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ORIGINAL PAPER

G. L. Coaker · D. M. Francis

Mapping, genetic effects, and epistatic interaction of two bacterial canker resistance QTLs from *Lycopersicon hirsutum*

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Abstract Two quantitative trait loci (QTL) from *Lycopersicon hirsutum*, Rcm 2.0 and Rcm 5.1, control resistance to *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). To precisely map both loci, we applied interval mapping techniques to 1,056 individuals in three populations exhibiting F_2 segregation. Based on a 1-LOD confidence interval, Rcm 2.0 mapped to a 14.9-cM interval on chromosome 2 and accounted for 25.7–34.0% of the phenotypic variation in disease severity. Rcm 5.1 mapped to a 4.3-cM interval on chromosome 5 and accounted for 25.8–27.9% of the phenotypic variation. Progeny testing of recombinant plants narrowed the QTL location for Rcm 2.0 to a 4.4-cM interval between TG537-TG091 and to a 2.2-cM interval between CT202-TG358 for Rcm 5.1. A population of 750 individuals exhibiting F_2 segregation was used to detect epistasis between both loci using ANOVA and orthogonal contrasts (P=0.027), suggesting that resistance was determined by additive gene action and an additive-by-additive epistatic interaction. A partial diallel mating design was used to confirm epistasis, advance superior genotypes, randomize genetic backgrounds, and create recombination opportunities. This crossing scheme created a more balanced population (n=112) containing the nine F_2 genotypic classes. Parents in the diallel were selected from the previous population based on resistance, genotype at the Rcm 2.0 and Rcm 5.1 loci, and horticultural traits. A replicated trial using the diallel population confirmed additive-by-additive epistasis (P<0.0001). These results validate the gene action, intra -locus interaction, and map position of two loci controlling resistance to *Cmm*.

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G. L. Coaker · D. M. Francis (✉)
Department of Horticulture and Crop Science,
The Ohio State University,
Ohio Agricultural Research and Development Center,
1680 Madison Ave., Wooster, OH 44691, USA
e-mail: Francis.77@osu.edu
Tel.: +1-330-263-3893
Fax: +1-330-263-3887

Introduction

Bacterial canker of tomato, caused by the gram-positive bacterium *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), is a devastating disease that proliferates in the xylem vessels of infected plants. The complex symptoms of bacterial canker include leaflet necrosis, unilateral leaf wilt, open stem cankers, plant defoliation, and plant death (Gleason et al. 1993). Fruit symptoms are characterized by small, raised, necrotic spots surrounded by a white halo and can contribute to economic losses beyond that attributed to decreased yield (Medina-Mora et al. 2001).

Although potential sources of resistance to *Cmm* have been identified, the use of uncharacterized bacterial isolates and a poor understanding of the genetic basis of resistance have hampered breeding progress. Resistant accessions have been found within the wild species of tomato—in particular, the green-fruited species *Lycopersicon hirsutum* Humb. and Bunpl. (Francis et al. 2001) and *L. peruvianum* (L.) Miller accession LA2157 (Sandbrink et al. 1995; Van Heusden et al. 1999). *L. hirsutum* accession LA407 is resistant to genetically diverse *Cmm* strains, and the magnitude of resistance appears to be similar to that of *L. peruvianum* (Francis et al. 2001).

Two quantitative trait loci (QTL) controlling resistance to *Cmm* from *L. hirsutum* LA407, Rcm 2.0 on chromosome 2 and Rcm 5.1 on chromosome 5, were identified using single marker analysis. These loci were introgressed into a *L. esculentum* Mill. genetic background using inbred backcross breeding (Kabelka et al. 2002). We hypothesized that both loci confer resistance through different mechanisms (Coaker et al. 2002; Kabelka et al. 2002). Rcm 2.0 and a QTL from LA407 that controls an increased rate of stem vascular development and maturation both map to the distal portion of chromosome 2 (Coaker et al. 2002). Lines carrying the LA407 QTL on chromosome 2 appear to have a more rapid maturation of the vascular tissue. A longer incubation period and decreased rate of symptom progression have been documented for bacterial canker with increasing plant age (Chang et al. 1992; Forster and Echandi 1973). Thus, it is

appealing to hypothesize that this locus confers resistance to *Cmm* due to a faster maturing and therefore more resistant vascular system. Rcm 5.1, on the other hand, is linked to the *Pto* resistance gene cluster on chromosome 5, and this locus was identified using a resistance gene analog molecular marker (Kabelka et al. 2002). Therefore, we hypothesized that Rcm 5.1 may lie within the *Pto* cluster and may control resistance to *Cmm* by mounting a classical resistance gene-mediated defense response. Ultimately it will be necessary to clone Rcm 2.0 and Rcm 5.1 in order to test mechanistic hypotheses.

The availability of high-resolution genetic maps, large-insert genomic libraries, and suitable mapping populations has enabled map-based cloning of qualitative traits in various plant species. The feasibility of quantitative trait map-based cloning has been established in tomato, Arabidopsis, and rice. In tomato, the QTLs *fw*2.2 (Alpert and Tanksley 1996; Frary et al. 2000) controlling fruit size, OVATE (Liu et al. 2002) controlling the transition from round to pear-shaped fruit, and *Brix*9-2-5 (Fridman et al. 2000) affecting fruit total soluble solids content have been cloned using a map-based approach. Rcm 2.0 and Rcm 5.1 control a large portion of the phenotypic variance for *Cmm* resistance, and we have developed a *L. hirsutum* LA407 large-insert transformation competent artificial chromosome (TAC) library (Qu et al. 2003). The LA407 TAC library will permit chromosome walking towards the genes underlying Rcm 2.0 and Rcm 5.1, and it should therefore be feasible to clone both loci following the development of a sufficiently detailed genetic map.

