

# Screening Wild Tomatoes for Resistance to Bacterial Speck Pathogen (*Pseudomonas tomato*)

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## ABSTRACT

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Plants of 19 wild *Lycopersicon* accessions were tested in the greenhouse for resistance to *Pseudomonas tomato*, which causes bacterial speck. Plants of *L. pimpinellifolium* (PI 126430), *L. peruvianum* (PI 128643 and 128650), and *L. hirsutum* f. *glabratum* (PI 134417 and 134418) remained symptomless. Individual plants of *L. pimpinellifolium* (PI 126433, 126925, and 126939) and *L. peruvianum* (PI 126946 and 128652) also showed a high level of resistance to the pathogen. Genetic studies indicated that resistance of *L. pimpinellifolium* in accession PI 126430 to *P. tomato* was dominant and conditioned by a single gene.

Bacterial speck of tomato (*Lycopersicon esculentum* Mill.), caused by *Pseudomonas tomato* (Okabe) Alstatt, is characterized by foliar and fruit spotting (7). The disease was first detected in Israel in 1970 on tomato plants of cultivars used for processing (6), and since then it has become increasingly prevalent in fields of both fresh market and processing cultivars. Although diseased plants may be found throughout the year in commercial plots, bacterial speck is most commonly found in early spring and fall plantings.

*P. tomato* is a seedborne pathogen. Chambers and Merriman (2) and Bashan et al (1) reported isolating the pathogen from commercial seeds that produced severely infected plants in the field. Although fermentation extraction procedures and surface sterilization of seed appear to eliminate *P. tomato* (1,2), infection may still occur in the field because the pathogen can survive and the disease develop from inoculum in soils and plant debris (1,2,5).

Despite the use of recommended control measures, such as seed disinfection and foliar sprays with copper hydroxide preparations, bacterial speck continues to occur and sometimes presents a serious problem to local farmers. A search for sources of resistance to *P. tomato* from tomato germ plasm collections was thus initiated

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at the Volcani Center in 1977. First, a large number of *L. esculentum* cultivars and breeding lines from different genetic backgrounds were tested for resistance to *P. tomato*, but all were rated susceptible (Zutra and Pilowsky, unpublished). Therefore, a search for resistance to *P. tomato* in wild *Lycopersicon* material was conducted. In this paper, we report the presence of a high level of resistance to the bacterial speck pathogen in wild relatives of the cultivated tomato.

## MATERIALS AND METHODS

**Pathogen cultures.** Isolate 134, a single colony isolate of *P. tomato* from infected

tomato foliage collected in the field, was used in all tests. Cultures were tested for pathogenicity in the greenhouse on tomato seedlings of the susceptible cultivar VF 134-1-2 (Peto Seed Co., Inc., Saticoy, CA 93003). Stock cultures were maintained at room temperature on Difco nutrient agar slants containing 1% glycerol and renewed every 3-4 wk.

**Inoculum preparation.** Cultures were grown at 28 C on Difco nutrient agar containing 1% glycerol. Inoculum was prepared by suspending the growth of 24- to 48-hr agar slant cultures with sterile distilled water and 0.001% Tween-20. Cell concentration was determined by a standard curve calibrated by dilution plating and spectrophotometer procedure at 570 nm, and final inoculum concentration was adjusted to about  $10^8$  colony-forming units per milliliter with sterile distilled water.

**Inheritance of resistance in *L. pimpinellifolium* (PI 126430).** The inheritance of resistance to the bacterial speck pathogen was studied in the greenhouse. Crosses were made between accession PI 126430 and a susceptible line of *L. esculentum* (no. 10) to obtain seeds of the F<sub>1</sub> and F<sub>2</sub> generations. The F<sub>1</sub>

**Table 1.** Reaction of individual plants of 19 accessions of wild species of tomato to *Pseudomonas tomato*<sup>a</sup>

<i>Lycopersicon</i> sp.	No. of plants with disease rating <sup>b</sup> of				
	0	1	2	3	4
<i>L. pimpinellifolium</i>					
PI 79532	0	0	2	5	0
PI 124039	0	1	0	4	1
PI 126430	20	0	0	0	0
PI 126433	6	0	0	0	4
PI 126925	2	7	0	0	1
PI 126939	2	3	3	2	1
CIAS 27	0	0	4	3	8
Line 425	0	0	0	0	16
Line 426	0	0	1	10	6
Line 429	0	0	0	7	0
Line 752	0	0	1	10	6
LA 121	0	0	0	0	12
<i>L. peruvianum</i>					
PI 126946	2	3	0	8	0
PI 128643	9	0	0	0	0
PI 128650	8	0	0	0	0
PI 128652	5	5	0	0	0
<i>L. hirsutum</i> f. <i>glabratum</i>					
PI 126449	0	0	5	13	1
PI 134417	6	0	0	0	0
PI 134418	20	0	0	0	0

<sup>a</sup>Seeds were obtained from the North Central Regional Plant Introduction Station, Ames, IA; C. M. Rick, University of California, Davis; and the National Institute for Agricultural Research, Celaya, Mexico.

<sup>b</sup>Disease ratings from 0 = symptomless leaves to 5 = more than 40 lesions per plant. Twenty plants of the susceptible control *L. esculentum* VF 134-1-2 received a rating of 4.

hybrid was backcrossed to line 10 and to accession 126430.

