# Fine Mapping, Genetic Effects, and Epistatic Interaction of Two *Lycopersicon hirsutum* QTL Conferring Resistance to *Clavibacter michiganensis* subsp. *michiganesis*.

Gitta Coaker[1], Eileen Kabelka[2], and David M. Francis[1]

[1]Department of Horticulture and Crop Science, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691, USA. [2]Department of Crop Sciences, The University of Illinois at Urbana Champaign, 1101 W. Peabody, Urbana, IL 61801.

## ABSTRACT

The purpose of this study was to combine a recurrent selection breeding strategy with genetic mapping. Our goal was to map and determine the genetic effects of two quantitative trait loci (QTL) controlling resistance to bacterial canker of tomato caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). The two QTL from *L. hirsutum* LA407 had previously been separately introgressed into *L. esculentum* using inbred backcross breeding, and single marker-trait analysis positioned the QTL on chromosome 2 (Rcm2.0) and chromosome 5 (Rcm5.1). In this study, interval mapping techniques were applied to populations with $F_2$-like segregation that combined the two introgressions. Rcm2.0 accounted for 9.5-11.4% (LOD > 4) and Rcm5.1 accounted for 5-15% (LOD > 4) of the phenotypic variation. An Additive x Additive epistatic interaction between Rcm2.0 and Rcm5.1 was hypothesized (P = 0.027). Marker-assisted selection coupled with a diallele mating design was used to re-create the 9 possible $F_2$ genotypic classes and confirm epistasis (P < 0.0001).

## INTRODUCTION

Crosses between cultivated tomato and wild relatives offer a powerful approach for genetic mapping. However, breeding progress is often limited due to the negative affects of the wild species genome in $F_2$ populations. We were interested in exploring simultaneous genetic analysis and breeding through the use of recurrent selection. Previously, Rcm2.0 and Rcm5.1 were identified in an inbred backcross population with *L. hirsutum* LA407 as the donor parent and *L. esculentum* processing varieties as recurrent parents (Kabelka et al., 2002. Phytopathology 92: 504-510). In this study we combined the two loci, mapped the position of QTL, and determined their genetic effects while completing two rounds of recurrent selection.

## OBJECTIVES

•We hypothesized that two *L. hirsutum* loci could be accurately positioned using interval mapping techniques while studying both their genetic effects and interaction in recurrent selection populations.

•We hypothesized that a crossing scheme which re-created the $F_2$ genotypic classes would increase experimental power to detect epistasis by providing a more balanced design than 9:6:6:1 segregation while randomizing the genetic background for unlinked loci.

•We hypothesized that Rcm5.1 falls within the Pto resistance gene cluster.

## MATERIALS AND METHODS

### *Plant Materials and Breeding Scheme*

Populations segregating for both Rcm2.0 and Rcm5.1 used to map QTL and detect epistasis between the two loci are described in Figure 1. The lines 61037 and 53025 were selected from $F_2$ populations derived by crossing inbred backcross line (IBL) 2361 and IBL 2353 to Ohio 86120. Selections ($F_3$) were crossed to Ohio 9242 and the resulting $F_1$'s were crossed. Heterozygotes for markers surrounding both Rcm 2.0 and Rcm 5.1 were selected and selfed to generate a population with $F_2$-like segregation for both loci. This population was evaluated for resistance in a randomized complete block (RCB) design with two replications (2001). Selections homozygous for Rcm2.0, Rcm5.1 or both loci were crossed in a partial diallele to recreate all 9 genotypic classes. A set of these lines was evaluated for resistance in a RCB design with two replications (2002). Disease evaluations were performed in the field following transplant of infected seedlings. The IBL 2353 and 2361 $F_2$ populations were evaluated in the greenhouse. Plots were rated on a 0-5 scale, with mean of 5 being dead and a mean of 0 having no symptoms.