In the investigation reported here, we fine-mapped two QTL from LA407 controlling resistance to *Cmm* on chromosome 2 (Rcm 2.0) and 5 (Rcm 5.1). We also demonstrate that Rcm 2.0 and Rcm 5.1 interact epistatically. Progeny from recombinant individuals surrounding Rcm 2.0 and Rcm 5.1 were used to confirm the map position and narrow the marker intervals controlling resistance. These markers and recombinant individuals can be used in marker-assisted selection and as a starting point for the eventual map-based cloning of the genes underlying Rcm 2.0 and Rcm 5.1.

Materials and methods

Plant material and populations

Population design

Three populations were used to map Rcm 2.0 and Rcm 5.1. The 2361 and 2353 F_2 populations consisted of 148 and 158 individuals and segregated for Rcm 5.1 and Rcm 2.0, respectively. These populations were previously described in detail (Kabelka et al. 2002). Briefly, both populations were derived from crosses between inbred backcross line (IBL) 2361 (Rcm 5.1) or IBL 2353 (Rcm 2.0) to *Lycopersicon esculentum* cv. Ohio 86120. All three populations used to map the QTL are described in Fig. 1. The lines 61037 and 53025 were selected from the 2361 and 2353 F_2 populations and selfed to develop F_4 lines. Selections (F_4) were crossed to Ohio 9242, and the resulting F_1s were intercrossed (Fig. 1). Heterozygotes for markers surrounding both Rcm 2.0 and Rcm 5.1 were selected and selfed to generate a population with F_2 segregation for both loci (n=750) in 2001.

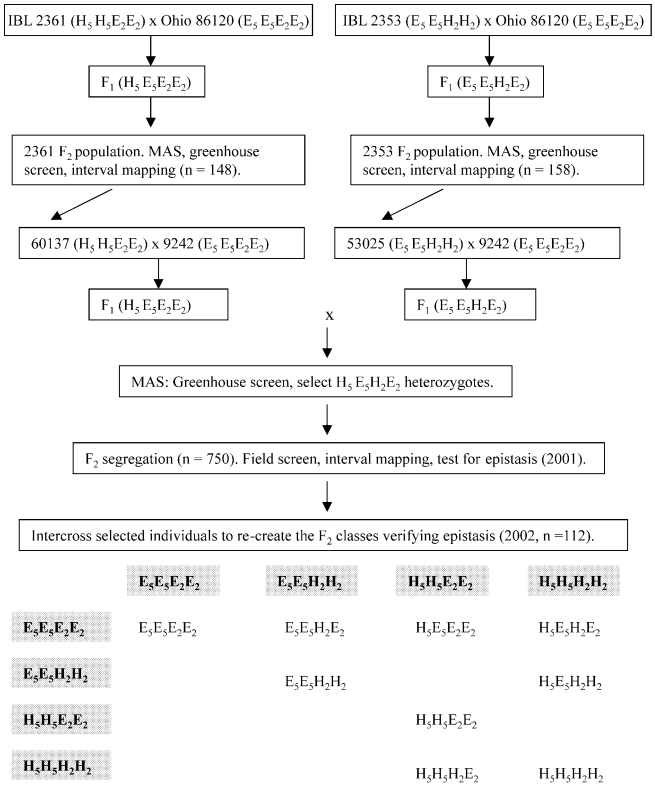

Fig. 1 Simultaneous breeding and mapping of QTLs for *Cmm* resistance. *H*, *E* are alleles from *Lycopersicon hirsutum* LA407 and *L. esculentum*, respectively, *subscripts* denote QTL Rcm 2.0 (*2*) and QTL Rcm 5.1 (*5*). *IBL* indicates inbred backcross line, *MAS* indicates marker-assisted selection. Lines 61094 and 53025 are F_4 individuals selected from the 2361 and 2353 F_2 populations and contain Rcm 5.1 and Rcm 2.0, respectively. Parental genotypes used to re-create the F_2 genotypic classes are shown in *gray boxes*

To verify and narrow the statistical map position for both loci, we carried out progeny testing. Heterozygous recombinant individuals were identified within the 2353 and 2361 F_2 populations. F_4 families were derived from individual F_2 recombinants surrounding Rcm 2.0 and Rcm 5.1. Recombinant plants were self-pollinated, and the two homozygous genotypic classes were selected for further analysis such that each family consisted of two genotypic classes. Eight F_4 families were analyzed for resistance due to Rcm 2.0, and 13 F_4 families were analyzed for resistance due to Rcm 5.1, with 10–12 individuals sampled per genotypic class.

To confirm epistasis detected in 2001 between Rcm 2.0 and Rcm 5.1, we crossed selections from the large F_2 population that were homozygous for either Rcm 2.0, Rcm 5.1, both loci, or neither locus in a partial diallel to re-create the nine possible F_2 genotypic classes (Fig. 1). For crosses resulting in more than 12 independent hybrids per genotype, random hybrids were selected for subsequent evaluation. A set of these hybrids was evaluated for resistance in the field during 2002 (n=112; 11–14 distinct hybrids per genotypic class).

