to 4, was used (0 = no symptoms; 1 = green upper foliage with small lesions on lower leaves, some lower leaf drop; 2 = green upper foliage with lesions mostly on lower and middle leaves, lower leaf-drop, restricted (<3mm) shallow stem lesions; 3 = lesions on most leaves, extensive leaf-drop, generally with deep stem lesions; 4 = extensive leaf and stem necrosis, most often the plants are dead). Plants with scores of 0 or 1 were considered resistant; those with scores of 2 or higher were considered susceptible. The DRS was calculated as it was for root-rot analysis.

Pepper-fruit traits as morphological markers

Inheritance of 3 fruit characteristics differentiating PSP-11 and PI201234 was evaluated in the PSP-11 × PI201234 cross. These traits were erect fruit habit (*up*), fruit shape (*fs*), and fasciculate fruit clustering at the plant's apex (*fa*). The inheritance study involved 5 populations: P_1 (PSP-11); P_2 (PI201234); F_1; F_2; and BCP_2 (F_1 × PI201234). Ten plants each of the parental lines, 9 plants each of F_1 and BCP_2, and 79 plants of the F_2 population were evaluated in the greenhouse. The traits were scored at fruit maturity. The RILs segregated and were scored for these traits. Segregation data were included in the linkage analysis of the molecular marker data, so that their positions on the linkage map could be determined.

Results

Phenotypic variation in isolates and disease syndromes

Figure 1 shows the frequency distributions of the RIL and the F_2 populations tested with the different *P. capsici* isolates. All disease reactions showed continuous distribution, indicating that resistance is polygenic. Asymmetric distribution toward the resistant parent (PI201234) was observed for

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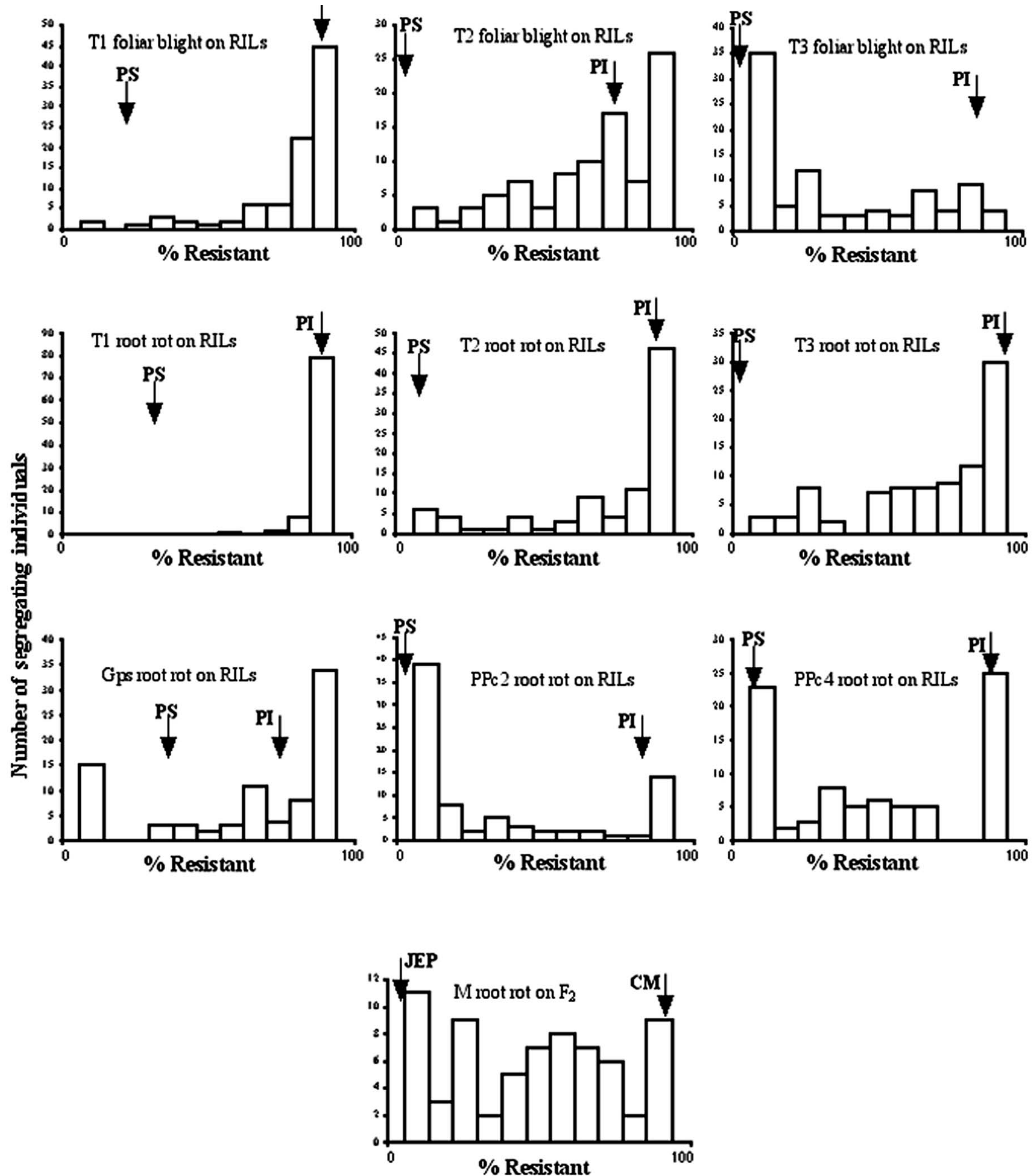
Fig. 1. Frequency distributions of the disease resistance score of the recombinant inbred lines (RILs) and the F_2 population challenged with 7 isolates of *Phytophthora capsici*. Arrows indicate the approximate positions of the parental values. PS, PSP-11; PI, PI201234; 'JEP', 'NuMex Joe E. Parker'; CM, 'Criolo de Morelos' ('CM334').



T1 and T2 isolates for both foliar-blight and root-rot disease syndromes. However, for isolate T3, the distributions skewed toward the susceptible and resistant parents for foliar blight and root rot, respectively, indicating that different factors may be responsible for inheritance of resistance to the 2 disease syndromes. The continuous distribution for root-rot disease syndrome observed for isolates Gps, PPc2, and PPc4 showed a bimodal trend, indicating that a major genetic factor may be involved in the polygenic inheritance of resistance to these isolates. Isolate M, used to test the F_2 population, showed a continuous distribution with 4 peaks, indicating that at least 4 major genes, as well as some minor ones, are probably involved in the genetics of resistance to this isolate. The difference in the reactions of the RILs to foliar-blight and root-rot inoculations of the 3 Taiwan isolates was evaluated using a pooled *t* test; the results are presented in Table 2. The reactions of the RILs differed in the 2 disease types for isolates T1 and T3 ($P \leq 0.001$), indicating that different genetic components may be involved in the inheritance of resistance to foliar blight and root rot in these isolates. The difference between the 2 disease types for isolate T2 was not significant. Table 3 shows the correlation

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Table 2. Mean scores, standard errors, and *t* values of reactions of recombinant inbred lines of the PSP11 × PI201234 cross to the root-rot and foliar-blight disease assay of 3 Taiwan *Phytophthora capsici* isolates.

