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Pto3 and *Pto4*: novel genes from *Lycopersicon hirsutum* var. *glabratum* that confer resistance to *Pseudomonas syringae* pv *tomato*

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Abstract Accessions of wild *Lycopersicon* germplasm were screened for resistance to *Pseudomonas syringae* pv *tomato* (*P.s. tomato*). Resistance to both race-0 and race-1 strains of *P.s. tomato* was identified in *L. pimpinellifolium*, *L. peruvianum* and *L. hirsutum* var. *glabratum*. Resistance to race-0 derived from *L. hirsutum* var. *glabratum* (*Pto3*) appeared to be inherited independently of *Pto1* and *Pto2*. Filial and backcross generations derived from interspecific crosses between *L. esculentum* and *L. hirsutum* var. *glabratum* revealed that *Pto3* resistance was inherited in a complex fashion and was incompletely dominant under conditions of high bacteria inocula. Resistance to *P.s. tomato* race-1 (*Pto4*) was also identified in *L. hirsutum* var. *glabratum*. *Pto3* and *Pto4* segregated independently of each other.

Key words Disease-resistance · *Lycopersicon* spp. Interspecific hybridization · Gene-for-gene · *Pto1*

Introduction

The gene-for-gene hypothesis states that an incompatible plant-pathogen interaction will occur when both the plant and pathogen possess complementary disease-resistance and avirulence genes, respectively (Flor 1956). When one of these corresponding genes is absent from the plant or pathogen, a compatible interaction manifested as a disease-susceptible phenotype occurs. The resistance gene/avirulence gene system has been documented in many plant pathogen interactions and is postulated to have resulted from plant-pathogen coevolution (Thompson and Burdon 1992).

In cultivated tomato, resistance to the bacterial speck pathogen *Pseudomonas syringae* pv *tomato* (*P.s. tomato*) race-0 is conferred by the single dominant gene *Pto1* (Pitblado and Kerr 1980) or *Pto2* (Pilowski and Zutra 1982). Race-1 isolates of *P.s. tomato* are pathogenic on *Pto1* cultivars (Lawton and MacNeill 1986). One feature that distinguishes race-0 strains from race-1 strains is the presence of the avirulence gene *avrPto* in all *P.s. tomato* race-0 isolates. When the cloned *avrPto* gene is introduced into race-1 strains of *P.s. tomato*, an incompatible reaction occurs with tomato plants that possess the *Pto1* gene. Deletion of *avrPto* from race-0 strains, however, does not convert them from incompatible to compatible (Ronald et al. 1992). These results suggest that there may be a more complex interaction between the tomato plant and *P.s. tomato* than a simple gene-for-gene interaction. The resistance gene/avirulence gene interaction between race-1 strains of *P.s. tomato* and the tomato plant has not been characterized to date.

In the search for additional sources of genotypic resistance to pathogens, plant breeders frequently rely on germplasm collected from the wild relatives of cultivated plants (Leppik 1970; Lunne and Wood 1991). In tomato, there are numerous examples of pest-resistance mechanisms that have been transferred from wild or exotic *Lycopersicon* germplasm into cultivated tomatoes (Rick et al. 1987). By screening accessions of wild tomatoes for resistance to mixed populations of *P.s. tomato*, numerous disease-resistant accessions were discovered (Pilowski and Zutra 1982). In the studies reported here, 12 accessions of wild *Lycopersicon* were tested for resistance to race-0 and race-1 isolates of *P.s. tomato*. The *L. hirsutum* var. *glabratum* PI 134417 was one of several accessions that showed resistance to both races of *P.s. tomato*. Resistance to *P.s. tomato* was previously identified in *L. hirsutum* var. *glabratum* (Pitblado and Kerr 1980; Pilowski and Zutra 1982; Sotirova and Bogatsevska 1990). Lawson and Summers (1984) reported that this disease-resistance gene was allelic to

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Pto1. However, sensitivity to the organophosphate insecticide fenthion, which is tightly linked to the *Pto1* resistance gene from *L. pimpinellifolium*, is not associated with disease-resistance from *L. hirsutum* var. *glabratum* (G. B. Martin, personal communication). These conflicting reports, the identification of disease-resistance to race-1, and the relative ease which hybrids between *L. esculentum* and *L. hirsutum* var. *glabratum* are obtained (Rick 1979) made a genetic study with *L. hirsutum* var. *glabratum* attractive.