Field and greenhouse design

The 2361 and 2353 F_2 populations and F_4 recombinant families were evaluated in the greenhouse in a completely randomized design (Kabelka et al. 2002). The 2001 population was grown in a completely randomized design in the field, and the 2002 population

was grown in a randomized complete block design with two replications also in the field. Trials for both years were conducted in Wooster, Ohio. Selections and disease severity ratings were based on individual plants for the 2361 and 2353 F_2 populations, F_4 recombinant families, and the 2001 field population. In 2002, selection and disease severity ratings were based on plot means. Each trial included *L. esculentum* cvs. Ohio 86120, Ohio 9242 and Ohio 88119 (all susceptible checks), Heinz 9144 (moderately resistant check), and IBLs 2353 and 2361 (resistant checks).

Inoculum preparation and disease evaluation

Two strains of *Cmm* obtained from naturally infected tomato plants in Ohio, USA were used in this study. Both strains had been previously characterized by Rep-PCR and belong to the A and C DNA fingerprint classes, respectively (Francis et al. 2001; Louws et al. 1998). A loop full of *Cmm* A226 and C290 cells was transferred to two separate flasks containing 5 ml of nutrient broth yeast extract without glucose (NBY; Hausbeck et al. 2000; Schaad 1980) and incubated at 25°C, 250 rpm, for 48 h. Each 5-ml culture was then inoculated into 1 l of NBY and incubated at 25°C, 250 rpm, for 24 h. The cells were pelleted at 10,000 rpm, 4°C, for 10 min, resuspended once in ddH_2O, centrifuged at 10,000 rpm, 4°C, for 10 min, and the remaining pellet resuspended in 25 ml ddH_2O. The bacterial concentrations were estimated by measuring the transmittance of a 1:4 dilution using a Beckman DU 640 spectrophotometer. By adjusting the cell concentration to 50% transmittance at 600 nm an approximate concentration of 3×10^8 cells/ml was obtained.

Populations undergoing field disease screens were spray-inoculated until runoff using a Preval Spray gun (Preval Sprayer, Yonkers, N.Y.) 1 week prior to transplanting in the field. Populations undergoing disease screens in the greenhouse were inoculated by petiole clip 2 weeks after transplanting. The second fully expanded true leaf was cut at a 45° angle, and 20 µl of inoculum was placed onto the cut surface.

Disease was rated on a weekly basis for a total of 8 weeks beginning 2 weeks post-inoculation in the greenhouse and 2 weeks post-transplant in the field. An additive scale was employed, with 0 indicating no visible symptoms and 5 indicating death. Plants were assessed for the presence of stem canker, secondary canker, wilt, and collapsed stems (Francis et al. 2001). For each individual or plot, disease progression was assessed on the basis of percent maximal disease (PMD). PMD is similar to the area under the disease progression curve, with the exception that the 0–5 scale is adjusted to 0–100%. PMD was calculated as:

$$PMD = 100\sum_{i=1}^{n-1}[(D_{i+1}+D_{i+1})/2][T_{i+1}-T_i]/[(T_n-T_1)/M]$$

Where n = total number of observations, D_i= individual disease rating score at the ith observation, T_i = time (days) at the ith observation, and M = maximum score of the disease rating scale (Francis et al. 2001).

Evaluation of parthenocarpy and seed set

L. hirsutum LA407 DNA surrounding Rcm 5.1 is associated with parthenocarpic fruit (Kabelka et al. 2002). Fruit that set without fertilization are small and seedless. To identify the marker interval controlling parthenocarpy, we evaluated homozygous F_3 recombinant families derived from the 2361 F_2 population for fruit size, fruit number, and total seed set. Seed set per plant provided a normally distributed quantitative scale by which to evaluate families for the expression of parthenocarpy. A total of 13 informative recombinant families were evaluated in the greenhouse, with two to four individuals sampled per genotypic class.

Marker generation, map construction, and QTL position

Marker analysis

Restriction fragment length polymorphism (RFLP) markers flanking Rcm 2.0 and Rcm 5.1 were converted to PCR-based cleaved amplified polymorphic sequence (CAPS) or indel (insertion/deletion) markers (Konieczny and Asubel 1993). Sets of PCR primers were generated from DNA sequences of genomic or cDNA clones associated with RFLP loci. Restriction-endonuclease detectible polymorphisms between *L. hirsutum* LA407 and *L. esculentum* cv. Ohio 86120 were determined by sequencing PCR products derived from each parent. Creation of the TAC-end clone marker, TACL2, has been described previously (Qu et al. 2003). Marker sequences, PCR conditions, and specific restriction endonucleases used to genotype all populations are shown in Table 1. Electrophoresis of molecular markers TG537, CT59ST, and TG337 was conducted on a 4% agarose gel, while all other markers were run on a 2% agarose gel. DNA was extracted from 4-week old seedlings using a mini CTAB procedure (Kabelka et al. 2002).

Map construction

Populations were genotyped with the molecular markers shown in Table 1, and a genetic linkage map was constructed with the computer program JOINMAP v. 3.0 (Van Ooijen and Voorrips 2001). Recombination fractions were converted to map distances in centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944).

QTL positioning

The populations used in this study segregated for *L. hirsutum* LA407 DNA surrounding only Rcm 2.0 and Rcm 5.1, and the *L. esculentum* parents used in population development are closely related. Therefore, composite interval mapping (CIM) was not possible due to the lack of unlinked segregating markers. Simple interval mapping (SIM) analysis, using the computer program MAPQTL v. 4.0 (Van Ooijen et al. 2002), was utilized to identify marker intervals on chromosomes 2 and 5 containing QTLs for *Cmm* resistance. To identify the appropriate threshold LOD score for QTL detection, given the population size and number of markers used, we conducted a 1,000× permutation test (Churchill and Doerge 1994) using the MAPQTL v. 4.0 computer program. The permutation test resulted in LOD threshold values of 3.5 and 3.0 for the 2353 and 2361 F_2 populations, respectively, and 3.4 for the large (n=750) 2001 field population. QTL likelihood plots were constructed from the LOD scores obtained in SIM analysis with the computer program MAPCHART v. 2.1 (Voorrips 2002).