Isolate	Root rot	Foliar blight	*t* value	*P*
T1	98.83±0.49	88.57±2.26	–4.44	<0.001
T2	80.56±3.22	75.56±2.75	–1.19	0.24
T3	75.00±3.11	36.68±3.87	–7.80	<0.001

coefficients of 9 disease scores in the RIL population in which QTLs were detected. Isolates T1 and Gps had a significant but weak correlation for root rot (r = 0.236; P = 0.05); they did not correlate with any other isolates, indicating their uniqueness. Root rot of PPc4 significantly correlated with root rot of T2, T3, and PPc2, and foliar blight of T2 and T3. Root rot of isolate PPc2 correlated significantly with root rot and foliar blight of isolates T2 and T3.

Polymorphism between parental lines

Of the 501 RAPD primers screened for polymorphism between PSP-11 and PI201234, 46 (9.2%) generated between 1 and 4 polymorphic fragments. Between 'JEP' and 'CM334', 34 of 192 RAPD primers (17.7%) produced polymorphic bands. Forty-six AFLP primer combinations were used to search for polymorphism between PSP-11 and PI201234. These produced 195 polymorphic bands, with an average of 4.33 polymorphic bands per primer combination. 'JEP' and 'CM334' were differentiated by 65 polymorphic bands, produced by 7 AFLP primer combinations (9.28 polymorphic bands per primer combination). A total of 83 SSR primers was used to screen the 2 parental pairs for polymorphism; 14 primers (16.9%) generated polymorphic bands between 'JEP' and 'CM334', and 15 primers (18.1%) were polymorphic between PSP-11 and PI201234.

Linkage maps

RIL map

A total of 183 markers (123 AFLPs, 41 RAPDs, 15 SSRs, 1 SCAR, and 3 morphological) were used to genotype the RIL population. Of these markers, 144 (78.7%; 91 AFLPs, 34 RAPDs, 15 SSRs, 1 SCAR, and 3 morphological markers) met the minimum requirement for linkage at LOD ≥ 2.0 and were used for map construction (Fig. 2). The markers were distributed on 17 linkage groups, as opposed to not the 12 expected based on the haploid chromosome number of pepper. The number of markers per linkage group ranged from 2 to 22, and the map covered 1466.1 cM of the pepper genome. Of the 144 markers placed on the map, 122 (84.7%) were used as framework markers because they mapped with LOD ≥ 3.0. The remaining 22 markers were placed in the intervals of framework markers at LOD ≥ 2.0. Distances between adjacent framework markers ranged from 1.4 to 39.0 cM, with an average of 13.83 cM.

F_2 linkage map

The F_2 population was assessed with a total of 146 markers: 78 AFLPs, 51 RAPDs, 14 SSRs and 3 SCARs Of these, 113 (77.4%; 51 AFLPs, 45 RAPDs, 14 SSRs, and 3 SCARs) were placed on the F_2 map distributed over 16 linkage groups (Fig. 3). The number of markers per linkage group varied from 2 to 21. Of the 113 markers, 91 were ordered at LOD ≥ 3; this formed the framework map for the F_2. The remaining markers were placed on the map with 2 ≤ LOD ≤ 3. The F_2 map covered a total of 1089.2 cM of the pepper genome, with an average interval between adjacent framework markers of 14.52 cM.

Assignment of linkage groups to pepper chromosomes

Some linkage groups of both maps were assigned pepper chromosome numbers, because they shared common markers with already published linkage maps. This included 9 and 10 linkage groups from the RIL and F_2 maps, respectively.

QTL identification on the RIL map

Several QTLs were detected for the 2 disease syndromes of *P. capsici* on the RIL map (Fig. 2). Any 2 or more QTLs with LOD peaks falling within 20 cM of each other were assigned to a *Phyto* group, as described by Grube et al. (2000). All the QTLs were therefore organized into 16 *Phyto* groups on the RIL map; letters *A* to *P* were used to distinguish each group. *Phyto.G* contained 3 QTLs within the marker interval $E35/M48_{350}$–$E33/M48_{71}$ on linkage group PP3. The following groups contained 2 QTLs each: *Phyto.A* and *Phyto.C* on linkage group PP1, *Phyto.D* on linkage group PP2, *Phyto.M* on linkage group PP9, and *Phyto.P* on linkage group PP15.

Isolate T1

Two QTLs were detected for foliar blight of isolate T1, 1 each on linkage groups (LGs) PP2 and PP7, together explaining 23.8% of the phenotypic variation. The 2 QTLs individually have negative additive effects. The QTL on LG PP7, *T1f-2* (*Phyto.J*), is unique to foliar blight of isolate T1, whereas the second QTL, *T1f-1*, fell in the same region (*Phyto.D*), with a QTL controlling root rot of isolate PPc2 (*PPc2r-2*). One QTL, *T1r-1* (*Phyto.L*), was detected for root rot of isolate T1. This QTL is unique to T1 root rot and explained 11.22% of the total phenotypic variation (Table 4).

Isolate T2

Two QTLs were detected by CIM for foliar blight of isolate T2, 1 each on LGs PP2 and PP9. The 2 QTLs together explained 51.9% of the total phenotypic variation in resistance to T2 foliar blight. QTL *T2f-2* (*Phyto.N*), on LG PPI1, is unique to foliar blight of isolate T2, whereas *T2f-1* on LG PP9 shared the same chromosomal region with *PPc2r-6* (*Phyto.M*). Two QTLs, *T2r-1* (*Phyto.B*) and *T2r-2* (*Phyto.E*), were identified for resistance to root rot of isolate T2 on LGs PP1 and PP2, together explaining 30.9% of the total variation. Both were unique to isolate T2 root rot.