We report here the identification and analysis of two novel genes, *Pto3* and *Pto4*, which conferred resistance to *P.s. tomato* race-0 and race-1, respectively. Inheritance of the *Pto3*- and *Pto4*-resistance was complex. This complexity may be due to the wide interspecific nature of the crosses which affected the expression of resistance to the bacterial speck pathogen. The *Pto3* and *Pto4* disease-resistance genes were backcrossed into a *L. esculentum* background to permit more extensive genetic analysis. *Pto3* was not allelic to *Pto1* and *Pto2*, and *Pto3* and *Pto4* segregated independently. These studies showed that extensive genetic characterization of the *P.s. tomato* resistance from *L. hirsutum* var. *glabratum* will require additional backcrossing to distinguish the segregation of the *Pto3* disease-resistance gene from the extraneous genetic contributions of the wild parent which complicated genetic analyses.

Materials and methods

Tomato accessions, growth conditions and crosses

Fourteen accessions representing five different species of *Lycopersicon* were tested for resistance to two *P. syringae* pv *tomato* strains (Table 1). Peto 238R (disease-resistant) and Peto 238S (disease-susceptible) are inbred lines obtained from J.C. Watterson (Petoseed Company, Saticoy, Calif.). Peto 238R possesses the *Pto1* gene which confers resistance to race-0 isolates of *P. syringae* pv *tomato*. The *Pto1* resistance gene was introgressed into cultivated tomatoes from *Lycopersicon pimpinellifolium* PI 370093 (Pitblado and Kerr 1980). PI 370093 was obtained from R. Brammall (Horticultural Experiment Station, Simcoe, Ontario, Canada). LA716 was obtained from S.D. Tanksley (Cornell University, Ithaca, N.Y.). All remaining USDA Plant Introduction (PI) accessions were obtained from the Plant Introduction Station at Geneva, N.Y. Except for PI 126449, the PI accessions were reported to have resistance to race-0 isolates of *P.s. tomato* (Pilowski and Zutra 1982). It was not known whether there was resistance to race-1 strains of *P.s. tomato* in any of the accessions obtained.

Tomato seeds were germinated in flats containing University of California soil mix III. Seedlings were transplanted one per one-gallon pot or 25 per 1 800 cm^2 flat. Plants were grown at temperatures between 21°C and 27°C in the greenhouse under ambient sunlight and were watered regularly with 1×Foliage-Pro (Dyna Gro, Novato, Calif.). In all interspecific crosses, *L. esculentum* served as the female parent. Flowers of the female parent were emasculated and pollinated 1 or 2 days preanthesis as described by Rick (1980). F_1 seeds were collected from a Peto 238R×PI 134417 cross and a Peto 238S×PI 134417 cross. F_1 plants were allowed to self and the F_2 seed were collected. For the production of the *Pto3* backcross-1 (BC_1) progeny, the recurrent parent Peto 238S was emasculated and pollinated by the F_1 hybrid (Peto 238S×PI 134417). The BC_2 generation was produced from a single disease-resistant BC_1 plant backcrossed to Peto 238S.

Bacterial strains and growth conditions

The *P.s. tomato* race-0 strain *pt*11 was isolated from a bacterial speck-infested tomato field in Mexico (D.A. Cooksey, UC Riverside, Riverside, Calif.). The *P.s. tomato* race-1 strain *pt*14D46 was isolated in California by Clarence Kado and was provided by D.L. Coplin (Ohio State University, Columbus, Ohio). The strain *pt*14D46 has also been referred to as *pt*156 (D.L. Coplin, personal communication). Single-colony isolates of *P.s. tomato* were suspended in water, spread on King's B media plates (King et al. 1954), and allowed to grow for 48 h at 25°C. Bacteria were removed from the plate, suspended in sterile water, and diluted to the concentration specified in the text. An OD_{590}=0.4 of the bacteria was equivalent to 2×10^8 colony forming units per ml (cfu/ml) which gave maximal bacterial speck symptoms on Peto 238S without producing any visible bacterial speck symptoms on Peto 238R. Sterile cotton swabs soaked in the bacterial suspension were gently rubbed on the upper leaf surface of young tomato plants at the 3–4 leaf stage unless otherwise specified. Inoculations were performed in the greenhouse. Plants were scored disease-resistant or disease-susceptible 72 h post-inoculation. For diagnosis of bacterial speck phenotypes, young expanding leaves were inoculated. To avoid possible systemic acquired resistance, the time interval between successive inoculations was spaced such that the leaves treated at each succeeding inoculation had not been visibly initiated at the time of the preceding inoculation.