Statistical analysis

Verification of QTL position and seed set using recombinant families

Recombinant F_4 families for Rcm 2.0 and Rcm 5.1 were evaluated for resistance to *Cmm*. Recombinant F_3 families for Rcm 5.1 were also evaluated for seed set as described above. Analysis of variance (ANOVA) and mean separations for genotypic differences were performed with the SAS GENERAL LINEAR MODELS (GLM; SAS Institute, Cary, N.C.) procedure. Mean separations were based on the least significant difference (LSD) only after a significant F test was detected. The statistical model for genotypic differences within a family was:

$$X_{ij} = \mu + G_i + F_j + \varepsilon_{ij}$$

where X_{ij} = phenotypic value for the ith genotype in the jth family, μ = population mean, G_i = the effect of the ith genotype, F_j = the effect of the jth family, and ε_{ij}= residual error term.

Table 1 Molecular markers and PCR conditions

Marker	Primer	Annealing Temp (°C)	Cycles	Size[a] (bp)	Enzyme	Chromosome
TG337	F 5′-TCCACAGTTATTGCTTCTTGTTTC-3′ R 5′-GGGTGTGTCTGTTTGACAGC-3′	57	36	450	*Mnl*I	2
TG537	F 5'TACCCGAGGCTCAGAAACAC-3′ R 5′-CATCAACAGGAGATCGGTTTT-3′	57	36	446	*Hin*fI	2
TG492	F 5′-TGGAGAAGGTTCAAAGGGAACG-3′ R 5′-GGGCCAAGGAATATTTCTCAAGG-3′	57	36	900	*Mnl*I	2
TG091	F 5′-TGCAGAGCTGTAATATTTAGAC-3′ R 5′-CFFTCTCAGTTGCAACTCAA-3′	60	36	600	*Dra*I	2
CT059	F 5′-TTGTAGTGGCAAGTGTCACGGG-3′ R 5′-TCATATCGGGAGAAGGTGCGTG-3′	60	36	400 &390	None	2
CT094	F 5′-GGGAAAAACTGGCAGCTAA-3′ R 5′-TGCCCAATTATGTGGCAGTA-3′	57	36	1000	*Dra*I	2
TG620	F 5′-CTCTGTGCCAGAGCTCGAA-3′ R 5′-TTTTACCTGGCGGAGAACTG-3′	57	36	503	*Eco*RI	2
TG441	F 5′-TGTCAGCATAGGCTTTTCCA-3′ R 5′-CGGTCGGGAAAAATGACA-3′	60	36	550	*Rsa*I	5
TPT	F 5′-ATCCCCGAGGGTAGGATATG-3′ R 5′-GGATAGAACGCGCCTACTTG-3′	57	38	600	*Rsa*I	5
CD064	F 5′-CCCATCAGCATCAGCTACAG-3′ R 5′-ATAACACCGGTGCTTCAGGA-3′	60	36	550	*Mnl*I	5
TG503	F 5′-GGCTTCAGAATCCCTAACCA-3′ R 5′-GATTTTGGGGCAAATCAAGA-3′	57	36	600	*Hin*fI	5
CD031	F 5′-ATCTCGGGATCATGGTTGAC-3′ R 5′-ATFFCCAFAGAAATTCCAAA-3′	57	33	501	*Hin*fI	5
TACL2	F 5′-GAGCGGACATTAGAGCGAGA-3′ R 5′-TGTGGGTAGCTGAAGGGTTC-3′	57	36	550	*Mnl*I	5
PTO	F 5′-ATCTACCCACAATGAGCATGAGCTG-3′ R 5′-GTGCATACTCCAGTTTCCAC-3′	62	36	700	*Rsa*I	5
TG538	F 5′-CCAAGTGCAGAGAGTACTGGA-3′ R 5′-TGAATGAACATGATCAAAGTATGC-3′	55	36	1500	*Rsa*I	5
CT202	F 5′-TAATCCGAGAAGGTGATCCG-3′ R 5′-GGCTTATAACCCATGCCAAAAG-3′	60	35	180	*Tsp*45I	5
TG318	F 5′-CAAGCCATAGAAATTGCCGTA-3′ R 5′–TGCTCTCTCTGTGATGGAAGC-3′	57	38	580	None	5

[a] Size of undigested PCR product

Detection of epistasis using two-way ANOVA

Data from the 2001 and 2002 field were analyzed using the SAS GLM procedure for ANOVA and mean comparisons of genotypes (SAS Institute). The statistical model used to detect the main effects of either locus and epistasis in 2001 was

$$X_{ij} = \mu + M1_i + M2_j + \left(M1_i * M2_j\right) + \varepsilon_{ij}$$

where X_{ij} = the phenotypic value for the genotype in the i^{th} and j^{th} marker classes, μ is the population mean, $M1_i$ = the effect of the i^{th} marker on chromosome 5, $M2_j$ = the effect of the j^{th} marker on chromosome 2, and ε_{ij} = the residual error term. Degrees of freedom were calculated by default with the Satterthwaite option (Neter et al. 1990). The appropriate F test for epistasis between molecular markers was

$$\left(MSM1_i * M2_j\right) / \left(MS\varepsilon_{ij}\right)$$

The statistical model used to detect the main effects of either locus and epistasis in 2002 was

