Resistance to *Pseudomonas syringae* pv. *tomato* in Wild *Lycopersicon* Species

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ABSTRACT

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In 1981, 540 PI accessions and named cultivars of tomato were evaluated for resistance to *Pseudomonas syringae* pv. *tomato*, causal agent of bacterial speck of tomato. Resistance was found in *Lycopersicon esculentum, L. glandulosum, L. hirsutum, L. peruvianum,* and *L. pimpinellifolium* and in several accessions classified as known or suspected species crosses. Both symptomless reactions and symptoms of an intermediate degree of resistance were observed. The hypersensitive resistance to *P. tomato* in accessions PI 112215 of *L. pimpinellifolium*, PI 129157 of *L. hirsutum* f. *glabratum*, and *L. esculentum* 'Ontario 7710' was conditioned by a single dominant gene common to each of the three lines.

Bacterial speck of tomato is caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye & Wilkie (*P. tomato*). The pathogen has become both widespread and economically important (8). Free moisture, high relative humidity, and temperatures of 15–25 C are optimum conditions for disease development (16,19). Under these conditions, control methods such as crop rotation, sanitation, disease-free seed and transplants, and sprays of antibiotics or copper compounds have been inadequate. Resistance to bacterial speck has been reported by Gitaitis et al (7), Pilowsky and Zutra (14), Pitblado and Kerr (15), and Yunis et al (18). Pitblado and Kerr (15) reported that the resistance in *Lycopersicon esculentum* 'Ontario 7710' was expressed by a single dominant gene. Pilowsky and Zutra (13) found evidence that resistance in *L. pimpinellifolium* accession PI 126430 also was governed by a single dominant gene, different from that found in Ontario 7710.

The identification of resistant genotypes can be of value in breeding programs and in the control of bacterial speck. The results of screening 540 cultivars and PI accessions under greenhouse conditions for bacterial speck resistance are presented along with the nature and inheritance of bacterial speck resistance in two wild *Lycopersicon* spp. compared with Ontario 7710.

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MATERIALS AND METHODS

All PI accessions were obtained from the North Central Regional Plant Introduction Station, Ames, IA 50011. Seed of Chico III was obtained from A. L. Castle, Inc., Morgan Hill, CA 95037, and seed of Ontario 7710 from E. A. Kerr, Horticulture Experiment Station, Sincoe, Ontario, Canada N3Y 4N5. Seed was sown in wooden flats containing loam, peat, and perlite (1:1:1). Each flat consisted of five rows divided in half to produce 10 four-hill plots. A susceptible

check (Chico III) and nine PI lines were included per flat. Hills were thinned to one plant when the first true leaf emerged. Plants were grown on greenhouse benches until they reached the two- to three-leaf stage. At this time, the flats were placed in an inoculation chamber, a portion of the greenhouse bench equipped with water misters and enclosed in Monsanto 602 plastic.

A strain of *P. tomato* was isolated from tomato foliage lesions collected in a commercial production field at Muscatine, IA. The strain accepted a negative Gram stain and produced a green fluorescent pigment on King's B medium (10). In addition, a cytochrome oxidase reaction was observed. Pathogenicity was tested by spray inoculation and injection infiltration (11) of Chico III tomato plants.

For each of the 20 times plants were screened, *P. tomato* was reisolated from a Chico III source plant and increased for 24 hr at 25 C on Difco nutrient agar plates. Inoculum was prepared by gently scraping culture material from plates and suspending the bacteria in sterile distilled water. A Bausch & Lomb Spectronic 20 with the wavelength set at 540 nm was used to adjust the final inoculum concentration to provide an absorbance reading of 0.13. Suspensions at this optical density contained approximately 10^8 colony-forming units per milliliter, confirmed by dilution plate counts.

Inoculations were made during the winter when chamber temperatures averaged 22 ± 2 C and relative humidities were 95–98%. Plants were exposed to these conditions for 24 hr before and 48 hr after inoculation. Inoculum was applied with a DeVilbiss atomizer connected to a compressed-air line set at 5.98 kg/cm^2. The atomizer was held 10.2–15.2 cm above the plant canopy and moved so that all foliage was finely wetted. The atomizer reservoir contained 42 ml of inoculum, which was sufficient to inoculate two flats, or 80 plants; on the average, each plant received 0.52 ml of inoculum. Lines with no visible symptoms were classified as resistant. Additional plantings of resistant lines were tested for leaf hypersensitivity. A 30-gauge hypodermic needle was used to infiltrate a mature leaf's intercellular spaces with inoculum (10^8 cfu/ml). Readings were made 24 hr after infiltration. Rapid cellular collapse was classified as a positive reaction (12).