Results

Resistance to *P.s. tomato* in the genus *Lycopersicon*

To confirm resistance to *P.s. tomato* race-0 and to test for resistance to race-1, *pt*11 (race-0) and *pt*14D46 (race-1) were inoculated onto all *Lycopersicon* accessions listed in Table 1. This collection of *Lycopersicon* accessions included representatives from *L. esculentum*, *L. pennelli*, *L. pimpinellifolium*, *L. hirsutum*, and *L. peruvianum*. Table 1 displays the number of plants from each accession that exhibited a resistant or susceptible phenotype with the

Table 1 *Lycopersicon* accessions tested for resistance to race-0 and race-1 *Pseudomonas syringae* pv *tomato*

Accession	*Lycopersicon* spp.	*P.s. tomato* strains[a]	
		pt11 (race-0)	pt14D46 (race-1)
Peto 238S	*L. esculentum*	R(0), S(10)	R(0), S(10)
Peto 238R	*L. esculentum*	R(10), S(0)	R(0), S(9)
LA716	*L. pennellii*	R(0), S(1)[b]	R(0), S(4)
PI 370093	*L. pimpinellifolium*	R(10), S(0)	R(0), S(9)
PI 126433	*L. pimpinellifolium*	R(4), S(6)	R(1), S(9)
PI 126449	*L. hirsutum*	R(1), S(9)	R(1), S(8)
PI 126925	*L. pimpinellifolium*	R(1), S(9)	R(3), S(7)
PI 126939	*L. pimpinellifolium*	R(3), S(7)	R(4), S(4)
PI 126946	*L. peruvianum*	R(3), S(4)	R(7), S(2)
PI 128643	*L. peruvianum*	R(1), S(9)	R(1), S(9)
PI 128650	*L. peruvianum*	R(2), S(5)	R(1), S(7)
PI 128652	*L. peruvianum*	R(7), S(1)	R(0), S(8)
PI 134417	*L. hirsutum*	R(7), S(1)	R(8), S(0)
PI 134418	*L. hirsutum*	R(7), S(3)	R(6), S(4)

[a] The number of plants exhibiting a disease-resistant (R) or disease-susceptible (S) phenotype in each population of plants is indicated in parentheses

[b] This inoculation was repeated with 12 plants. All were S

Table 2 Resistance to *P.s. tomato* race-0 in the BC_1 and BC_2 generation

Inoculation date	Inoculum (cfu/ml)	BC_1 Plants				BC_2 Plants			
		Phenotype[a]		χ^2	*P*-Value	Phenotype		χ^2	*P*-Value
		R	S			R	S		
8/18/92	2×10^6	16	21	0.68	0.3–0.5				
8/25/92	2×10^6	17	15	0.13	0.7–0.8	8	15	2.13	0.1–0.2
9/6/92	2×10^6	39	33	0.50	0.3–0.5	11	13	0.17	0.5–0.7
9/18/92	2×10^8	29	42	2.38	0.1–0.2	9	15	1.5	0.2–0.3

[a] Plants were classified as resistant (R) or susceptible (S). An intermediate phenotype (nine plants) was observed on the 8/25/92 inoculations and was pooled with R plants. A 1R:1S ratio was tested for significance

race-0 strain of *P.s. tomato*. When inoculated with *pt*11 the race-0 isolate of *P.s. tomato*, most accessions were shown to have both disease-resistant and disease-susceptible plants in the populations. Only with the inbred lines Peto 238S and LA716 were all plants susceptible to bacterial speck disease. The race-1 strain of *P.s. tomato pt*14D46 caused severe bacterial speck symptoms in all accessions tested except for the *L. hirsutum* accession PI 134417 (Table 1). As with resistance to the race-0 isolate *pt*11, most populations contained plants that were resistant and susceptible to the race-1 strain *pt*14D46; only the inbred lines Peto238S, Peto238R, LA716 and the accessions PI 370093 and PI 128652 had plants that were uniformly susceptible to *pt*14D46.

Eight *Lycopersicon* accessions had individuals resistant to both *pt*11 (race-0) and *pt*14D46 (race-1) *P.s. tomato*. The accession *L. hirsutum* var. *glabratum* PI 134417 was chosen for further genetic characterization. The genes that conferred resistance to the race-0 and race-1 strains of *P.s. tomato* derived from this accession will be referred to as *Pto3* and *Pto4*, respectively.