Isolate T3

Two QTLs were detected for resistance to foliar blight of isolate T3, 1 each on LGs PP1 (*T3f-1*; *Phyto.C*) and PP3 (*T3f-2*; *Phyto.G*), together explaining 62.3% of the phenotypic variation. Neither QTL is exclusive to foliar blight of isolate T3. For the root-rot analysis of isolate T3, 3 QTLs, *T3r-1* (*Phyto.A*) on LG PP1, *T3r-2* (*Phyto.G*) on LG PP3, and *T3r-3* (*Phyto.P*) on LG PP15, were identified, together explaining 49.8% of the observed phenotypic variation.

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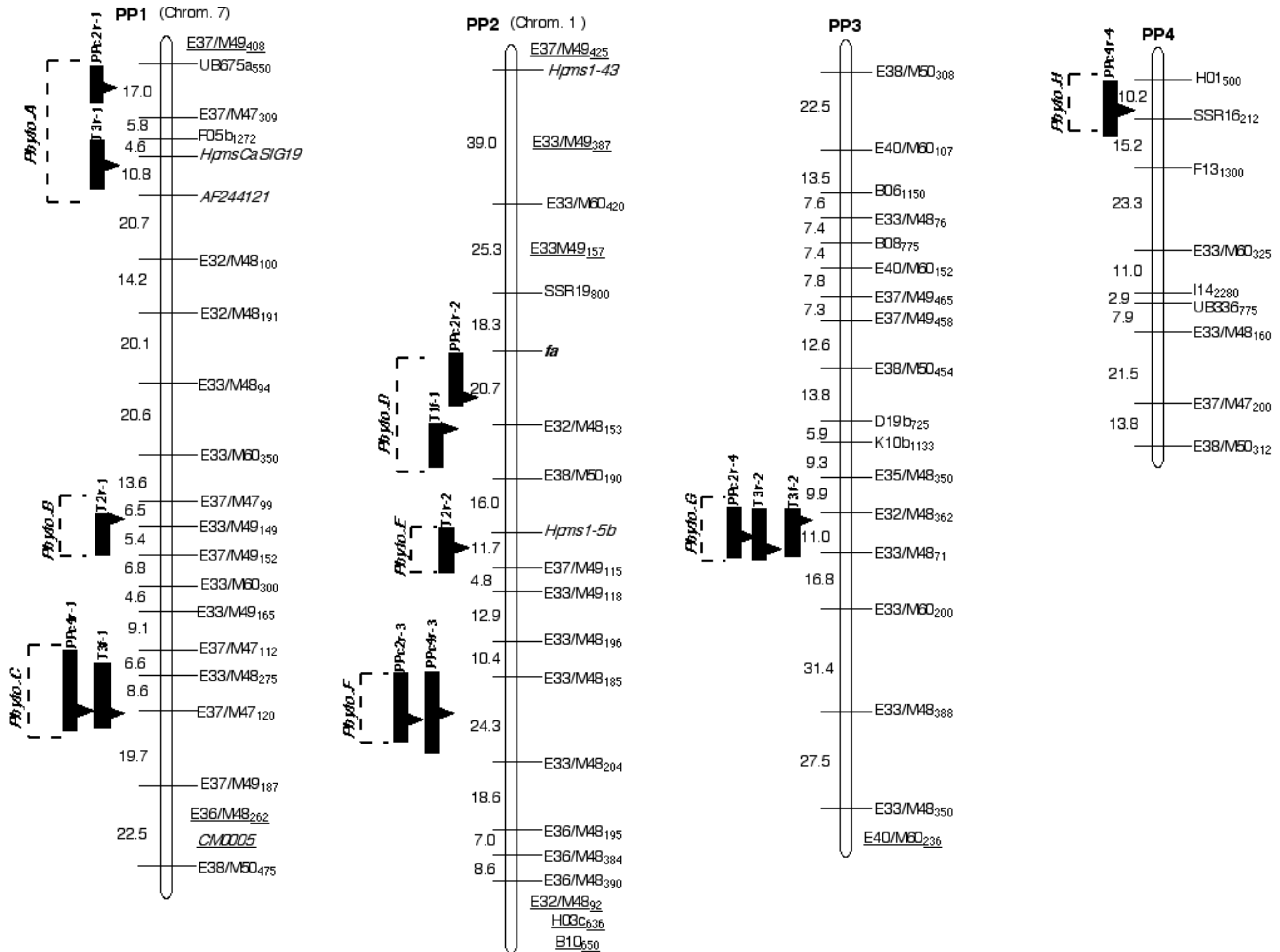
Fig. 2. The RIL (PSP-11 × PI201234) pepper linkage map showing the positions of the quantitative trait loci (QTLs) that affects resistance to root rot and foliar blight caused by *Phytophthora capsici*. Assignment of numbers to linkage groups (PP) is random. Numbers in parentheses correspond to pepper chromosome assignment in previously published pepper linkage maps. Genetic markers are to the right of each linkage group; genetic distances (cM) between adjacent markers are to the left. Morphological markers are in bold italics (***up***, erect fruit habit, ***fs$_e$***, elongated fruit shape, ***fa***, fasciculate fruit clusters at the apical nodes). Underlined markers were placed on the map at 2 ≤ log of odds ratio (LOD) ≤ 3 and were not included in the QTL interval mapping analysis. Italicized markers are simple sequence repeat (SSR) markers in common with a previously published pepper linkage map. QTL intervals are represented with solid bars to the left of the linkage groups; the QTL symbols appear on top of the bars. Arrowheads on the bars show the approximate positions of the peak LOD scores for the QTLs. The dashed lines are used to group closely placed individual QTLs into *Phyto* QTL clusters.



Isolate Gps

Two QTLs, *Gpsr-1* (*Phyto.I*) and *Gpsr-2* (*Phyto.P*) on LGs PP5 and PP15, respectively, were identified for resistance to root-rot disease of isolate Gps. These 2 QTLs together explained 36.9% of the observed variation in the resistance to root rot of Gps.

Isolate PPc2

A total of 6 QTLs with effects on resistance to root-rot disease of isolate PPc2 were detected by CIM, together explaining 73.5% of the phenotypic variation. These are *PPc2r-1* (*Phyto.A*) on LG PP1, *PPc2r-2* (*Phyto.D*) and *PPc2r-3* (*Phyto.F*) on LG PP2, *PPc2r-4* (*Phyto.G*) on LG PP3, *PPc2r-5* (*Phyto.J*) on LG PP6, and *PPc2r-6* (*Phyto.M*) on LG PP9. Of these 6 QTLs, only *PPc2r-5* (*Phyto.J*) was unique to isolate PPc2. This QTL also has the highest LOD peak (of 9.07), and alone explained 51.84% of the phenotypic variation (Table 4). The other 5 QTLs shared the same chromosomal regions with at least 1 other isolate.