$$X_{ijkl} = \mu + M1_l + M2_j + B_k + M1_i * M2_j + M1_i * B_k + M2_j * B_k + G_l\left(M1_i * M2_j\right)$$

where X_{ijkl} = phenotypic value for the lth genotype in the kth block with the ith and jth marker classes, μ = population mean, $M1_i$ = the effect of the ith marker on chromosome 5, $M2_j$ = the effect of the jth marker on chromosome 2, B_k = the effect of the kth block, G_l = the effect of the lth genotype, and ε_{ijkl} = the residual error term. Degrees of freedom were calculated via the Satterthwaite option (Neter et al. 1990). The appropriate F test for epistasis between molecular markers was

$$\left[\left(MSM1_i * M2_j\right) + \left(MS\varepsilon_{ijkl}\right)\right] / \left[\left(MSG_i\left(M1_i * M2_j\right)\right) + \left(MSB_k\left(M1_i * M2_j\right)\right)\right]$$

The phenotypic variance due to Rcm 2.0, Rcm 5.1, and epistasis was estimated from the variance components for each term in the statistical model using the SAS PROCMIXED (SAS Institute) procedure with restricted maximum likelihood specified.

Detection of epistasis using orthogonal contrasts

To verify the gene action of each locus and test for all types of diallel epistatic interactions, we employed orthogonal contrast mean separation statistics (Cockerham and Zeng 1996; Table 2). The F test used to detect epistasis with orthogonal contrasts is only appropriate for completely balanced data sets. A T-test was conducted to test for all contrasts individually such that each contrast had one degree of freedom, where $T^2 \approx F$.

Table 2 Orthogonal contrast codes used to determine gene action and type of epistasis. The alleles denoted H and E are from *LA407* and *Lycopersicon esculentum*, respectively; subscripts denote QTL Rcm 2.0 (2) and Rcm 5.1 (5). The final disease rating in 2001 and 2002 was used for orthogonal contrast analysis

Contrast[a]	H_2H_5 H_2H_5	H_2H_5 H_2H_5	H_2E_5 H_2E_5	H_2H_5 E_2H_5	H_2H_5 E_2E_5	H_2E_5 E_2E_5	E_2H_5 E_2H_5	E_2H_5 E_2E_5	E_2E_5 E_2E_5	2001 *P*-value	2002 *P*-value
A Rcm 2.0	–1	–1	–1	0	0	0	1	1	1	<0.0001	<0.0001
D Rcm 2.0	1	1	1	–2	–2	–2	1	1	1	0.005	1
A Rcm 5.1	–1	0	1	–1	0	1	–1	0	1	<0.0001	<0.0001
D Rcm 5.1	1	–2	1	1	–2	1	1	–2	1	0.400	1
$A_2 \times A_5$	1	0	–1	0	0	0	–1	0	1	0.027	0.001
$A_2 \times D_5$	–1	2	–1	0	0	0	1	–2	1	0.250	1
$D_2 \times A_5$	–1	0	1	2	0	–2	–1	0	1	0.050	1
$D_2 \times D_5$	1	–2	1	–2	4	–2	1	–2	1	0.800	1

[a] A indicates additive gene action; D indicates dominant gene action

Results

Genetic linkage map, QTL identification, and QTL validation

Kabelka et al. (2002) identified Rcm 2.0 and Rcm 5.1 by single marker analysis and introgressed these QTL into an *L. esculentum* genetic background. We placed additional molecular markers surrounding both loci onto three populations containing a total of 1,056 plants exhibiting F_2 segregation to construct genetic linkage maps and position QTL. The genetic linkage map surrounding Rcm 5.1 was created using the 2361 F_2 population (*n*=148), which exhibited segregation for Rcm 5.1 only, and a large 2001 field population (*n*=750) that exhibited segregation for both loci. Ten molecular markers on chromosome 5 were placed onto both populations, spanning 14.0 cM (the approximate limit of the *L. hirsutum* LA407 introgression), with an average distance of 1.4 cM between markers (Fig. 2). CAPS marker TG441 segregated only in the 2361 F_2 population and not in the larger population due to a recombination event. Thus, TG441 is not included in the genetic linkage map of Rcm 5.1 illustrated in Fig. 2.

The genetic linkage map surrounding Rcm 2.0 was constructed using the 2353 F_2 population (*n*=158) that segregated for Rcm 2.0 only and the 2001 field population (*n*=750) segregating for both loci. Seven molecular markers on chromosome 2 were placed onto both populations, spanning a distance of 19.9 cM, with an average distance of 2.84 cM between markers (Fig. 2). The order of the markers agreed with the high-density RFLP map of tomato previously constructed using an *L. esculentum* × *L. pennellii* F_2 population (Pillen et al. 1996).

Marker order, genetic distance, and QTL location were similar across populations. The QTL results for the largest mapping population (2001 field, *n*=750) are shown in Fig. 2. Rcm 2.0 accounted for 25.7–34.0% (LOD>4) and Rcm 5.1 accounted for 25.8–27.9% (LOD >4) of the

