Differential Resistance of Tomato Cultigens to Biovars I and III of *Pseudomonas solanacearum*

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ABSTRACT

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The reaction of six tomato cultigens (Yoshimatsu 4-11, Rotam-4, Rodade, Hawaii 7998, CL 1131-0-0-13-0-6, and Irat L3) to *Pseudomonas solanacearum* biovars I and III was evaluated in a greenhouse trial. Seedlings were root-inoculated with 11 strains of biovar I and nine strains of biovar III. The plants were individually scored 15 days after inoculation for wilt severity on a scale from 1 (no symptoms) to 5 (dead plant). Cluster analysis grouped strains of biovar III in a lower virulent cluster than those of biovar I. Yoshimatsu 4-11, Rotam-4, and Rodade had specific resistance to biovar III but not to biovar I.

Bacterial wilt caused by *Pseudomonas solanacearum* (Smith) Smith is one of the most damaging diseases of tomato (*Lycopersicon esculentum* Mill.) worldwide. It is particularly limiting when the crop is grown in humid climates at low and medium elevations in tropical and subtropical regions.

One of the most striking characteristics of *P. solanacearum* is the high variability observed among its strains, which can differ in host range, geographic distribution, pathogenicity/virulence, transmissibility by insects, physiological properties, and adaptation to different temperatures (7,11,12). Because no better classification is yet available (7), strains of *P. solanacearum* are separated into races or into biovars, even though these subdivisions are informal groupings, i.e., not accepted by the International Code of Nomenclature of Bacteria (23).

Buddenhagen et al (5) proposed three races; the number was later expanded to five when ginger and mulberry (1,13) were reported as susceptible hosts. The five known biovars were defined on the basis of different abilities of strains to utilize and/or oxidize several hexose alcohols and disaccharides (10,12). Races and biovars are poorly correlated, except for race 3, which may be equivalent to biovar II (4,8). This lack of concordance is not surprising, since races are defined by host range (ecological characters) and biovars are defined through biochemical tests (phenotypic characters) (9). The race subdivision for *P. solanacearum* is not taxonomically valid, since it is not based on the pathogenic differential ability to induce disease on cultivars of the same species (25), and the biovar system is sometimes considered of little biological meaning because it does not represent the pathogenic potential of the strain or its geographic origin (4).

Tomatoes are cultivated throughout Brazil, and *P. solanacearum* is widely distributed as different biovars/races (21). However, the crop has been affected only by biovars I and III (*unpublished*). Northern and northeastern Brazil are the regions most affected because of the high ambient temperatures. A national survey on vegetable crops indicated that biovar III prevails in those regions, even though biovar I could also be isolated from tomato, pepper, and eggplant.

For breeding purposes, it is essential to know if tomato cultigens react differently when infected by different pathogen biovars. The objective of this work was to establish if tomato resistance to bacterial wilt is strain- or biovar-dependent.

MATERIALS AND METHODS

Bacterial cultures. Eleven strains of *P. solanacearum* biovar I and nine of biovar III (Table 1), from different locations in Brazil, were recovered from a culture collection in CNPH, Brasília, DF, where they were stored in sterile tap water in screw-cap tubes at room temperature (25 C). From these tubes, cultures were streaked on tetrazolium-chloride (TZC) medium (14), and fluidal colonies, typical of virulent specimens, were selected after 72 hr of incubation at 28 C. The selected colonies were restreaked on the same medium devoid of TZC and incubated for 48 hr at the same temperature. The inocula were then prepared by flooding the plates with tap water (pH 7.0), suspending the cells, and adjusting the concentration to approximately 10^8 cfu/ml, according to a previously calibrated absorbance curve, with a spectrophotometer at 600 nm.

Host plants. The tomato cultigens assayed for bacterial wilt resistance were Yoshimatsu 4-11, Hawaii 7998, Irat L3, CL 1131-0-0-13-0-6, Rotam-4, and Rodade. The first four were obtained, respectively, from the National Institute for Research in the Amazon (INPA/

Table 1. Strains of *Pseudomonas solanacearum* biovars I and III from different host plants and locations in Brazil