Isolate PPc4

Four QTLs were identified for resistance to root-rot disease of isolate PPc4. These QTLs are *PPc4r-1* (*Phyto.C*) on LG PP1, *PPc4r-2* (*Phyto.F*) on LG PP2, *PPc4r-3* (*Phyto.H*) on LG PP4, and *PPc4r-4* (*Phyto.O*) on LG PP11. Together they explained 69.60% of the total variation observed for resistance to root rot of PPc4. *PPc4r-3* and *PPc4r-4* were unique to isolate PPc4.

QTL detection in the F_2 population

The *P. capsici* isolate M, used to assess the resistance response of the F_2 population to root-rot inoculation, identified

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Fig. 2 (*concluded*).

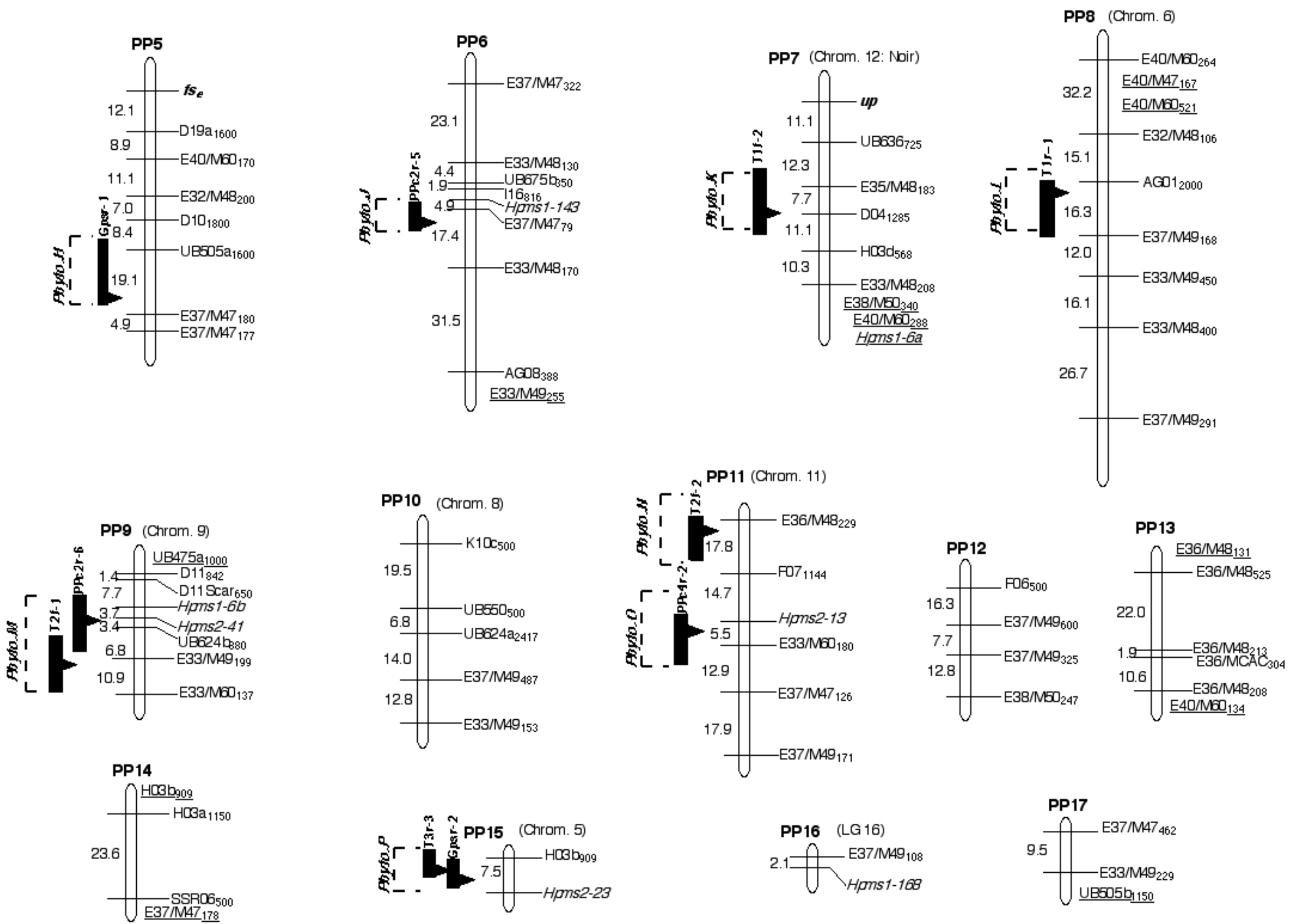

Table 3. Pearson's correlation coefficients of disease reactions (foliar blight and root rot) from the recombinant inbred line population tested with different *Phytophthora capsici* isolates.

	T1f	T2f	T3f	T1r	T2r	T3r	Gps	PPc2
T2f	–0.151							
T3f	–0.063	0.519**						
T1r	–0.042	0.336**	0.170					
T2r	–0.108	0.716**	0.499**	0.124				
T3r	–0.113	0.628**	0.687**	0.130	0.700**			
Gps	0.029	0.002	–0.099	0.236*	–0.053	–0.107		
PPc2	0.036	0.307**	0.340**	0.200	0.370**	0.278**	0.085	
PPc4	–0.083	0.262**	0.306**	–0.040	0.404**	0.373**	0.164	0.321**

*significant at 0.05; **significant at 0.01.

5 resistance QTLs: *Mr-1* and *Mr-2* on LG JC1, and *Mr-3*, *Mr-4*, and *Mr-5* on LGs JC2, JC4, and JC5, respectively (Fig. 3). The chromosomal regions where these QTLs fell were assigned *Phyto* names, as were the RIL resistance QTLs, differentiated with letters *Q* to *U*. *Mr-5* (*Phyto.T*) had the highest LOD (6.22), with an R^2 value of 35.29%. Together, the 5 QTLs explained 89.6% of the phenotypic variation (Table 4).

QTL epistatic interactions

Through the multiple interval mapping analysis, one significant digenic epistatic interaction (in the RIL population)

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Table 4. Features of pepper quantitative trait loci (QTLs) that control resistance to *Phytophthora capsici* root rot and foliar blight detected using composite interval mapping of the recombinant inbred line (RIL) (PSP-11 × PI201234) and F_2 ('Joe E. Parker' × 'Criollo de Morelos 334') mapping populations.