Phenotypic diversity of the disease-susceptible response

When plants derived from a single accession of *Lycopersicon* were compared to each other, plants could easily be classified as disease-resistant or -susceptible. In contrast, when bacterial speck lesions on different plant accessions were compared significant variation was observed. The size, presence of the chlorotic halo, and the degree of necrosis varied extensively from accession to accession. The chlorotic halo with race-0 and race-1 was always very pronounced in *L. esculentum*, but was reduced in the wild *Lycopersicon* accessions. For example, bacterial speck lesions that formed on the leaves of the *L. pimpinellifolium* accession PI 126433 lacked a chlorotic halo. In addition, the necrotic lesions caused by *pt*14D46 (race-1) was distinct from those caused by *pt*11 (race-0) on 238R and 238S. While strain *pt*11 induced brown necrotic lesions that were 0.5–1.0 mm in diameter and circumscribed by a large chlorotic halo, *pt*14D46 induced black necrotic lesions of similar size that were circumscribed by a smaller chlorotic halo. In contrast, the necrotic lesions on PI 126433 were grayish white in color. The variability in the appearance of the bacterial speck lesions suggested that *Lycopersicon* spp. might utilize different strategies to combat *P.s. tomato* infection and other factors may contribute to the development of disease symptoms.

Effect of bacterial concentration on expression of *Pto3*-mediated resistance to *P.s. tomato*

Earlier studies with interspecific *Lycopersicon* spp. hybrids had demonstrated that the concentration of *P.s. tomato* inoculum influenced expression of the disease-resistance gene *Pto1* (Stockinger and Walling, unpublished results). Homozygous *Pto1/Pto1* plants could be distinguished from *Pto1/pto1* plants by their degree of resistance to different inoculation concentrations of *P.s. tomato*. To establish bacterial concentrations that distinguish the *Pto3/pto3* heterozygotes and *Pto3/Pto3* homozygotes, Peto 238S (*pto3pto3*), PI 134417 (*Pto3Pto3*) and their F_1 hybrid (*Pto3pto3*) were inoculated with 4×10^{10}, 2×10^8, 2×10^7, or 2×10^6 cfu/ml of *pt*11 (race-0). The disease-susceptible parent Peto 238S (*pto3pto3*) displayed bacterial speck symptoms at all concentrations of *P.s. tomato* race-0 tested. The homozygous Peto 238R (*Pto1Pto1*) and PI 134417 (*Pto3Pto3*) were resistant at all concentrations of *P.s. tomato* tested. The F_1 hybrid (Peto 238S×PI 134417; *Pto3pto3*) plants were resistant when inoculated with bacterial concentrations of up to 2×10^6 cfu/ml. Increasing the bacterial concentration to 2×10^7 cfu/ml caused distinct pinpoint necrotic lesions circumscribed by chlorotic halos on the F_1 plants. Thus like *Pto1pto1* heterozygotes from a *L. esculentum* × *L. pennellii* cross (Stockinger and Walling, submitted), the interspecific background in the *Pto3pto3* heterozygotes (*L. esculentum* × *L. hirsutum* var. *glabratum*) influenced the expression of *Pto3*.

Genetic Characterization of *Pto3*

Genetic complementation between Pto1 and Pto3

To test for genetic complementation between *Pto1* and *Pto3*, the F_1 and F_2 plants from the Peto 238R (*Pto1Pto1*) × PI 134417 (*Pto3Pto3*) cross were inoculated with the *P.s. tomato* race-0 strain *pt*11. The F_1 hybrids (*Pto1pto1*/

Pto3pto3) were asymptomatic when inoculated with 2×10^6 cfu/ml but exhibited pinpoint necrotic lesions when inoculated with 2×10^8 cfu/ml. The fact the F_1 plants exhibited a disease-susceptible phenotype at the higher *P.s. tomato* inoculum indicated that the interspecific *L. esculentum/L. hirsutum* var. *glabratum* hybrid background influenced the expression of both *Pto1* and *Pto3* disease-resistance genes. These data also indicated that the disease-resistance mechanisms of *Pto1* and *Pto3* were not additive nor mechanistically similar.