No.	CNPH accession no.	Biovar	Host	Origin[a]	Year
1	8	I	Tomato	Amazonas (N)	1987
2	13	I	Potato	Distrito Federal (MW)	1987
3	16	I	Tomato	Amazonas (N)	1987
4	19	I	Tomato	Pará (N)	1987
5	31	I	Tomato	Amapá (N)	1988
6	42	I	Tomato	Amapá (N)	1988
7	74	I	Tomato	Amapá (N)	1990
8	100	I	Tomato	Pernambuco (NE)	1992
9	101	I	Tomato	Pernambuco (NE)	1992
10	102	I	Tomato	Pernambuco (NE)	1992
11	43	I	Tomato	Amapá (N)	1988
12	18	III	Tomato	Amazonas (N)	1987
13	20	III	Pepper	Pará (N)	1987
14	33	III	Tomato	Amapá (N)	1988
15	41	III	Tomato	Amapá (N)	1988
16	47	III	*Solanum gilo*	Amazonas (N)	1981
17	53	III	Eggplant	Maranhão (NE)	1984
18	55	III	Pepper	Pernambuco (NE)	1989
19	56	III	Eggplant	Pernambuco (NE)	1989
20	62	III	Tomato	Pernambuco (NE)	1990

[a] Letters in parentheses represent geographic regions within Brazil: N = north, MW = midwest, NE = northeast.

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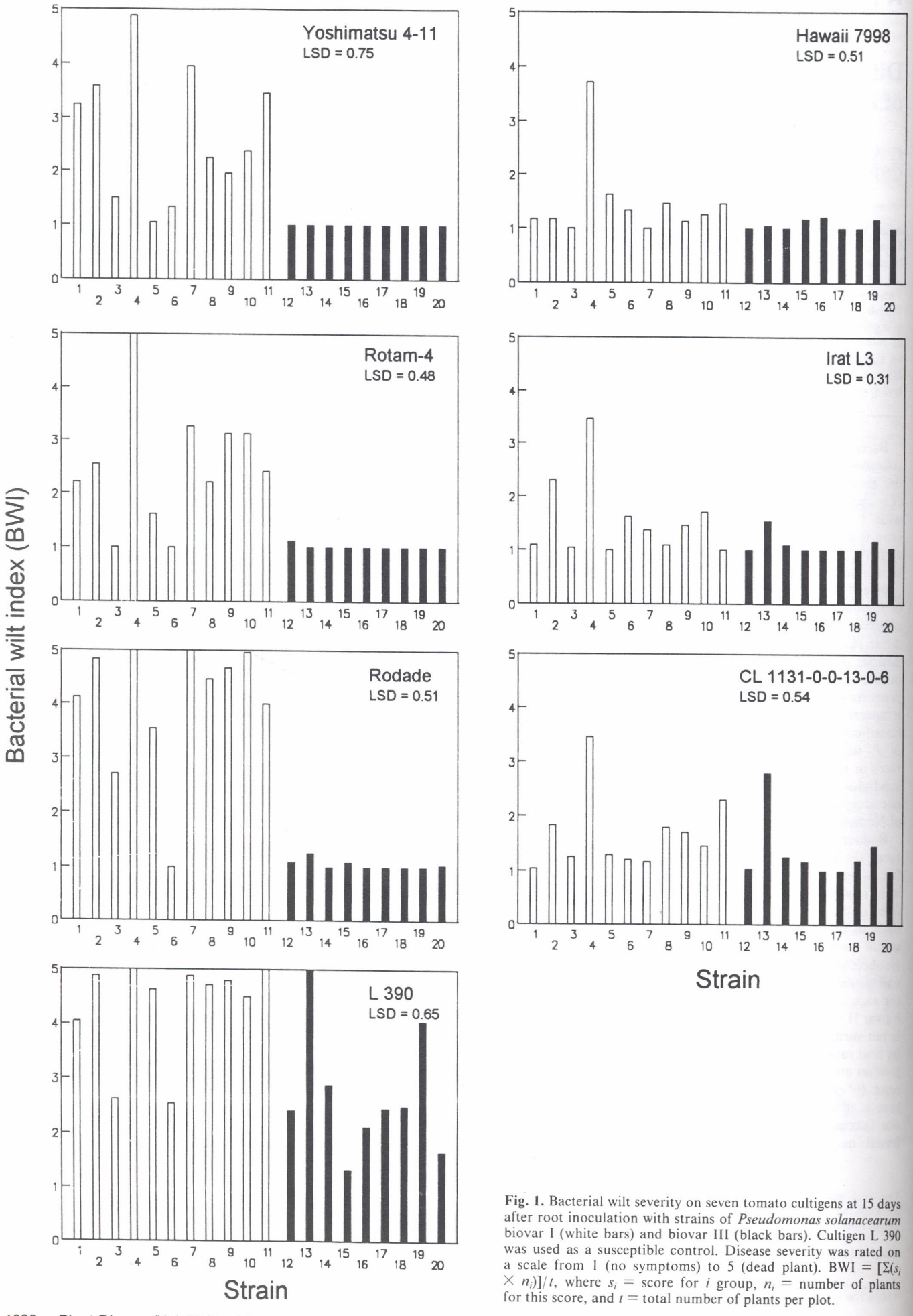

Fig. 1. Bacterial wilt severity on seven tomato cultigens at 15 days after root inoculation with strains of *Pseudomonas solanacearum* biovar I (white bars) and biovar III (black bars). Cultigen L 390 was used as a susceptible control. Disease severity was rated on a scale from 1 (no symptoms) to 5 (dead plant). BWI = $[\Sigma(s_i \times n_i)]/t$, where s_i = score for i group, n_i = number of plants for this score, and t = total number of plants per plot.