### *Molecular Markers and Statistical Analysis*

PCR-based cleaved amplified polymorphic (CAP) markers were used to create a genetic linkage map and position QTL using MAPMAKER. Additive and dominant gene action was determined by orthogonal contrasts. Epistasis was tested using both contrasts and analysis of variance (ANOVA) models. ANOVA and mean comparisons of genotypes were performed with the SAS General Linear Models procedure.

To determine if Rcm5.1 resides within the Pto disease resistance cluster, a transformation competent artificial chromosome (TAC) library containing LA407 DNA was screened to identify a clone containing Prf and a parolog of Pto, LimPth4 (Fig 2). The left border end sequence was converted to a CAP marker (TACL2) and placed onto the IBL 2361 $F_2$ and the 2001 field populations.

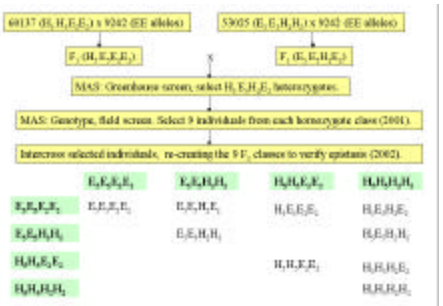


**Figure 1. Simultaneous breeding and mapping of QTLs for *Cmm* resistance.** The scheme allows multiple rounds of selection and multiple opportunities for recombination while generating populations to map genes and confirm genetic models. Parents used to re-create the $F_2$ genotypic classes are shown in green boxes. The alleles denoted H and E are from *L. hirsutum* and *L. esculentum*, respectively, subscripts denote QTL Rcm 2.0 and QTL Rcm5.1, and MAS = marker-assisted selection.

## RESULTS

### *Mapping Rcm 2.0 and 5.1 Using Interval Mapping Techniques*

Marker order, genetic distance, and QTL results were consistent across populations (Fig 3-4). Rcm2.0 accounted for 9.5-11.4% of the phenotypic variation ($4 \geq$ LOD $\leq 14.7$), while Rcm5.1 accounted for 5.6-15% of the phenotypic variation ($4.0 \geq$ LOD $\leq 5.0$; Fig 3). The gene action of both loci was additive in nature (P < 0.0001). To help eliminate negative horticultural traits associated with Rcm 2.0 and 5.1, recurrent selection for horticultural attributes was employed in conjunction with MAS and phenotypic selection for disease resistance.

A TAC library was screened and a positive clone containing Prf and a paralog of Pto (LpimPth4) was identified (Fig 2). The TAC clone and a CAP marker based on Pto map within the one LOD confidence interval surrounding Rcm5.1 (Fig 3C, 3D). As the confidence interval for Rcm5.1 spans multiple markers, we were unable to determine if this locus falls within the Pto cluster or is merely linked to the Pto cluster. Informative recombinants suggest that Rcm5.1 falls between TG538 and CT202, an observation that is being verified in progeny tests.

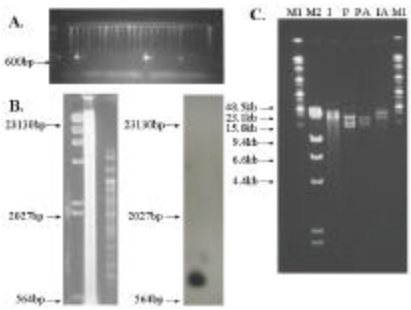


**Figure 2. Screening of *L. hirsutum* TAC library.** Amplification of a 600 bp fragment in TAC pools was used as a primary screen. Lane 2 contains control DNA **(A)**. Agarose Gel and Southern blot of *Bam*HI/*Hind*III digested positive clone **(B)**. PFGE analysis of the positive clone. I, *Isce-I*; P, *PmlI*; A, *Asc*; M1, midrange PFGE ladder; M2, λDNA *Hind*III. The 33.4 Kb clone contains Prf and a parolog of Pto **(C)**.