Independent assortment of Pto1 and Pto3

To test for independent assortment of *Pto1* and *Pto3*, 56 F_2 progeny from Peto 238R (*Pto1Pto1*)×PI 134417 (*Pto3Pto3*) were inoculated with 2×10^6 cfu/ml of *P.s. tomato*. Only two disease-susceptible plants were observed. A chi-square test for two independently segregating genes (54R:2S=15R:1S) yielded values of 0.27 ($0.5<P<0.7$); these values were well within theoretical limits prescribed for two independent loci. However, these data were also consistent with the segregation of three independent loci (54R:2S=63R:1S) with chi-square test values of 0.25 ($0.5<P<0.7$). In this case, a third locus (××) would influence the expression of the disease-resistance gene and the triple mutant, *pto1pto1/pto3pto3/xx*, would exhibit the disease-susceptible phenotype. With either the two- or three-locus models, these data show that *Pto1* and *Pto3* segregate independently.

Pto3 inheritance in the F_2 and backcross generations

To further characterize *Pto3*, the cross Peto 238S×PI 134417 was performed. The F_2, BC_1, and BC_2 populations of plants were tested for resistance to *P.s. tomato* race-0 and race-1 strains. To follow *Pto3* inheritance in the F_2 progeny, two populations of F_2 were inoculated with *P.s. tomato pt*11 (race-0). Inoculation of 120 F_2 plants at 2×10^6 cfu/ml produced 95 disease-resistant and 25 disease-susceptible plants. A chi-square test for Mendelian single-gene inheritance (95R:25S=3R:1S) yielded a value of 1.2 ($0.2<P<0.3$) which suggested that *Pto3* was inherited as a single gene. Inoculation of 47 F_2 plants at 2×10^8 cfu/ml produced a gradation of disease-resistance phenotypes. The two discrete disease-resistant and -susceptible classes seen at the 2×10^6 cfu/ml *P.s. tomato* inoculations were not observed. Only plants that were homozygous for *Pto3* exhibited complete resistance to *P.s. tomato*; *Pto3pto3* plants gave intermediate degrees of bacterial speck symptoms and *pto3pto3* plants were fully susceptible to this pathogen (see above). For segregation analysis, asymptomatic plants (*Pto3Pto3*) and plants with intermediate phenotypes (*Pto3pto3*) were grouped (31 plants total). The observed data were not statistically different from the 3:1 ratio expected for single gene segregation.

To reduce the phenotypic ambiguity observed in the F_2, and to better establish *Pto3* inheritance, successive inoculations of 72 BC_1 progeny [Peto 238S×(Peto 238S×PI 134417)] and 20 BC_2 progeny were performed (Table 2). The first inoculation was made when the plants were at the 3–4 leaf stage using a *P.s. tomato* concentration of 2×10^6 cfu/ml. Subsequent inoculations of 2×10^6 cfu/ml and 2×10^8 cfu/ml on newly developed leaves occurred 7, 12, and 24 days after the first inoculation, respectively (Table 2). The phenotypic variability that was observed with the 2×10^8 cfu/ml inoculations of F_2 plants was greatly reduced in the BC_1 and BC_2 populations. Only in one inoculation was an intermediate class of phenotypes noted; but the fact that this variability was not eliminated reinforced the idea that the interspecific background of *L. esculentum*×*L. hirsutum* var. *glabratum* profoundly affected the disease-resistant phenotype. When resistant and susceptible plants were counted for each of the successive inoculations, a 1R:1S ratio was observed (Table 2). This ratio was consistent with single-gene inheritance.

Characterization of *Pto4*

To determine if resistance to the race-0 and race-1 strains of *P.s. tomato* segregated independently, 60 BC_1 and 22 BC_2 progeny plants were inoculated with both *P.s. tomato* races. One half of a leaflet was inoculated with pt11 (race-0) and the other side of the leaflet was inoculated with *pt*14D46 (race-1). When segregation of the *Pto3* (race-0 resistance) and *Pto4*(race-1 resistance) genes was monitored 11 *Pto3pto4*, 8 *Pto3Pto4*, 8 *pto3Pto4*, and 6 *pto3pto4* plants were counted. This distribution was consistent with a 1:1:1:1 ratio of *Pto3pto4:Pto3Pto4:pto3Pto4:pto3pto4* (chi square value=1.67; $0.1<P<0.2$) and indicated that resistance to race-0 and to race-1 *P.s. tomato* isolates segregated independently. BC_2 plants that were resistant to both race-0 and race-1 *P.s. tomato* strains have been further backcrossed to Peto 238S and also allowed to self-pollinate for future genetic characterization.