Brazil), the University of Florida (USA), INRA (France), and AVRDC (China); Rotam-4 and Rodade were provided by the Vegetable and Ornamental Plant Research Institute (South Africa). All were reported previously as having some resistance to bacterial wilt (2,3,16,19, 22,24). The cultigen L 390, also from AVRDC, was used as a susceptible control. The seeds were sown in polystyrene trays containing sterilized field soil and placed in a greenhouse.

Inoculation. The seedlings were inoculated at the two-true-leaf stage (15 days after sowing) by dipping the roots, which had been washed in tap water and severed at one-third from the lower extremity, for 1 min into the bacterial suspension (26). The seedlings were then transplanted promptly to sterile soil in 0.5-L plastic pots. During the first 5 days after inoculation, the greenhouse temperature at night was maintained above 20 C; the day temperature was always maintained at 20–40 C.

Disease assessment. The wilt severity was recorded on individual plants at 15 days after inoculation. The following scale, modified from Winstead and Kelman (26), was used: 1 = no symptoms, 2 = up to one-third of the leaves wilted, 3 = from one-third to two-thirds of the leaves wilted, 4 = all of the foliage wilted with the possible exception of the terminal bud, and 5 = dead plant. A bacterial wilt index (BWI) was calculated for each plot: $BWI = [\Sigma(s_i \times n_i)]/t$, where s_i = score for i group, n_i = number of plants for this score, and t = total number of plants per plot.

Experimental design and statistical analysis. A completely randomized two-way factorial design (tomato cultigens and bacterial strains) was used with three replications. The experimental unit consisted of two pots each with four seedlings. Analysis of variance of BWI was done with MSTAT by Russel P. Feed, Michigan State University. The data from all cultigens collectively were submitted to cluster analysis with the simple linkage method and the computer package FITOPAC by George Shepherd, University of Campinas/UNICAMP, Brazil.

RESULTS

Analysis of variance for BWI revealed significant differences ($P < 0.05$) among tomato cultigens, bacterial strains, and the interaction between cultigens and strains, with a coefficient of variation of 16.44%. When the BWIs for each cultigen were plotted against strains of the two biovars in separated groups, the virulence of biovar III was generally lower than that of biovar I (Fig. 1). Since strains and the interaction between cultigens and strains were significantly different, a cluster analysis was performed to compare the virulence of bacterial strains and to determine whether strains of the same biovar followed a defined pattern of clustering. Within three clusters, the strains of the same biovar were placed in the same group, except for strains 3 and 6 of biovar I. Because these were the least virulent to all the tomato cultigens, they were grouped into the cluster of biovar III. Alternatively, the most virulent strain from biovar I, strain 4, was separated into a third group (Fig. 1). To avoid confusion about group comparisons among strains of different biovars, we recommend that the virulence of the strains be evaluated previously in a universal susceptible cultigen such as L 390 (Fig. 1). The cultigens Hawaii 7998, Irat L3, and CL 1131-0-0-13-0-6 were resistant to most of the strains of both biovars I and III except for strain 4. In contrast, Yoshimatsu 4-11, Rotam-4, and Rodade were generally resistant to the strains of biovar III but susceptible to the strains of biovar I. Moreover, the reaction of Rodade to biovar I strains was similar to that of the susceptible control, L 390.

DISCUSSION

Variability in virulence among strains of *P. solanacearum* has been mentioned by many authors (13,15,20,24). Martins et al (17) previously observed variability in virulence among strains of biovar I and biovar III from different locations in Brazil, but they could not compare biovar differential virulence because their trials were conducted at different times. Prior et al (20) suggested a linkage between virulence to tomato cultigens and biovar classification. Strains that were more virulent on the cultigen Capitan (moderately resistant) belonged to biovar I, whereas the less virulent strains belonged to biovar III. Cultigen Caraïbo (resistant) was consistently resistant to strains of both biovars.

Our results support the hypothesis that biovars I and III differ pathogenically and therefore support the division of *P. solanacearum* into two or more subspecies governed by the International Code of Nomenclature of Bacteria. Hayward (12) suggested that biovars I and III belong to different groups because biovar I is less nutritionally versatile than biovar III, the two biovars are distinct on the basis of DNA probes and RFLP analysis, and the two biovars may be of separate evolutionary origin based on their geographic distribution (6). These criteria support separation into distinct phenons by numerical taxonomy, according to Hayward (12).