Trait[a]	QTL[b]	Marker interval[c]	LG[d]	LOD[e]	R^2 (%)[f]	Add[g]	Dom[h]	DR[i]
RIL								
T1f	*T1f-1*	$E32/M48_{153}$ – $E38/M50_{190(PS)}$	PP2	7.22	56.06	–28.62	—	—
	T1f-2	$D04_{1285(PI)}$ – $H03d_{568(PS)}$	PP7	7.72	43.11	–30.20	—	—
					23.80			
T2f	*T2f-1*	$UB624b_{880(PS)}$ – $E33/M49_{199(PI)}$	PP9	3.19	40.05	17.21	—	—
	T2f-2	$E37/M49_{187(PS)}$ – $E36/M48_{229(PI)}$	PP11	3.43	12.53	–9.47	—	—
					51.90			
T3f	*T3f-1*	$E37/M47_{112(PI)}$ – $E37/M47_{120(PI)}$	PP1	2.74	47.95	32.15	—	—
	T3f-2	$E32/M48_{362}$ – $E33/M48_{71}$	PP3	3.22	48.05	–31.83	—	—
					62.30			
T1r	*T1r-1*	$AG01_{2000(PS)}$ – $E37/M49_{168(PS)}$	PP8	2.55	11.22	1.70	—	—
					11.22			
T2r	*T2r-1*	$E37/M47_{99(PI)}$ – $E37/M49_{152(PI)}$	PP1	11.28	69.83	34.93	—	—
	T2r-2	*Hpms1–5* – $E37/M49_{115(PS)}$	PP2	12.01	54.93	–35.07	—	—
					30.90			
T3r	*T3r-1*	*HpmsCaSIG19* – *AF244121*	PP1	4.19	50.98	26.31	—	—
	T3r-2	$E32/M48_{362(PI)}$ – $E33/M48_{71(PS)}$	PP3	2.50	35.89	20.57	—	—
	T3r-3	$H03b_{909(PI)}$ – *Hpms2–23*	PP15	2.50	15.64	11.88	—	—
					49.80			
Gpsr	*Gpsr-1*	$D10_{1800(PI)}$ – $E37/M47_{180(PS)}$	PP5	2.88	14.90	14.54	—	—
	Gpsr-2	$H03b_{909(PI)}$ – *Hpms2–23*	PP15	4.34	21.59	–17.36	—	—
					36.90			
PPc2r	*PPc2r-1*	$UB675a_{550(PI)}$ – $E37/M47_{309(PI)}$	PP1	3.01	16.73	–18.59	—	—
	PPc2r-2	*fa* – $E32/M48_{153(PS)}$	PP2	6.96	51.84	34.23	—	—
	PPc2r-3	$E33/M48_{196(PI)}$ – $E33/M48_{204(PS)}$	PP2	6.12	46.54	34.98	—	—
	PPc2r-4	$E32/M48_{362(PI)}$ – $E33/M48_{71(PS)}$	PP3	8.42	49.72	–35.82	—	—
	PPc2r-5	*Hpms1–143* – $E33/M48_{170(PS)}$	PP6	9.07	51.84	–34.64	—	—
	PPc2r-6	$D11Scar_{650}$ – $E33/M49_{199(PI)}$	PP9	2.99	12.02	14.81	—	—
					73.50			
PPc4r	*PPc4r-1*	$E33/M49_{165(PI)}$ – $E37/M47_{120(PI)}$	PP1	7.84	55.79	33.06	—	—
	PPc4r-2	$E33/M48_{196(PI)}$ – $E33/M48_{204(PS)}$	PP2	3.68	55.49	32.93	—	—
	PPc4r-3	$H01_{500(PS)}$ – $SSR16_{212}$	PP4	3.06	13.43	–15.80	—	—
	PPc4r-4	$F07_{1144(PI)}$ – *Hpms2–13*	PP11	3.16	19.94	19.23	—	—
					69.60			
F_2								
Mr	*Mr-1*	*Hpms2–24* – $E35/M62_{202(CM)}$	JC1	2.93	37.08	6.21	–44.92	7.23
	Mr-2	$E35/M62_{134(CM)}$ – $E35/M62_{119(CM)}$	JC1	3.09	44.96	–12.72	–51.57	4.05
	Mr-3	$E35/M62_{76(\text{'JEP'})}$ – $E35/M62_{96(\text{'JEP'})}$	JC2	2.58	19.56	11.56	51.89	4.49
	Mr-4	$AG02_{1338(\text{'JEP'})}$ – $E38/M50_{437(\text{'JEP'})}$	JC4	3.20	33.35	9.20	–39.61	4.30
	Mr-5	$J12b_{1480 (CM)}$ – $E33/M49_{434(CM)}$	JC5	6.22	35.29	24.06	19.82	0.82
					89.60			

[a]Isolate/disease syndrome combination (r = root rot, f = foliar blight).
[b]Symbols given to QTLs associated with each trait, as in Figs. 2 and 3.
[c]Markers flanking the peak log of odds ratio (LOD) of the QTL; the parental source of each marker is in parenthesis (PI = PI201234, PS = PSP-11, CM = 'CM334', 'JEP' = 'NuMex Joe E. Parker').
[d]Linkage group.
[e]Log of odds ratio, as described in Materials and methods.
[f]Percentage of phenotypic variation explained by the QTL under consideration; the sum of R^2 of markers linked to the QTLs (in bold) was calculated with multiple regression analysis of the flanking markers.
[g]Additive estimate of the QTL.
[h]Dominance estimate of the QTL.
[i]Dominance ratio; it's significance is DR < 0.2 (additive), 0.2 < DR > 0.8 (partially dominant), 0.8 < DR > 1.2 (dominant), DR > 1.2 (overdominant).

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was observed for a pair of QTLs controlling resistance to pepper root-rot disease caused by *P. capsici* isolate PPc2. QTLs *PPc2r-3* and *PPc2r-4* of isolate PPc2 had significant additive × additive effect, with LOD of 3.83 and R^2 value of 10.1%. No significant digenic epistasis was detectable in the F_2 population at the LOD threshold of 2.5.

Single-marker QTLs

Regression analysis (1-way ANOVA) of DRSs with markers that were placed on the map at LOD < 2 and those that did not link at all identified 12 markers (10 AFLPs and 2 RAPDs) and 2 markers (1 AFLP and 1 RAPD) in the RIL and F_2 populations, respectively. These markers are presented in Table 5. The R^2 values of the markers ranged from 7% (P = 0.014) to 22% ($P \leq 0.001$). Markers *E33/M49*$_{350}$, *E37/M47*$_{322}$, and *E37/M49*$_{529}$ were each significantly associated with resistance to 2 isolates of *P. capsici*.