Severity of disease symptoms was determined 10–14 days after inoculation. The top two fully expanded and uniformly infected leaves of each plant were used to make the following ratings: 0 = no lesions, 1 = 1–3% of leaf area necrotic, 2 = 3–6% of leaf area necrotic, 3 = 6–12% of leaf area necrotic, 4 = greater than 12% of leaf area necrotic. These disease ratings were used to obtain a mean disease rating along with its standard deviation and a standardized disease index (DI). The DI was computed by dividing the mean disease rating of each line in a flat by the mean disease rating of susceptible Chico III grown in the same flat and multiplying the result by 100. A weighted mean for DI was calculated when a line was grown in more than one flat. All lines were compared with the susceptible check (Chico III, DI = 100).

To determine whether resistance to *P. tomato* in PI 112215 of *L. pimpinellifolium*, PI 129157 of *L. hirsutum* f. *glabratum*, and *L. esculentum* 'Ontario 7710' was based on different genes, a diallel crossing scheme was initiated that included the susceptible *L. esculentum* 'Chico III.' All possible crosses were attempted. PI 129157 showed a unilateral incompatibility with the other species and was crossed successfully only when used as a female parent. Parent, F_1, and F_2 seedlings from the diallel were tested for resistance by spray inoculation. Parent and F_1 plants were also tested by injection infiltration.

Table 1. Reaction of 540 PI accessions representing 13 *Lycopersicon* spp. to *Pseudomonas syringae* pv. *tomato*

Population	No. of lines	Mean score[a]	Mean DI[b]	No. of symptomless lines
Tetraploids	1	0.18	6	1
Lycopersicon glandulosum	9	0.82	34	4
L. hirsutum	13	0.11	4	12
L. hirsutum f. *glabratum*	8	0.05	2	8
L. peruvianum	30	0.67	29	17
L. peruvianum var. *humifusum*	3	1.58	63	0
L. cheesmanii f. *minor*	3	2.25	114	0
L. pimpinellifolium	210	0.90	35	117
L. esculentum × *L. hirsutum*	4	1.18	43	2
L. esculentum × *L. peruvianum*	4	1.39	54	0
L. esculentum × *L. pimpinellifolium*	2	2.71	94	0
L. esculentum × *L. pimpinellifolium* (suspected)	153	1.96	76	14
L. esculentum	100	1.75	66	11

[a] Weighted mean: 0 = no lesions, 1 = 1–3% leaf necrosis, 2 = 3–6% leaf necrosis, 3 = 6–12% leaf necrosis, 4 = greater than 12% leaf necrosis.
[b] Weighted mean of a standardized disease index (DI): Chico III = 100.

Table 2. Large-fruited *Lycopersicon* accessions and selected commercial cultivars resistant to *Pseudomonas syringae* pv. *tomato*

PI code	Source	Species[a]	Fruit diameter (cm)
Niagara 315 VF	United States	17	...[b]
Sweet 100	United States	17	...
Ontario 7710	Canada	17	...
303726[c]	Canada	17	3.8
358815	Malaysia	17	2.5
375937	United States	12	2.0
205018	United States	16	2.0
251322	Ecuador	16	2.0
370080[d]	Canada	17	2.0
269140	Netherlands	13	1.9
126443	Peru	6	1.7
134418	Ecuador	8	1.7
128660	Peru	9	1.5
129156	Ecuador	16	1.5

[a] 6 = *L. glandulosum*, 8 = *L. hirsutum* f. *glabratum*, 9 = *L. peruvianum* including var. *humifusum*, 12 = *L. pimpinellifolium*, 13 = *L. esculentum* × *L. hirsutum*, 16 = *L. esculentum* × *L. pimpinellifolium* (suspected), 17 = *L. esculentum* including f. *pyriforme*.
[b] Data not available.
[c] Earlinorth.
[d] Sub-Arctic Delight.

RESULTS AND DISCUSSION

Bacterial speck resistance was prevalent among the tested lines (Table 1); 140 (26%) of the lines had a DI of 10 or less, whereas 237 (44%) had a DI of 50 or less. Many of these lines had symptomless reactions, similar to those of Ontario 7710, when inoculated with *P. tomato*. Numerous accessions were heterogeneous in their response to inoculation. These lines could often be identified by their large standard deviations for mean score. PI accessions that had symptomless reactions to spray inoculation were found in *L. esculentum*, *L. glandulosum*, *L. hirsutum* (including f. *glabratum*), *L. peruvianum* (including varieties *dentatum* and *humifusum*), and *L. pimpinellifolium*; several lines were also identified as species crosses. All three evaluated accessions of *L. cheesmanii* had ratings similar to those of susceptible Chico III.