Discussion

Characterization of the genetic and molecular interaction between tomato and the causal agent of tomato bacterial speck disease, *P.s. tomato*, offers an exciting and challenging area of research into plant-pathogen interactions. *Pto1* resistance to *P.s. tomato* is well characterized genetically (Pitblado and Kerr 1980) and at the molecular level (Martin et al. 1993); the *Pto1* gene encodes a protein kinase that is likely to mediate early events in the perception and transduction of signals to activate the battery of defense-related genes that are important to the curtailment of *P.s. tomato* infection. In addition to *Pto1*, other disease-resistance mechanisms to *P.s. tomato* are found in *Lycopersicon* spp. (Pilowski and Zutra 1986). The study of these additional resistance mechanisms will allow a complete understanding of the diversity of strategies that are utilized by disparate tomato species to curb *P.s. tomato* infection. Towards

this end, we have initiated work to elucidate the interaction between two races of *P.s. tomato* and five species of *Lycopersicon*. Results of this study have suggested that the interaction between tomato and *P.s. tomato* may be complex. When representatives of different *Lycopersicon* species were analyzed for their response to *P.s. tomato*, they showed extensive variation in size, number, and severity of the bacterial speck lesions. This variation could have been accounted for by allelic differences at one locus (*Pto1*), however the results presented here suggest that the response to *P.s. tomato* is a complex event controlled by multiple factors. A new source of resistance to both race-0 and race-1 *P.s. tomato* was identified in *L. hirsutum* var. *glabratum* PI 134417 and designated *Pto3* and *Pto4*, respectively. Classical complementation analysis demonstrated that *Pto3* segregated independently of *Pto1*. *Pto3* is also distinct from the *Pto2* locus (Pilowski and Zutra 1986); *Pto1* and *Pto2* map to tomato chromosome 5 and these loci are separated by a maximum of 9 cM (Stockinger and Walling, unpublished results).

Although *Pto3* was inherited as a single gene, its expression appeared to be influenced by other modifying factors. Interspecific hybrid plants from crosses between members of the red-fruited *Lycopersicon* (*L. esculentum* and *L. pimpinellifolium*) and the green-fruited Lycopersicon (*L. pennellii* and *L. hirsutum* var. *glabratum*) failed to exhibit the same level of resistance as the parental species. In order to observe the disease-resistance phenotype in the interspecific hybrids, the bacterial inoculum had to be reduced 100-fold from 2×108 cfu/ml to 2×106 cfu/ml. This was observed not only for *Pto3* in *L. esculentum*×*L. hirsutum* var. *glabratum* hybrids (this study), but also for Pto1 in *L. esculentum*×*L. pennellii* hybrids (Stockinger and Walling, unpublished results). The impact of the modifying factors were conspicuous when F_2 plants were inoculated with 2×10^8 cfu/ml *P.s. tomato*. Rather than the detection of discrete disease-resistant or disease-susceptible plants, a continuous distribution of phenotypes was observed. The intermediate phenotypes were most likely due to genic factors from *L. hirsutum* var. *glabratum* that modified the expression of resistance in the *Pto3pto3* plants. The intermediate disease-resistance phenotypes observed with race-0 were also observed with race-1. The intermediate disease-resistance phenotypes may be due to genes that regulate or alter the initial recognition events or else accelerate the induction of defense-related genes important for curtailing pathogen invasion (Bowles 1990). Multiple controlling factors have been implicated in many host-pathogen relationships in tomato including resistance to viruses (Martin 1970; Hassan et al. 1984; Banerjee and Kalloo 1987), fungi (Maxon-Smith 1977) and nematodes (Watts 1947; Gilbert and McGuire 1952, 1956; Maxon-Smith 1977).

Since *Pto1* and *Pto3* were found to be mechanistically dissimilar and not alleles of one another, and *Pto4* is a novel gene conferring resistance to race-1 isolates of *P.s. tomato*, further investigation of these genes is warranted. Plants that exhibited an asymptomatic response to both *P.s. tomato* races have been further backcrossed to Peto 238S, as well as being allowed to self-pollinate for future genetic analysis. It is possible that once completely introgressed into the *L. esculentum* background, *Pto3* and *Pto4* will act as a completely dominant resistance genes. This will facilitate placement of *Pto3* and *Pto4* onto the tomato molecular map (Tanksley et al. 1992) and further their characterization at the molecular level.

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