Inheritance and genomic location of 3 pepper-fruit trait loci

PSP-11 has elongate fruit shape, erect fruit habit, and fasciculate fruit clustering, whereas PI201234 has triangular fruit shape, pendant fruit habit, and nonclustering fruit. All 9 F_1 plants had triangular fruit shape, pendant fruit habit, and no fruit clustering at the plant apex. This indicates dominance of triangular fruit shape, pendant fruit habit, and nonclustering of fruit over elongate fruit shape, erect fruit habit, and fasciculate fruit clustering, respectively. This was confirmed by the backcross to PI201234 (BCP_2). The fruits of all 9 plants of BCP_1 were triangular, pendant, and not clustered. Of the 75 F_2 individuals, 51 had triangular fruits, whereas 24 had elongate fruits. This segregation fit a 3:1 ratio, with a χ^2 value of 1.96 (P = 0.20). Sixty-one F_2 plants had pendant fruit habit and 14 had erect fruit habit, supporting a 3:1 segregation ratio with a χ^2 value of 1.6 (P = 0.20). The F_2 plants also segregated in a 3:1 ratio for nonclustering fruit habit (χ^2 = 0.36; P = 0.50), with 21 plants bearing fruit in clusters and 54 plants bearing nonclustered fruit. These data indicate that single genes control these traits. Segregation data in the RIL population were included in molecular marker linkage analysis, with fruit shape (elongate) designated as fs_e, erect fruit habit as *up*, and fasciculate fruit clustering as *fa*. These were placed on the RIL linkage map as follows: fs_e on LG PP3, *fa* on LG PP2 (Chrom. 1), and *up* on LG PP7 (Chrom. 12 Noir).

Discussion

Two intraspecific linkage maps were constructed for pepper, with the aim of locating QTLs that control resistance to foliar blight and root rot caused by *P. capsici*. A low level of polymorphism was observed for all DNA markers between the 2 pairs of parental lines of our mapping populations. This level of polymorphism was lower than other reported DNA marker analysis in pepper (Kang et al. 2001; Lefebvre et al. 1995), but not unexpected for intraspecific crosses. We did not score faint or ambiguous polymorphic bands in the RAPD and AFLP analyses, and this certainly decreased the level of polymorphism detected. However, these maps were successfully used to locate QTLs with strong effects on resistance to *P. capsici*.

Table 5. Markers identified with 1-factor ANOVA having significant association ($P \leq 0.05$) with resistance to *Phytophthora capsici* root rot and foliar blight.

Marker[a]	Trait	R^2 (%)[b]	P[c]	LG
RIL map				
$F05a_{2545(PI)}$	PPc2r	12	0.002	ng
$AG08_{388(PI)}$	T3f	8	0.007	ng
$E33/M48_{259(PS)}$	T3r	9	0.003	ng
$E33/M49_{350(PI)}$	T3f	9	0.004	ng
	T2f	8	0.007	ng
$E37/M47_{178(PI)}$	T2r	10	0.002	PP13
$E37/M47_{250(PI)}$	Gpsr	8	0.01	ng
$E37/M47_{322(PI)}$	T2r	7	0.008	ng
	T3r	7	0.012	ng
$E37/M47_{525(PI)}$	T1f	7	0.009	ng
$E37/M49_{529(PI)}$	Gpsr	9	0.006	ng
	PPc2r	7	0.014	ng
$E38/M50_{386(PI)}$	GPsr	16	0.001	ng
$E40/M60_{521(PI)}$	T1r	7	0.01	ng
F_2 map				
$J12a_{2428(CM)}$	Mr	22	0.001	JC1
$E38/M50_{208(CM)}$	Mr	11	0.006	ng

Note: ng, marker not linked to any linkage group at LOD ≥2.0.
[a]Markers that were either placed on the map at LOD ≥2, or did not link at all. The parental source of each marker is in parentheses. PI, PI201234; PS, PSP-11; CM, CM334.
[b]Coefficient of determination, equivalent to the amount of variation explained by the marker.
[c]Probability value.

Linkage maps

One of the mapping populations used in this study is a set of recombinant inbred lines. This mapping population is very useful, because it enables the transfer of information from one laboratory to another. We were able to combine information from resistance studies carried out on this population in 2 locations (Taiwan and California). This is important because of restrictions on the transportation of plant pathogens across different geographic locations and for the confirmation of QTLs. The RIL population could also be used to develop a consensus linkage map for pepper that combines information from all published genetic linkage maps with common markers. Currently, we are able to assign chromosome numbers to some of the linkage groups on our maps because of the markers they share in common with published pepper linkage maps. Most of these were 10 SSR markers from the recently published pepper map of Lee et al. (2004). Others were RAPD, AFLP, and morphological markers. The *up* locus allowed us to assign LG PP7 of the RIL map to pepper chromosome 12: Noir (Lefebvre et al. 2002). LG PP1 of the RIL was assigned to chromosome 7 because of the presence of the SSR marker *HpmsCaSIG19*, and LG PP2 was assigned to chromosome 1 because of marker *Hpms1–43*. LGs JC10 and JC11 were both assigned