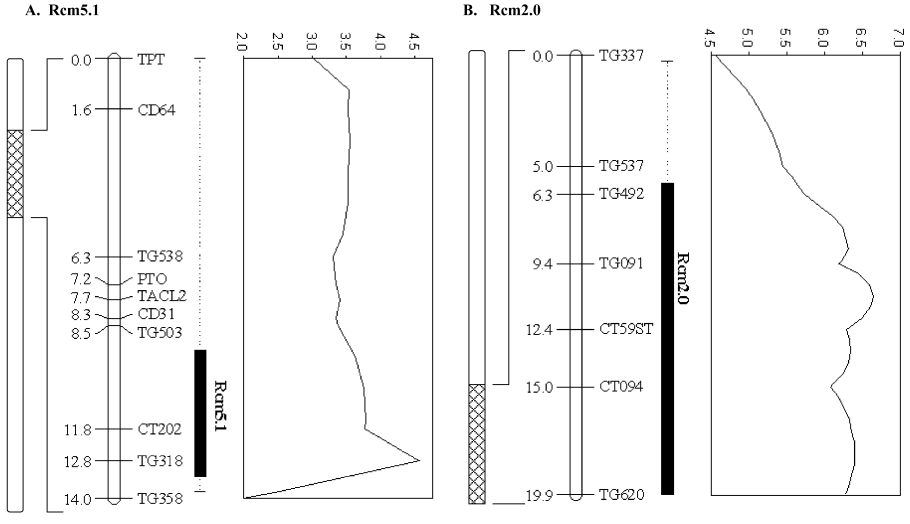

Fig. 2 A linkage map of chromosome 5 (**A**) and 2 (**B**). *Hatched lines* Region of *LA407* introgression for each chromosome. The names of the markers and the map distance between them are shown to the *right* and *left* of the introgressed region, respectively. The LOD (log 10 of the odds ratio) plots for PMD (percent maximal disease) to the *right* of the chromosomes indicate the most likely positions of QTLs controlling resistance. The 1- and 2-LOD intervals are displayed as *black bars* and *dotted lines*, respectively

Table 3 Experimental verification of Rcm 2.0's genetic location. Molecular markers used in the genotypic analysis are shown in the first row. (*PMD* percent maximal disease)

Family	TG337	TG537	TG492	TG091	CT059	CT094	TG620	PMD[a]
1	H[b]	H	H	H	E	E	E	10.5a
1	E	E	E	E	E	E	E	26.1b
2	E	H	H	H	E	E	E	7.9a
2	E	E	E	E	E	E	E	16.5b
3	E	H	H	H	H	H	H	12.5a
3	E	E	E	E	H	H	H	22.4b
4	E	E	E	E	H	H	H	31.1a
4	E	E	E	E	E	E	E	29.2a

[a] Means followed by different letters are significantly different
[b] H indicates *LA407* DNA, E indicates *L. esculentum* DNA

Table 4 Experimental verification of Rcm 5.1's genetic location and the genetic location associated with parthenocarpic fruit. Molecular markers used in genotypic analysis are shown in the first row (*PMD* percent maximal disease)

Family	TG441	TPT	CD64	TG538	PTO	TACL2	CD31	TG503	CT202	TG318	TG358	PMD[a]	Seed[a]
1	H[b]	H	H	H	H	H	H	H	H	H	H	7.2a	66.0a
1	H	E	E	E	E	E	E	E	E	E	E	20.3b	244.0b
2	H	H	H	H	H	H	H	H	H	H	H	7.6a	53.7a
2	H	H	H	E	E	E	E	E	E	E	E	20.0b	300.0b
3	E	E	E	H	H	H	H	H	H	H	H	9.5a	126.0a
3	E	E	E	E	E	E	E	E	E	E	E	28.5b	300.0b
4	E	E	E	H	H	H	H	H	H	H	H	10.6a	66.0a
4	H	H	H	H	H	H	H	H	H	H	H	7.7a	58.5a
5	H	H	H	E	E	E	H	H	H	H	H	11.2a	300.0a
5	E	E	E	E	E	E	E	E	E	E	E	19.3b	300.0a
6	H	H	H	H	H	H	H	H	H	H	H	11.2a	3.5a
6	H	H	H	E	E	E	H	H	H	H	H	6.2a	169.0a
7	H	H	H	H	H	H	H	H	H	H	H	6.7a	13.0a
7	E	E	E	E	E	E	E	E	H	H	H	4.8a	250.5b
8	E	E	E	E	E	E	E	E	H	H	H	12.3a	192.5a
8	E	E	E	E	E	E	E	E	E	E	E	20.1b	258.6a

[a] Means followed by different letters are significantly different
[b] H indicates *LA407* DNA; E indicates *L. esculentum* DNA

phenotypic variation across populations. The 1-LOD confidence interval for Rcm 2.0 spanned 14.9 cM between markers TG537-TG620, while the 1-LOD confidence interval for Rcm 5.1 spanned 4.3 cM between markers TG503-TG358.

The precise genomic location of Rcm 2.0 and Rcm 5.1 was determined by progeny-testing recombinant plants within the 2353 and 2361 F_2 populations. Heterozygous F_2 recombinant plants were self-pollinated, and the two homozygous classes present in each F_3 family were selected and further selfed. Homozygous F_4 families were then screened for the presence or absence of resistance to *Cmm*, and comparisons were made between genotypic classes within each family. Families containing *L. hirsutum* LA407 DNA for the 4.4-cM TG537-TG091 marker interval surrounding Rcm 2.0 retained *Cmm* resistance, while families lacking LA407 DNA for this interval did not. These results are summarized by family in Table 3. The analysis of recombinant progeny verified the genomic location of Rcm 2.0 and narrowed the marker interval controlling *Cmm* resistance from 14.9 cM to 4.4 cM. Families containing LA407 DNA for the 2.2-cM CT202-TG358 marker interval surrounding Rcm 5.1 retained resistance to *Cmm*, while families lacking LA407 DNA for this interval were susceptible to *Cmm* (Table 4). The analysis of recombinant progeny also verified the genomic location of Rcm 5.1 and narrowed the marker interval controlling resistance from 4.3 cM to 2.2 cM. Thus, Rcm 5.1 is not a member of the *Pto* cluster and is located 4.6–4.1 cM distal to molecular markers based on *Pto* and *Prf* sequences. The results obtained from progeny testing of recombinant plants clearly position Rcm 2.0 between TG537 and TG091 and Rcm 5.1 between CT202 and TG358.

