

Genetic resistance to strains of the bacterial canker pathogen (*Clavibacter michiganensis* subsp. *michiganensis*) in tomato

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Introduction:

Bacterial canker of tomato (*Lycopersicon esculentum* Mill.) caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) accounts for severe economic loss (Gleason et al, 1993). The causal organism has pathogenicity traits that include systemic infection, wilt, canker, marginal necrosis, stunting, and fruit spots (Jones et al, 1993). In North America, yield losses have been estimated as high as 84% in commercial fields and 93% in controlled studies (Poysa, 1993). In the Great Lakes Region, surveys rank *Cmm* as the most important disease of processing tomatoes (Francis et al, 1995).

Although traditional breeding in the United States has targeted resistance to *Cmm* for over twenty years (e.g. Emmatty and Johns, 1973), the development of commercial cultivars for use in the Great Lakes Region has been slow (Gleason et al, 1991). This may, in part, be due to: (1) evaluating sources of resistance with strains of *Cmm* that are not genetically defined, (2) working with strains of *Cmm* not endemic to the region, or (3) not fully understanding the genetic basis or mechanism(s) of resistance against *Cmm*.

To address these issues, our approach uses: (1) genetically classified strains of *Cmm* endemic to the Great Lakes Region, (2) traditional and molecular genetic analysis of host resistance and (3) a breeding population structure that facilitates the identification of quantitative traits and allows evaluation in replicated trials. Utilizing this approach facilitates the development of reliable genetic resistance and contributes to a better understanding of the interaction between *Cmm* and its host.

Objectives:

- I To identify the number and chromosome location of the gene(s) that confer(s) resistance to *Cmm* in greenhouse and field trials by combining traditional evaluation and restriction fragment length polymorphism (RFLP) marker analysis.
- II Evaluate PCR-based molecular markers based on the conserved domains of plant resistance genes to test the hypothesis that members of resistance gene families mediate resistance to *Cmm*.
- III Develop tomato germplasm and processing varieties conferring resistance to *Cmm* utilizing marker-assisted selection.
- IV Use genetic information obtained to form hypotheses about the underlying mechanism(s) controlling resistance to a systemic pathogen.

Material & Methods:

Plant material: An accession of *Lycopersicon hirsutum*, LA407, has been shown to exhibit markedly reduced canker symptoms and low titers of bacteria above the inoculation site when infected with two distinct genetic types of *Cmm*, designated "A" and "C" (Louws et al, 1994; 1995; Francis et al, in progress). An inbred backcross population (IBC), consisting of 65 BC₂S₅ lines, derived from a cross between *L. esculentum* and *L. hirsutum* (LA407) was used to evaluate resistance to these strains. Populations were created (F₂ and F₃) from crossing resistant IBC lines with a susceptible genotype, Ohio 86120, for genetic confirmation and for germplasm/variety advancement.

***Cmm* inoculation procedures:** Two prevalent and distinct genetic types of *Cmm*, types "A" and "C", found in the Great Lakes Region (Louws et al, 1994; 1995) were used for this study. In greenhouse studies, plants were inoculated by clipping a petiole with scissors dipped in 3 x 10⁸ cfu/ml inoculum (Pine et al, 1955; Ricker et al, 1996). Field trials are established using greenhouse transplants previously inoculated using a Preval[®] spray gun to apply a 3 x 10⁸ cfu/ml suspension. Spray inoculation in the greenhouse, prior to transplant, mimics the epidemiology of the disease under grower conditions.

Genetic analysis: An extensive RFLP library (Tanksley et al, 1992) is available to identify the number and chromosome location of gene(s) that confer(s) resistance to *Cmm*. Preliminary studies have found that over 55% of the RFLP markers available are polymorphic between *L. esculentum* and *L. hirsutum* (Kabelka, unpublished). In addition, primer sequences (Francis, unpublished) designed from conserved domains of previously cloned resistance genes were used. The use of this strategy is based on the observation that resistance genes in plants tend to be clustered (McMullen and Simcox, 1995; Michelmore et al, 1992).

Experimental design and data analysis: Greenhouse and field studies to evaluate response to petiole clip or spray inoculation of *Cmm* were performed in replicated trials using a randomized complete block design. Linkage relationships between DNA-based markers and chromosomal segments conferring resistance to *Cmm* were determined by analysis of variance (PROC GLM; SAS). Mean values of disease ratings for the tested genotypes were compared to appropriate control genotypes using mean separation statistics.

Results:

Scoring for Disease:

Symptom rating of *Cmm* in tomato is complicated by diverse symptoms, which include unilateral wilt, marginal necrosis, upward curling of leaflets, stunting, stem cankers and reddish-brown discoloration of vascular tissue.