**Seedling preparation, inoculation, and evaluation of resistance.** Seeds of 19 wild *Lycopersicon* accessions (listed in Table 1) and *L. esculentum* (VF 134-1-2) were sown in the greenhouse in flats containing a 1:2:1 (v/v) mixture of peat, loam, and vermiculite. Seven days after sowing, the seedlings were transplanted to 5-cm-diameter plastic pots (one plant per pot) containing the same mixture. When plants reached the four-leaf stage, six to 20 plants of each accession were individually inoculated with *P. tomato* by atomizing the prepared bacterial suspension onto the upper and lower surfaces of all the leaves with an electric sprayer. Three to five seedlings of each accession were sprayed in a similar manner with distilled water as uninoculated control plants.

After inoculation, the plants were placed in a plant growth chamber at 25 C under a misting device for 72 hr and then transferred to a greenhouse chamber set at 25–28 C and illuminated daily for 12 hr.

Ten days after inoculation, lesions were counted on all the leaves of the test plants. Plants were scored according to the following scale: 0 = no lesions; 1 = 1–10 lesions per plant; 2 = 11–20 lesions; 3 = 21–40 lesions; and 4 = more than 40 lesions per plant (2).

## RESULTS

**Screening test.** The data of the screening test (Table 1) indicate the existence in the wild material of a type of high resistance to *P. tomato*, ie, symptomless reaction. Symptomless plants were recorded in 10 of 19 accessions tested. Five accessions contained only symptomless plants: PI 126430 (*L. pimpinellifolium*), PI 128643 and 128650 (*L. peruvianum*), and PI 134417 and 134418 (*L. hirsutum* f. *glabratum*). Five additional accessions, in which distinctly different responses were found, had symptomless individuals: PI 126433, 126925, and 126939 (*L. pimpinellifolium*); and PI 126946 and 128652 (*L. peruvianum*).

The five accessions in which all the inoculated plants were completely symptomless were retested in the greenhouse with 15–25 plants of each accession inoculated again with *P. tomato*. No lesions appeared on inoculated leaves in plants of any of these accessions, whereas 45–80 lesions per plant were counted in the susceptible *L.*

**Table 2.** Reaction of F<sub>1</sub>, F<sub>2</sub>, and backcross (BC) progenies from the cross between *Lycopersicon esculentum* (line 10) and *L. pimpinellifolium* (PI 126430) to *Pseudomonas tomato*<sup>a</sup>

Generation	Parental line or cross	Number of observed plants		Chi square	P value
		Resistant <sup>b</sup>	Susceptible <sup>c</sup>		
P <sub>1</sub>	PI 126430	60	0		
P <sub>2</sub>	Line 10	0	60		
F <sub>1</sub>	Line 10 × PI 126430	65	0		
F <sub>2</sub> <sup>d</sup>	F <sub>1</sub> selfing	227	85	0.837	0.25–0.50
BC <sub>1</sub> F <sub>1</sub> <sup>e</sup>	F <sub>1</sub> × line 10	102	104	0.019	0.75–0.90
BC <sub>1</sub> F <sub>1</sub>	F <sub>1</sub> × PI 126430	35	0		

<sup>a</sup>Plants growing in 5-cm-diameter pots were inoculated at the four-leaf stage.

<sup>b</sup>Resistant = plants with symptomless leaves.

<sup>c</sup>Susceptible = plants with 50–90 lesions.

<sup>d</sup>Expected ratio of resistant:susceptible is 3:1, or 234:78.

<sup>e</sup>Expected ratio of resistant:susceptible is 1:1, or 103:103.

*esculentum* control, VF 134-1-2. Recovery tests made later from infected plants of this cultivar confirmed the presence of the pathogen.

**Inheritance of resistance in *L. pimpinellifolium* (PI 126430).** The *L. esculentum* × *L. pimpinellifolium* F<sub>1</sub> plants inoculated with *P. tomato* responded with a symptomless reaction, similar to the resistant parent, accession 126430 (Table 2). F<sub>2</sub> progenies segregated in a 3:1 ratio of three resistant (symptomless) to one susceptible. Plants of the backcross to the susceptible parent, line 10, segregated in a 1:1 ratio of one resistant to one susceptible. Backcross progenies to the resistant parent, accession 126430, were all resistant. These data suggest the dominance of a single factor for resistance to *P. tomato*.

## DISCUSSION

Rick (4) has emphasized the potential value of natural variability in wild species of tomato as sources of germ plasm for varietal improvement, including disease resistance. The discovery of a high degree of resistance to *P. tomato* in 10 of 19 wild accessions evaluated in this study (Table 1) clearly supports this idea. It is not known whether the type of high resistance (symptomless reaction) found in the 10 accessions was caused by the same or a different set of genes. It seems probable from the diverse origin of most of these accessions that different factors of resistance were involved.

Genetic data obtained from the F<sub>1</sub>, F<sub>2</sub>, and backcross generations indicate that resistance to *P. tomato* in accession PI 126430 of *L. pimpinellifolium* (the most closely related of the tomato species to the cultivated tomato, *L. esculentum*) was dominant and conditioned by a single

gene (Table 2). Recently, Pitblado and Kerr (3) reported a high level of resistance to *P. tomato* in Ont. 7710, an experimental cultivar of *L. esculentum* with a complex ancestry. They suggested that resistance might be simply inherited. When plants of Ont. 7710 were inoculated with the same isolate of *P. tomato* used in this study (isolate 134), the plants did not show any symptoms. On the other hand, *L. esculentum* cultivars Rehovot-13 and Hosen-Eilon, which are reported to have resistance to the pathogen (8), are susceptible to isolate 134 (Zutra and Pilowsky, unpublished). Studies are under way to determine whether resistance to *P. tomato* in Ont. 7710 and in accession 126430 of *L. pimpinellifolium* is based on the same or different genes. Trials will be carried out to test their reaction to *P. tomato* under field conditions.

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