Some plants produced lesions with very small necrotic areas surrounded by a large chlorotic halo. Campbell 28 (DI = 60) and Ohio 736 (DI = 42) were

Table 3. Evaluation of parent, F_1, and F_2 diallel progenies of tomato for resistance to *Pseudomonas syringae* pv. *tomato*

Population	No. of plants	Percent of plants by disease ratings[a] 0	1	2	3	Observed R:S	Expected R:S	Model	Chi-square	*P* value
Chico III (P_1)	143	0	1	17	82	0:143	0:143	0:1	...	...
Ontario 7710 (P_2)	56	100	0	0	0	56:0	56:0	1:0	...	...
PI 112215 (P_3)	47	96	4	0	0	45:2	47:0	1:0	...	...
PI 129157 (P_4)	32	94	6	0	0	30:2	32:0	1:0	...	...
($P_1 \times P_2$)	6	100	0	0	0	6:0	6:0	1:0	...	...
($P_2 \times P_1$)	8	100	0	0	0	8:0	8:0	1:0	...	...
($P_1 \times P_3$)	4	100	0	0	0	4:0	4:0	1:0	...	...
($P_3 \times P_1$)	8	88	12	0	0	7:1	8:0	1:0	...	...
($P_1 \times P_4$)	2	100	0	0	0	2:0	2:0	1:0	...	...
($P_2 \times P_3$)	8	100	0	0	0	8:0	8:0	1:0	...	...
($P_3 \times P_2$)	8	88	12	0	0	7:1	8:0	1:0	...	...
($P_2 \times P_4$)	8	100	0	0	0	8:0	8:0	1:0	...	...
($P_3 \times P_4$)	2	100	0	0	0	2:0	2:0	1:0	...	...
($P_1 \times P_2$) PRF_2[b]	191	75	1	9	15	143:48	143.25:47.25	3:1	0.002	0.97
($P_1 \times P_3$) PRF_2	193	75	3	7	15	145:48	144.75:48.25	3:1	0.002	0.97
($P_1 \times P_4$) F_2	173	78	5	9	8	136:37	129.75:43.25	3:1	1.204	0.27
($P_2 \times P_3$) F_2	98	97	3	0	0	95:3	98:0	1:0	...	...
($P_3 \times P_2$) F_2	97	100	0	0	0	97:0	97:0	1:0	...	...
($P_2 \times P_4$) F_2	138	98	2	0	0	135:3	138:0	1:0	...	...
($P_3 \times P_4$) F_2	96	100	0	0	0	96:0	96:0	1:0	...	...

[a] Plants with a score of 0 were considered resistant.
[b] PRF_2 = pooled reciprocal F_2 population data.

two such cultivars. DI scores for these lines were quite variable from test to test; this type of resistance may be strongly influenced by the environment. Recently, Gitaitis et al (7) reported that Campbell 28 and Ohio 7663 possessed a level of field resistance to bacterial speck in tomato transplant fields; both Ohio 736 and Ohio 7663 have Campbell 28 in their parentage (2,3).

Since 20% of the randomly chosen commercial cultivars and accessions in this study were resistant, a systematic screening of additional cultivars and PI accessions for resistant globe-fruited lines of large size, up to 8 cm in diameter, could speed the incorporation of these genes into fresh-market tomato lines (Table 2). In addition, resistant sources genetically different from that found in Ontario 7710 should be sought and used. The combination of two different sources of resistance might be more durable if new pathogenic races of the bacterial speck pathogen become prevalent.

Numerous scattered leaf spots typical of bacterial speck infections were found on Chico III after spray inoculation. Ontario 7710, PI 112215, PI 129157, and all F_1 plants remained free from these symptoms. Necrotic areas developed on the youngest expanding leaf of a few plants at the time of inoculation. These atypical symptoms account for the 1 ratings that caused segregation ratios to deviate from the ratios expected (Table 3). In this genetic study, only plants completely free from symptoms (0 ratings) were considered resistant. In contrast, all resistant parents and all F_1 progeny produced a hypersensitive reaction after infiltration inoculation with the bacterium. A plant defense mechanism may operate at the cellular level in these lines (17).

Resistant × susceptible F_2 populations produced a good fit to a 3:1 resistant:susceptible model (Table 3). Therefore, the resistance to bacterial speck observed in Ontario 7710, PI 112215, and PI 129157 was controlled by a single, dominant gene. Furthermore, because susceptible segregates were not found in resistant × resistant crosses, the three different sources of resistance are based on the same gene. Either PI 112215 or PI 129157 could serve as a source of multiple disease resistance (5). PI 112215 possesses resistance to early blight (1), target leaf spot (4), Cladosporium leaf mold (9), and bacterial wilt (1). PI 129157 carries resistance to both the carmine and the two-spotted spider mite (6) and to early blight (1). No evidence was found, however, that the bacterial speck resistance in these two lines was superior to or different from that found in Ontario 7710.

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