- Scoring for response to *Cmm* petiole clip or spray inoculation is based on visual assessment of symptom severity.
- An additive disease rating scale of 0-5 is used. A value of "1" would be given for each type of symptom with subtraction of "1/2" point or addition of "1" point, depending on degree of symptom severity (slight or severe). A plant exhibiting unilateral necrosis and slight canker formation (Figure 1a) would be given a rating of "1.5". A plant exhibiting severe stem canker and wilting (Figure 1b) would be given a rating of "3". A score of "0" would represent a symptom-free rating while "5" would indicate death of plant (Figure 1c). Under field evaluation, individual plant ratings were made to calculate plot average. For comparison, the average plot ratings in (Figure 1d) would be "4.5" (left side of image) vs. "1" (right side of image). See examples following.



Figure 1a



Figure 1b



Figure 1c

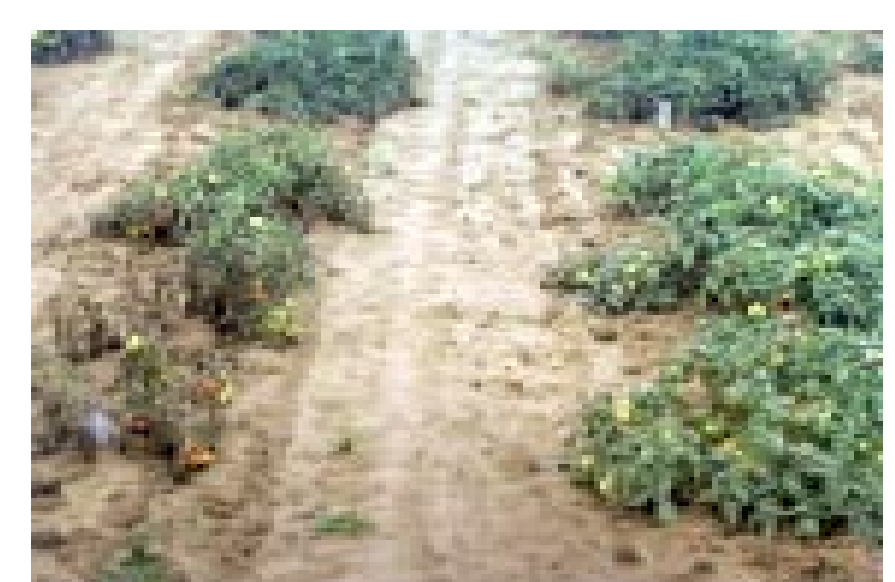


Figure 1d

Greenhouse & Field Studies:

An IBC population, consisting of 65 BC₂S₅ lines, derived from a cross between *L. esculentum* and *L. hirsutum* (LA407) was used to evaluate resistance to *Cmm*, types "A" and "C", in replicated greenhouse and field trials.

Resistance to *Cmm*, types “A” and “C”, was identified in select lines of the IBC population:

Line 2353:

- Resistance to *Cmm*, type “A” (Table 1) and type “C” (Table 2) was identified in an IBC line 2353 which performed significantly better than susceptible controls when evaluated at an individual rating point (5-6 weeks) or area under the disease progression curve (AUDPC) in both greenhouse and field trials.
- The choice of 2353 as a source of resistance is based on its consistent low disease rating.

(A) 1998 Greenhouse Trial					
Genotype	Description	Week 6	AUDPC		
2312	IBC line	nd	nd		
8245	S control	5.0	D	17.13	C
86120	S control	5.0	D	17.75	C
2314	IBC line	4.75	C D	17.25	C
2361	IBC line	4.25	B C D	15.74	B C
2332	IBC line	4.25	B C D	15.0	B C
97744	R control	4.25	B C D	14.63	B C
2348	IBC line	4.25	B C D	14.5	B C
88119	S control	4.0	B C	14.25	B C
97743	R control	3.75	B	12.5	B C
9144	R control	3.5	B	12.0	B C
2357	IBC line	1.75	A	6.5	A
2353	IBC line	1.5	A	4.63	A
LSD		0.93		4.01	
IBC population mean		4.43		15.92	
(B) 1998 Field Trial					
Genotype	Description	Week 5	AUDPC		
88119	S control	3.27	D E	8.03	D E F
8245	S control	3.09	D E	7.9	D E F
86120	S control	3.08	D E	7.37	D E F
97743	R control	3.08	D E	7.27	C D E F
97744	R control	3.04	D E	7.27	C D E F
2314	IBC line	2.87	C D E	6.87	C D E F
2332	IBC line	2.75	B C D E	6.81	C D E F
9144	R control	2.71	B C D E	5.76	B C D
2312	IBC line	2.32	A B C D	4.39	A B C
2348	IBC line	2.23	A B C D	6.34	B C D E
2361	IBC line	1.87	A B C	5.0	B C
2357	IBC line	1.87	A B C	4.06	A B
2353	IBC line	1.75	A B C	3.08	A
LSD		0.57		1.55	
IBC population mean		2.85		6.73	