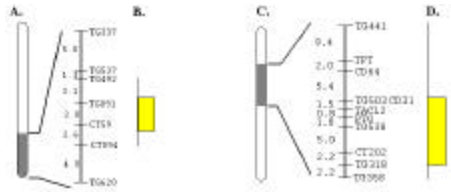


**Figure 3. Genetic map and confidence interval for QTL conferring resistance to Cmm.** Location of introgressions surrounding Rcm 2.0 (**A**) and Rcm 5.1 (**C**) are indicated in gray. Adjacent genetic maps provide a reference for the 1 LOD (yellow box) and 2 LOD (black line) confidence intervals for Rcm2.0 (**B**) and Rcm5.1 (**D**). The relationship between genetic and physical distance within the Pto cluster is 0.01 to 0.03 cM per Kb.

**Figure 4. Phenotypic variation between genotypes in the field**. Top: Left plot contains Rcm 5.1 and 2.0 from *L. hirsutum* while the right plot is homozygous for *L. esculentum* loci. Bottom: Individual plants showing resistant and susceptible phenotypes (Right) and stem canker on susceptible plant (Left).

### *Detection of Epistasis Between Rcm 2.0 and Rcm 5.1*

A significant Additive x Additive (A x A) epistatic interaction was detected between both loci in 2001 (P = 0.027). To verify epistasis, a crossing scheme re-creating the 9 $F_2$ genotypic classes with respect to both loci was employed in 2002 (Fig 1). A significant A x A epistatic interaction was detected in 2002 using a 2-way ANOVA (Table 1) and contrasts (P <0.0001). Genotypic mean separations were consistent with epistasis (Table 2).

**Table 1. ANOVA using data from the 8 th disease rating and area under the disease progression curve (AUDPC) for 2002 field disease screen.**

| Source | DF | F value Rate 8 | P value Rate 8 | F value AUDPC | P value AUDPC |
|---|---|---|---|---|---|
| Rcm5.1 | 2 | 35.378 | <0.0001 | 11.02 | 0.0001 |
| Rcm2.0 | 2 | 42.68 | <0.0001 | 8.66 | 0.0006 |
| Block | 1 | 0.29 | 0.5950 | 0.20 | 0.6546 |
| Rcm5.1*Rcm2.0 | 4 | 7.00 | 0.0001 | 2.93 | 0.0296 |
| Rcm5.1*Block | 2 | 0.72 | 0.4927 | 0.97 | 0.3878 |
| Rcm2.0*Block | 2 | 1.77 | 0.1832 | 0.56 | 0.5757 |
| Rcm5.1*Rcm2.0*Block | 4 | 1.78 | 0.1474 | 0.30 | 0.8797 |
| Error | 45 | 1.99 | 0.0095 | 0.75 | 0.8334 |

**Table 2. Epistasis based on the of number of *L. hirsutum* alleles**. Alleles are in reference to *L. hirsutum* LA407. 0 has all *L. esculentum* alleles, while a 4 indicates all LA407 alleles. Means with the same letter for T Grouping are not significantly different and N = number of plots. Data is from the 8 th disease rating for 2002 field disease screen.

| T Grouping | Mean | N | Number of Alleles |
|---|---|---|---|
| A | 2.986 | 12 | 0 |
| B | 2.500 | 26 | 1 |
| C | 1.250 | 34 | 2 |
| D | 0.667 | 26 | 3 |
| D | 0.518 | 14 | 4 |

## CONCLUSIONS

•Recurrent selection coupled with MAS for *Cmm* resistance was successful in combining both loci into one genetic background.

•Interval mapping placed Rcm 5.1 in the 11 cM TG503-TG318 marker interval, and Rcm 2.0 in the 5.6 cM TG091-CT094 marker interval.

•Both loci act in an additive fashion, and an A x A epistatic interaction exists between Rcm2.0 and Rcm5.1.

•The crossing scheme used to confirm A x A epistasis increased experimental power by balancing class size and randomizing unlinked loci.

•Rcm5.1 is linked to Pto and Prf, but informative recombinants suggest that Rcm5.1 falls outside of the cluster.

•The relationship between genetic and physical distance surrounding Pto is 0.01 to 0.03 cM per Kb.