Progeny testing of recombinant plants was also employed to identify the genomic region linked to Rcm 5.1 associated with parthenocarpic fruit. The use of an IBC breeding strategy to introgress segments of *L. hirsutum* LA407 DNA into an *L. esculentum* genetic background has eliminated many negative horticultural attributes inherited from LA407. However, Rcm 5.1 was associated with parthenocarpic fruit and small fruit size (Kabelka et al. 2002). Heterozygous recombinant plants within the 2361 F_2 population were self-pollinated to generate F_3 families. Fruit from homozygous individuals within each F_3 family was analyzed for seed set. Families containing *L. hirsutum* LA407 DNA for the 2.2-cM TG538-TG503 marker interval possessed significantly lower seed set than those with *L. esculentum* DNA for this marker interval (Table 4). Thus, the marker interval containing Rcm 5.1 is located 3.3 cM distal to the marker interval controlling parthenocarpic fruit.

Table 5 Rcm 2.0 and Rcm 5.1 exhibit additive gene action. Mean separation was performed with data from the sixth disease score. Molecular markers TG091 and TG318 were used to determine the genetic effects of Rcm 2.0 and Rcm 5.1 using LSD mean separation

Genotype[a]	2353 (Rcm 2.0)[b]	2361 (Rcm 5.1)[b]	2001 Field[b]	2002 Field[b]
E_2E_2	2.32a	–	2.09a	1.58a
H_2E_2	1.95a,b	–	1.60b	0.90b
H_2H_2	1.56b	–	1.56b	0.53c
E_5E_5	–	2.45a	2.34a	1.61a
H_5E_5	–	2.09b	2.06b	0.79b
H_5H_5	–	1.46b	1.73c	0.53c

[a] The alleles denoted H and E are from *LA407* and *L. esculentum*, respectively; subscripts denote QTL Rcm 2.0 (2) and QTL Rcm 5.1 (5)
[b] Means followed by different letters are significantly different

We sought to determine the gene action of both loci using two separate statistical analyses. Mean separation statistics using least significant difference (LSD) and orthogonal contrast were used to differentiate between additive, dominant, or recessive gene action. Rcm 5.1 exhibited additive gene action, and significant mean separation occurred between the three genotypic classes in the 2001 and 2002 field trials (Tables 2, 5). In the 2361 F_2 population, the mean disease score for heterozygotes was less than that for homozygotes lacking Rcm 5.1, but significant mean separation was not observed between these two classes (Kabelka et al. 2002). Additive gene action was also detected for Rcm 2.0 in the 2002 field trial (Tables 2, 5). Although the mean disease score for heterozygotes was less than that for the homozygous class lacking Rcm 2.0, significant mean separation did not occur between in the 2353 F_2 population or in the 2001 field disease screen (Tables 2, 5). These results suggest that both loci exhibit additive gene action but that large population sizes with balanced genotypic classes may be necessary to obtain significant mean separation between the three genotypic classes for each locus.

Detection of epistasis

We sought to determine if Rcm 2.0 and Rcm 5.1 interact. The 2001 field data were analyzed using both ANOVA and orthogonal contrasts to test for interactions between Rcm 2.0 and Rcm 5.1. The use of an ANOVA model enabled the identification of other potential sources of variation in our experiment and allowed for the estimation of variance components. The use of orthogonal contrast statistics enabled us to test for the four possible types of complimentary diallel epistasis based on the gene action of either locus (additive-by-additive, dominant-by-additive, additive-by-dominant, and dominant-by-dominant). An epistatic interaction was detected in 2001 by ANOVA and orthogonal contrasts (P=0.027), leading to heightened resistance when both loci are present in one genetic background.

In F_2 populations, some genotypic classes are represented at lower frequencies than others, necessitating large population sizes in order to detect QTL interactions (Tanksley 1993). To create a more balanced experimental design while randomizing the genetic background not associated with resistance, we intercrossed selections from the 2001 field that were homozygous for Rcm 2.0, Rcm 5.1, both resistance loci, and both susceptible loci in a partial diallel. Using this crossing scheme, we were able to create a more balanced data set containing the nine possible F_2 genotypic classes with 11–14 independent hybrids per genotype while randomizing the genetic background for unlinked loci (Fig. 1). Epistasis between Rcm 2.0 and Rcm 5.1 was detected in 2002 for individual disease ratings using the ANOVA model (P=0.0001), and additive-by-additive epistasis was detected using orthogonal contrasts (P=0.001; Table 2). Using percent maximal disease calculations, we were also able to detect a significant epistatic interaction between both loci by ANOVA (P=0.0296), and additive-by-additive epistasis was detected using orthogonal contrasts (P=0.005). Mean separation statistics using LSD were also employed for the nine F_2 genotypic classes in 2001 and 2002. Significant mean separation occurred in both 2001 and 2002, with genotypes homozygous for both loci exhibiting more resistance to *Cmm* and lower disease scores than all other classes (Table 6). In addition, genotypes that did not contain Rcm 2.0 and Rcm 5.1 were susceptible to *Cmm* and exhibited the highest disease scores in 2001 and 2002 (Table 6). The epistatic interaction between both loci accounts for an additional 17.3–18.0% of the phenotypic variation for *Cmm* resistance. Thus, when both loci are homozygous and present in the same genetic background, they control a large portion of the variation for *Cmm* resistance (68.8–79.9%).