Line 2361:

- Line 2361 confers resistance to *Cmm*, type “C” (Table 2) performing significantly better than susceptible controls when evaluated at an individual rating point (5-6 weeks) or area under the disease progression curve (AUDPC) in both greenhouse and field trials. Line 2361 performed well in the *Cmm*, type “A” field trial but performed poorly in the *Cmm*, type “A” greenhouse trial (Table 1).
- The choice of 2361 as a source of resistance is based on an association between resistance to *Cmm*, type “C” and a resistance gene analog polymorphism in the IBC population.

(A) 1997 Greenhouse Trial					
Genotype	Description	Week 6	AUDPC		
8704	S control	5.0	D	7.67	D E F G
2348	IBC line	4.0	C D	7.5	C D E F G
2356	IBC line	2.5	B C D	6.25	B C D E F G
70214	R control	2.0	A B C	5.75	A B C D E F G
2357	IBC line	2.0	A B C	5.25	A B C D E F
2312	IBC line	1.0	A B C	5.25	A B C D E F
2361	IBC line	1.0	A B C	5.0	A B C D E
2314	IBC line	1.0	A B C	4.5	A B C D E
2332	IBC line	1.0	A B C	3.5	A B C D E
2353	IBC line	0.5	A B	3.75	A B C D
LSD		1.72		2.33	
IBC population mean		2.87		6.46	
(B) 1997 Field Trial					
Genotype	Description	Week 5	AUDPC		
88119	S control	4.25	G	11.44	H
2357	IBC line	3.0	C D E F	8.94	E F G
8245	S control	2.88	B C D E F	7.69	C D E F G
2332	IBC line	2.88	B C D E F	7.69	C D E F G
9144	R control	2.75	B C D E F	8.25	D E F G
97743	R control	2.75	B C D E F	7.38	B C D E F G
97744	R control	2.63	A B C D E F	7.12	B C D E F G
2312	IBC line	2.5	A B C D E F	5.84	A B C D E F
2314	IBC line	2.25	A B C D E F	6.03	A B C D E F
2356	IBC line	2.25	A B C D E F	6.5	A B C D E F
2348	IBC line	2.13	A B C D E	5.56	A B C D E F
2361	IBC line	2.0	A B C D	5.21	A B C D E
2353	IBC line	1.88	A B C	4.8	A B C D
LSD		0.79		2.3	
IBC population mean		2.88		7.72	

Genetic Analysis:

Genetic analysis of the IBC population with molecular markers has resulted in the identification of putative loci conferring resistance to *Cmm*, types “A” and “C”. These associations have been, and are currently being, confirmed in F_2 and F_3 population studies.

Resistance to *Cmm*, type “A” in IBC Line 2353:

- An F_2 population derived from a cross between 2353 (resistant) and Ohio 86120 (susceptible) was evaluated for response to *Cmm*, type “A.” Segregation ratios, based on phenotypic evaluation, suggest a single locus conferring resistance ($X^2 = 1.825$; $df = 2$; $P < 0.05$).
- We are currently evaluating RFLP molecular markers to test the hypothesis that a locus on chromosome 2 is associated with resistance to *Cmm*, type “A.”

Resistance to *Cmm*, type “C” in IBC Line 2361:

- An F_2 population derived from a cross between 2361 (resistant) and Ohio 86120 (susceptible) was evaluated for response to infection with *Cmm*, type “C”.
- DNA from F_2 individuals was used as template for PCR amplification with a resistance gene analog polymorphic marker from chromosome 5 (Figure 2). Detection of polymorphism required digestion of the amplified product with *Rsa*I. Alleles from the susceptible parent (Ohio 86120) cleave into two bands while alleles from the resistant parent (2361) are not digested. Determination of heterozygosity was based on the stoichiometric relationships between upper and lower bands.
- Analysis of variance was used to test the hypothesis that this molecular marker was associated with disease resistance. A highly significant association ($P = 0.002$) was obtained which was interpreted as evidence for linkage (Table 3).