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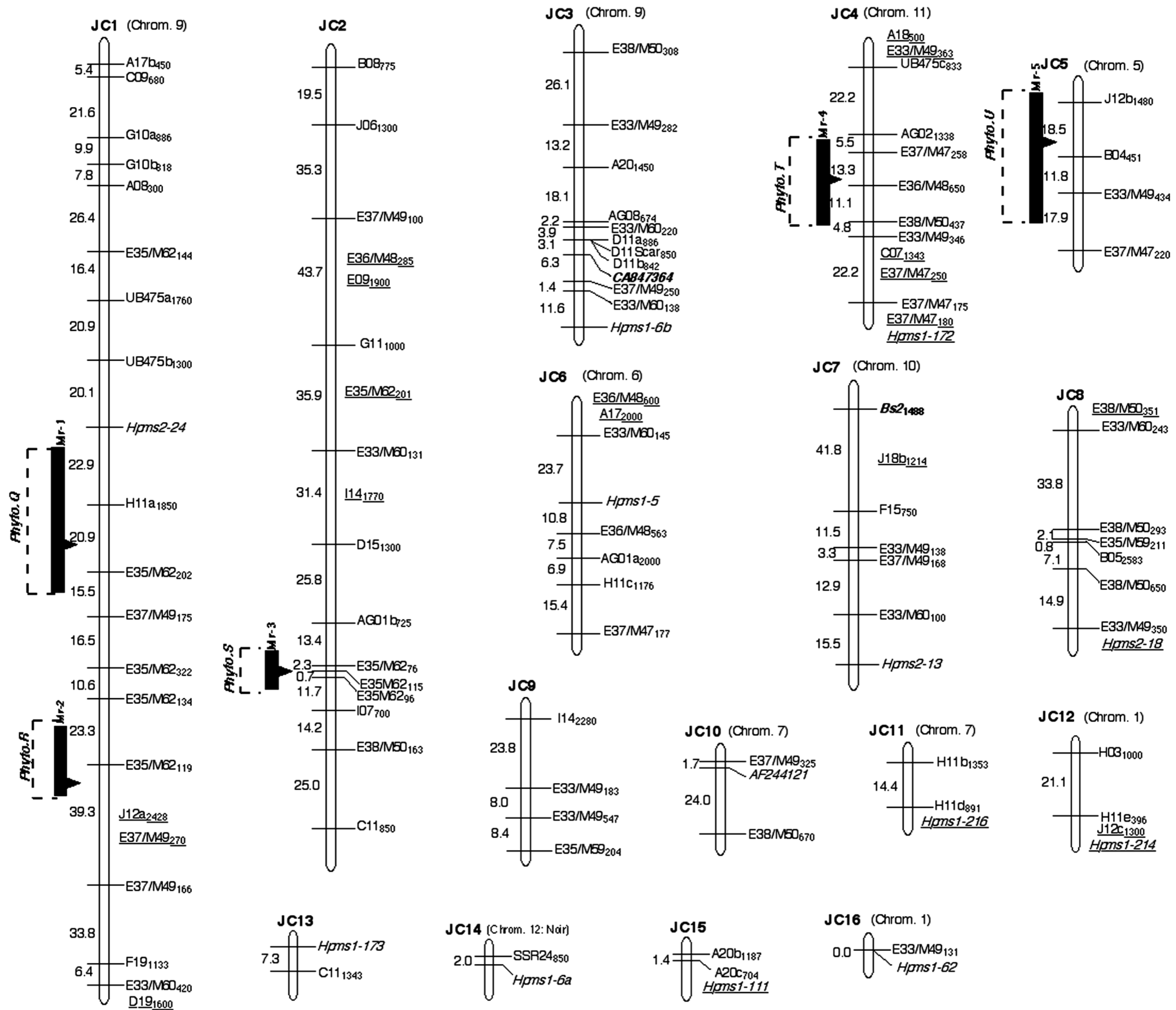
Fig. 3. The F_2 ('NuMex Joe E. Parker' ('JEP') × 'Criolo de Morelos 334' ('CM334')) pepper linkage map showing the positions of the QTLs that affects resistance to root rot caused by *P. capsici*. Assignment of numbers to linkage groups (JC) is random; names in parenthesis correspond to linkage groups of previously published pepper linkage maps. Genetic markers are to the right of each linkage group; genetic distances (cM) between adjacent markers are to the left. Markers in bold are candidate gene markers. Underlined markers were placed on the map at 2 ≤ LOD ≤ 2, and were not included in the QTL interval mapping analysis. Italicized markers are SSR markers in common with a previously published pepper linkage map. QTL intervals are represented with solid bars to the left of the linkage groups; the QTL symbols appear on top of the bars. Arrowheads on the bars show the approximate positions of the peak LOD score for the QTLs. The genomic regions where these QTLs fall were assigned *Phyto* names, as in Fig. 2.



to chromosome 7 of pepper because of the presence of markers *AF244121* and *Hpms1–216*, respectively. In addition, LGs JC12 and JC16 were assigned to pepper chromosome 1 because of markers *Hpms1–214* and *Hpms1–62*, respectively, and LGs JC1 and JC3 were assigned to pepper chromosome 9 because of markers *Hpms2–24* and $D11_{842}$, respectively. These LG pairs assigned to single chromosomes may coalesce in the future with additional markers on the F_2 map. Previously developed SSR markers (Lee et al. 2004) that were placed on our pepper maps are *AF244121* and *CM0005* (on LG PP1), *Hpms1–143* (PP6), *Hpms1–6a* (PP7 and JC14; Chrom. 12), *Hpms1–6b* and *Hpms2–41* (PP9; Chrom. 9), *Hpms2–18* (JC12), *Hpms1–173* (JC13), and *Hpms1–111* (JC15). SSR marker *Hpms2–13* was initially assigned at an LOD of 2 to chromosome 1. This marker is assigned to LG PP11 (Chrom. 11), 14.8 cM away from marker $F07_{1144}$, at LOD > 3. *Hpms2–13* is, therefore, probably on chromosome 11 of pepper.

It should be noted that there were more LGs in each of our linkage maps than the expected haploid chromosome number (12) of pepper. Some of these LGs may merge together as more markers are placed on the map. The current level of shared markers between the 2 maps does not allow us to develop a consensus map with a high level of confi-

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dence. This should be possible as more common markers are placed on each map.

Variations among *P. capsici* isolates and between root-rot and foliar-blight disease reactions

Variability was observed in resistance to the 6 *P. capsici* isolates used on the RIL population. Of the 16 genomic regions on the RIL linkage map that contain clusters of resistance QTLs arranged in *Phyto* groups, 7 regions had effects on resistance to at least 2 isolates and (or) conditioning of both root- and foliar-rot resistance. The remaining 9 were unique to individual isolates. The variability at the isolate level might result from the existence of different physiological races. Polach and Webster (1972) and Reifschneider et al. (1986) suggested that physiological races may exist within *P. capsici*. Oelke et al. (2003) confirmed this when they identified 9 different physiological races of *P. capsici* from 10 isolates collected from different locations. In addition, 14 physiological races of *P. capsici* were differentiated with a panel of pepper genotypes in a separate study that we conducted (unpublished). The presence of physiological races of this pathogen has great significance to the breeding of resistant pepper cultivars. Several QTLs with major effects were identified for most of the isolates in the RIL population, as well as in the F_2 population. Because all the *P. capsici* isolates used in this study were obtained from pure cultures, it can be concluded that resistance to *P. capsici* is polygenic.

The number of resistance QTLs detected for each isolate seems to correspond with the aggressiveness level of the isolates. The root-rot reaction of the RILs demonstrated that California isolate PPc2 was more aggressive than the other 5 isolates (Fig. 1). Six QTLs were detected for resistance to root rot of isolate PPc2, whereas other isolates had between 1 and 4 QTLs (Table 4). We also observed that isolate PPc2 is one of the most severe isolates in our collection, and it is insensitive to the chemical fungicide metalaxyl (unpublished data). In addition, Bartual et al. (1991) reported a significant correlation between the number of genes involved in the expression of resistance to *P. capsici* and the aggressiveness of the isolate used in the tests of resistance.