Table 6 LSD mean separations of digenic classes in 2001 and 2002. Mean separation was performed with data from individual disease ratings

Genotype[a]	2001 Field[b]	2002 Field[b]
$H_2H_2H_5H_5$	1.54a	0.52a
$H_2E_2H_5H_5$	1.66b,a	0.70a,b
$H_2H_2H_5E_5$	1.87b,c,a	0.63a
$H_2E_2H_2E_2$	1.92b,c,a	1.13b
$E_2E_2H_5H_5$	1.98b,c	1.06a,b
$H_2H_2E_5E_5$	2.13c	0.75a,b
$H_2E_2E_5E_5$	2.01b,c	2.49c
$E_2E_2H_5E_5$	2.58d	2.53c
$E_2E_2E_5E_5$	3.14e	2.90c

[a] The alleles denoted H and E are from *LA407* and *L. esculentum*, respectively; subscripts denote QTL location
[b] Means followed by different letters are significantly different

Discussion

The results from this study demonstrate that the QTL Rcm 2.0 is located to a 4.3-cM interval on the distal portion of chromosome 2 and that the QTL Rcm 5.1 is located to a 2.2-cM interval on chromosome 5 in *L. hirsutum* LA407. In the literature, some disagreement exists on the best method to position QTL. In our study, we were unable to use the CIM technique due to the very low frequency of polymorphic loci located outside of the regions surrounding Rcm 2.0 and Rcm 5.1 in all three populations. Therefore, progeny testing of recombinant families was conducted to verify the results obtained using SIM. This progeny testing also helped to identify recombinant individuals that narrow the region of LA407 DNA surrounding either locus. The results from progeny testing complimented the results obtained from interval mapping and increased the precision of the position of each QTL relative to the 1-LOD confidence interval for Rcm 2.0 and Rcm 5.1.

Previously, we hypothesized that Rcm 2.0 conferred resistance to *Cmm* by altering vascular morphology and development, while Rcm 5.1 might be a member of the *Pto* resistance gene cluster (Coaker et al. 2002; Kabelka et al. 2002). By precisely mapping Rcm 5.1, we were able to reject the hypothesis that this locus is a member of the *Pto* cluster. In fact, Rcm 5.1 lies 4.6–4.1 cM distal to the *Pto-Prf* region. Although it is interesting to speculate that Rcm 2.0 may act to decrease symptom progression due to an increased rate of vascular maturation (Coaker et al. 2002), statistical correlations do not confirm this function and further work will be necessary to determine if Rcm 2.0 and the vascular morphology QTL are controlled by the same gene(s). Cloning the genes underlying these loci will contribute to our understanding of the function of each QTL and thus mechanisms that contribute to quantitative disease resistance. The genetic mapping described in this paper will serve as a starting point for map-based cloning.

Although wild species possess a wealth of genetic diversity, they contain many undesirable traits such as small fruit size, poor taste, indeterminant growth, and low yield. Linkages between beneficial and undesirable loci are not uncommon when working with wild germplasm. Using IBC breeding to introgress Rcm 2.0 and Rcm 5.1 has eliminated some undesirable traits. Rcm 2.0 and Rcm 5.1 control a large portion of the phenotypic variance for resistance to *Cmm*, but they were also found to be associated with less than optimal fruit quality (Kabelka et al. 2002). In order to break the negative linkages from *L. hirsutum* LA407, recurrent selection for lines containing desirable horticultural attributes was employed in conjunction with marker-assisted selection and population development. The crossing design we employed allowed for multiple rounds of selection and opportunities for recombination while generating populations to map loci and confirm genetic models (Fig. 1). Progeny testing of recombinant families was also conducted to identify a genomic region linked to Rcm 5.1 that is associated with parthenocarpic fruit. We were able to demonstrate that Rcm 5.1 lies 3.3 cM distal to a marker interval contributing to parthenocarpy. Linkage drag associated with introgressed LA407 DNA can therefore be eliminated by marker-assisted selection using closely linked markers.

The majority of genes are not entirely independent in action and interact in intricate ways with other genes. Epistasis, or interlocus interaction, can be defined as the effect of alleles at one locus interacting and changing the effect of an allele at a second locus. Epistasis between a pair of loci affecting a quantitative trait can be detected by deviations in trait values from that expected based on the main effects of the two loci (Falconer 1989). By concentrating our efforts on populations where the majority of the trait variance was associated with two QTLs, we were able to detect and confirm additive-by-additive epistasis between Rcm 2.0 and Rcm 5.1, leading to heightened resistance when both loci are present in one genetic background. Our population design provided several advantages in investigating and detecting epistasis. Lines used to generate segregating populations contained single introgressions of LA407 DNA, harboring QTLs controlling the majority of the phenotypic variance for *Cmm* resistance. In addition, the F_2 segregation for both loci in the populations analyzed allowed for separate statistical tests for epistasis, orthogonal contrasts and two-way ANOVA, thereby increasing confidence in the results. A diallel crossing scheme was used to generate lines comprising the nine F_2 genotypic classes. This allowed for simple experimental analysis of a confirming population with balanced representation of genotypic classes. Furthermore, the F_2 genotypic classes enabled the analysis of all possible types of complimentary diallel epistasis.

In this study we were able to accurately position two loci controlling resistance to diverse *Cmm* strains. We demonstrated that both loci exhibit additive gene action and interact epistatically. The results presented will expedite breeding for bacterial canker resistance in tomato by the recurrent selection program employed during QTL detection and by enabling marker-assisted selection for two interacting QTLs.

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