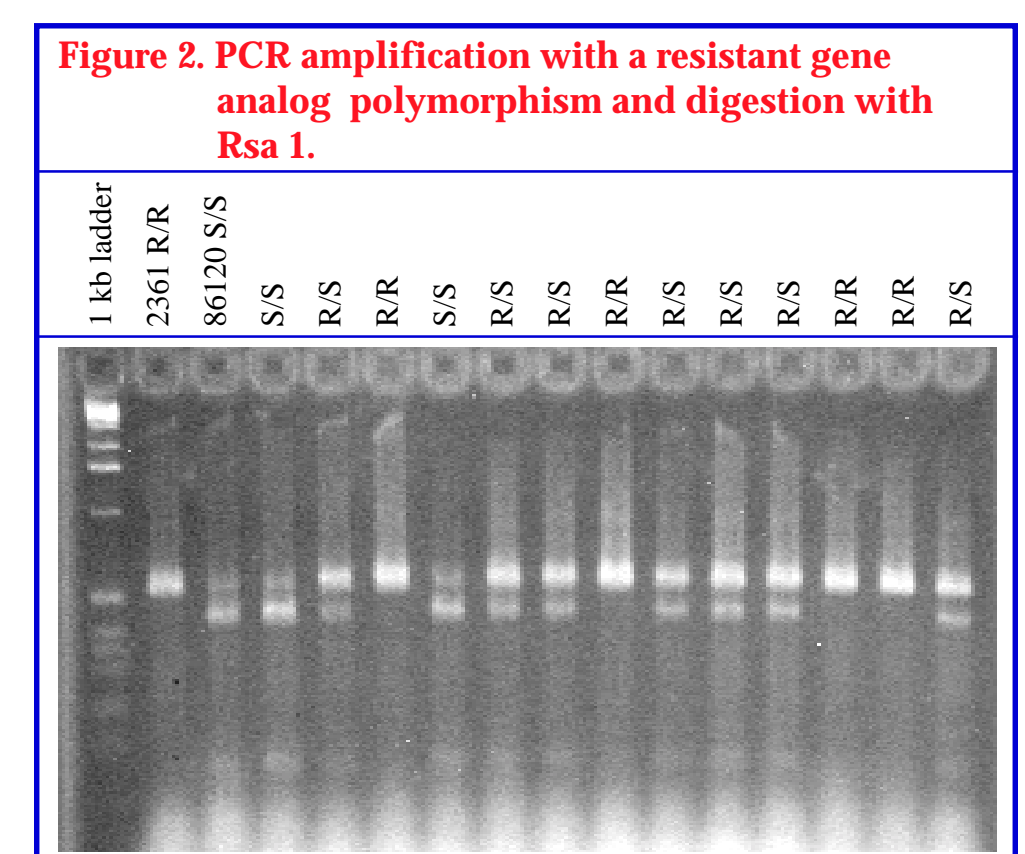


Table 3. Association between disease severity and a resistance gene analog polymorphism.

N	Class	Week 6	AUDPC	
43	S/S	2.5	B	8.3
72	S/R	2.0	A B	6.5
43	R/R	1.6	A	4.8
LSD		0.47		1.9
Mean		2.0		6.52

Conclusions:

- Resistance to *Cmm*, types “A” and “C”, has been identified in an IBC population derived from a cross between *Lycopersicon esculentum* and *Lycopersicon hirsutum*, LA407.
- IBC line 2353 confers resistance to *Cmm*, types “A” and “C”, performing significantly better than susceptible controls in both greenhouse and field trials.
- IBC line 2361 confers resistance to *Cmm*, type “C”, performing significantly better than susceptible controls in both greenhouse and field trials. However, it does not appear to perform well against *Cmm*, type “A”, in greenhouse trials.
- Genetic analysis with molecular markers has identified at least two loci from *Lycopersicon hirsutum*, LA407, that confer resistance to *Cmm*, types “A” and “C” - one on chromosome 5 and a second hypothesized to be on chromosome 2.

Discussion:

This study has utilized an IBC population to facilitate the identification of quantitative trait loci for resistance to *Cmm*. This population has many advantages as it allows studies to be replicated by location, by two different inoculation protocols and has allowed different strains of *Cmm* to be tested independently.

IBC line 2353 confers resistance to both *Cmm*, types “A” and “C”, while line 2361 seems to confer resistance only to *Cmm*, type “C”. This not only suggests different loci conferring resistance to *Cmm* but also may support the hypothesis that physiological races of *Cmm* exist.

The genetic information obtained from this study allows us to form hypotheses about the mechanism(s) of resistance to *Cmm* in tomato, which can be further tested by molecular and cellular approaches.

In the IBC population, a low proportion of donor parent characterizes each line such that, in any given plant, only 12.5% of the genome is *L. hirsutum*. Subsequent crosses to *L. esculentum* have identified resistant individuals that retain on average 6.25% of the *L. hirsutum* genome and may yield germplasm appropriate for incorporation into traditional breeding programs. Molecular markers associated with resistance identified in this study are currently being used to facilitate selection.

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