There was significant variation in the root-rot and foliar-blight disease syndromes caused by the 3 isolates from Taiwan, as demonstrated by the response of the RIL population. These differences were confirmed with a *t*-test analysis of 2 of the isolates. Although *t* tests did not detect significant difference between the means of the reaction of RILs to root-rot and foliar-blight disease syndromes caused by the third isolate (T2), the raw data showed that several RILs had differential reactions to the 2 disease syndromes of this isolate (data not shown). This result supports the earlier observations of Walker and Bosland (1999), that different genetic components confer resistance to root rot and foliar blight of *P. capsici*. This complex genetics of resistance can complicate breeding efforts, and attention has to be given to specific disease syndromes for effective breeding of resistant pepper cultivars.

P. capsici–resistance QTLs common to both maps

The F_2 linkage map yielded 5 QTLs controlling resistance to *P. capsici* root rot. The population was developed with 'CM334' serving as the resistant parent. This pepper accession is widely reported to have the strongest resistance to *P. capsici*. Of the 5 QTLs, *Phyto.Q* and *Phyto.U* on LGs JC1 (Chrom. 9) and JC5 (Chrom. 5) had the most significant effects on resistance (37.8 and 35.9, respectively). *Phyto.U* had the highest significance (LOD = 6.22) and highest additive effect (24.06). The peak LOD of *Phyto.U* is between 2 RAPD markers ($J12b_{1480}$ and $B04_{451}$). *Phyto.U* QTL corresponds with *Phyt1* (Grube et al. 2000), 1 of the 3 major QTLs detected across 3 intraspecific populations by Thabuis et al. (2003). This QTL, therefore, seems to be stable across *P. capsici* isolates, susceptible pepper genotypes, and different locations. A SCAR marker was recently developed for this QTL (renamed Phyto5.2) for use in marker-assisted selection (Quirin et al. 2005).

Phyto.U of the F_2 map and *Phyto.P* of the RIL map are probably the same QTL; the LGs on which they were detected were both assigned to chromosome 5 of pepper in this work. The remaining 4 resistance QTLs detected in the F_2 population could not be directly related with confidence to the QTLs detected in the RIL population, owing to the lack of common markers in the QTL regions. This mightchange as common markers are placed on the chromosomal regions harboring the QTLs. LG JC3 of the F_2 map and LG PP9 of the RIL map (both assigned to chromosome 9) share 3 markers in common. These markers are $D11_{842}$ (RAPD), $D11Scar_{650}$ (SCAR), and *Hpms1–6b* (SSR). *Phyto.M,* a QTL controlling resistance to foliar blight of isolate T2 and root rot of isolate PPc2, was detected on this chromosome on the RIL map. No QTL was detected on this chromosome on the F_2 map. However, marker *CA847364* was mapped within the clusters of the 3 common markers on chromosome 9 of the F_2 map. The *CA847364* marker is a responsive transcript (cDNA) obtained from *P. capsici*-challenged roots of *C. annuum* (acc. No. CA847364) (Richins, R., Alvarado, K., Leger, J. and O'Connell, M.A., 2002, unpublished data). Isolate M used on the F_2 population is probably different from isolates T2 and PPc2. This could account for the non-detection of a QTL in this orthologous region. The higher number of resistance QTLs detected in the RIL than in the F_2 population is probably related to the higher number of isolates used in testing the former. The RIL population affords the opportunity for multiple testing and replications. In view of the importance of the 'CM334' pepper accession in breeding for resistance to *P. capsici* and the presence of physiological races of this pathogen, we have begun the development of RILs for this accession. This will enable a detailed dissection of the different resistance factors in 'CM334' and help plant breeders track these QTLs.

Digenic epistatic interactions of *P. capsici*-resistance QTLs

One additive × additive epistatic interaction was observed in the RIL population between a pair of QTLs that control resistance to *P. capsici* in pepper. The RIL population was at the F_7 generation, where homozygosity was expected to be near unity, thereby precluding the calculation of dominant genetic effects. Therefore, dominance × dominance and additive × dominance epistasis could not be evaluated for QTLs controlling resistance to root rot and foliar blight of *P. capsici* in the RIL population. This means that there are probably

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more epistatic effects among the *P. capsici*-resistance factors in pepper than observed in this study. The presence of epistatic effects among resistance QTLs have a bearing on breeding programs designed to develop pepper varieties with resistance to *P. capsici*. Previous genetic analyses of resistance to *P. capsici* have indicated the presence of epistatic effects (Bartual et al. 1991, 1994). Also, Lefebvre and Palloix (1996) and Thabuis et al. (2003) reported digenic epistatic interactions among QTLs controlling various components of *P. capsici* resistance in pepper.

Potential for marker-assisted breeding of pepper with resistance to *P. capsici*

Molecular markers reported here should aid pepper breeders in marker-assisted breeding of *P. capsici*-resistant varieties. These markers include the ones bounding genomic regions on which resistance QTLs have been detected, as well as those obtained by simple marker regression analysis (1-factor ANOVA). Some resistance QTLs are nonadditive (Table 4). However, both additive and nonadditive (with favorable epistatic interaction with additive markers) QTL markers should be considered in marker-assisted selection. The possibility of favorable epistasis occurring not only between alleles of the same parent but also between alleles from different parents underscores the influence of genetic background on the susceptible parent. The loss of such nonadditive but favorable gene combinations can be responsible for the difficulties in introgression of resistance factors into susceptible cultivars through backcrossing (Lefebvre and Palloix 1996). Thabuis et al. (2001, 2004) demonstrated the value of marker-assisted selection using molecular markers linked to *P. capsici*-resistance QTLs in backcross and recurrent selection breeding programs. The availability of many markers associated with resistance to *P. capsici*, as we have reported in this study, is expected to give breeders a plethora of options to choose from. This is of importance because breeders work with populations that come from diverse genetic backgrounds. If a reported QTL marker is not polymorphic in the population a breeder is interested in, that marker is useless to his program. Although a majority of our resistance QTL markers are AFLPs and RAPDs, some of them are SSRs, and 1 is a SCAR. Primer sequence information is available for this set of markers, and efforts are underway to convert the other AFLP and RAPD markers of importance to specific SCAR markers.

Conclusion

The use of 2 intraspecific mapping populations of pepper, the different isolates of *P. capsici*, and the evaluation of 2 disease syndromes, root rot and foliar blight, led to the detection of several QTLs in pepper genome that confer resistance to *P. capsici*. It is hoped that the detection of these QTLs and the PCR-based DNA markers closely linked to them will facilitate breeding efforts designed to develop resistant pepper cultivars.

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