This study makes it clear that there are some tomato cultigens for which resistance is biovar-specific. We believe this is taxonomically meaningful, since it is linked directly to the epidemiology and control of bacterial wilt. This was apparent particularly in Yoshimatsu 4-11, Rotam-4, and Rodade (Fig. 1). The differential resistance of these cultigens to biovars I and III could be compared with that of cultigen Capitan, evaluated by Prior et al (20). Therefore, at least for these two biovars, Buddenhagen's statement (4) that "biovar subdivision appears to have little relation to the evolutionary or existing biology of the strains as pathogens" was not confirmed. These results were surprising, since we expected that tomato cultigen response would be only strain-dependent, and not biovar-dependent. Our expectation arose from Buddenhagen's comment on the bias of bacteriologists who attempt to rationalize their results with Hayward's biovars (4).

The breeding materials that possess resistance to only biovar III could have an important role in breeding for tomato resistance to bacterial wilt in the Brazilian environment if their resistance is also heat-stable, because biovar III prevails in regions where high temperatures predominate. Mew and Ho (18) suggested that bacterial wilt resistance can be either dependent or independent of soil temperature, with 32 C being the temperature that separates them. This was also observed by Prior et al (20) in relation to the cultigens Caraïbo and Capitan, which have heat-stable and heat-unstable resistance, respectively. The linkage between virulence of the strains belonging to biovar III and high temperatures, especially high soil temperatures, to some tomato cultigens is still to be determined.

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Characterization of Two Viruses Isolated from Patchouli in Japan

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ABSTRACT

Natsuaki, K. T., Tomaru, K., Ushiku, S., Ichikawa, Y., Sugimura, Y., Natsuaki, T., Okuda, S., and Teranaka, M. 1994. Characterization of two viruses isolated from patchouli in Japan. Plant Dis. 78:1094-1097.

Two sap-transmissible viruses were isolated from patchouli (*Pogostemon patchouli*) in Japan. The virus source plants showed faint mosaic, mottling, or no symptoms. In electron microscopic examinations, however, elongated (760 nm in length) or spherical (27 nm in diameter) viruslike particles were found. The spherical virus, designated patchouli mild mosaic virus (PaMMV), infected plants in seven families and was found to be serologically related to, but different from, broad bean wilt virus. The elongated virus, named patchouli mottle virus (PaMoV), had a narrower host range and was identified as a member of the Potyviridae on the basis of particle morphology, formation of cytoplasmic inclusions, and a distant serological relationship with turnip mosaic potyvirus.

Patchouli (*Pogostemon patchouli* Pellet. = *P. cablin* (Blanco) Benth.), a member of Labiatae, is vegetatively propagated in Indonesia, the Philippines, and other Southeast Asian countries for aromatic oils used in soaps and other toiletries. Many propagants have viruslike symptoms and reductions in yields within several years after first planting. Viruses reported in patchouli include patchouli mosaic virus (PaMV) (2), tobacco necrosis virus, and a rhabdovirus (3,4), but few studies have been conducted for viruses in patchouli in Asia.

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Patchouli plants from Southeast Asia are being grown in several botanical gardens and experimental farms in Japan, and many of the plantings have high incidences of viruslike symptoms. The identification and characterization of two viruses isolated from the plants and designated patchouli mild mosaic virus (PaMMV) and patchouli mottle virus (PaMoV) are reported here. A preliminary report was published (7).

MATERIALS AND METHODS

Sources of virus isolates. Patchouli plants originating from Southeast Asia were established in several locations in Japan. Many plants showed symptoms of mild mosaic or mottle while others appeared healthy. Plants used in this study were collected from two botanical gardens and maintained in glasshouses. On the basis of host reactions of some differential indicator plants to inocula derived from the patchouli collections and on electron microscopy of the patchouli and indicator plants, two virus isolates, PaMMV and PaMoV, were selected for further study.

Host range. All indicator plants were grown from seeds and maintained in aphid-free greenhouses or air-conditioned glass chambers at 20–25 C with a day length of about 12 hr. Inoculations were made by rubbing tissue extracts ground in 0.1 M phosphate buffer (pH 7.0) on indicator plants dusted with 600- or 800-mesh Carborundum. In some instances, a small amount of a Celite-bentonite mixture (1:1) was added directly to the extract. Indicator plants were observed for up to 2 mo for symptom development. Latent infections were detected by inoculation to *Chenopodium amaranticolor* Coste & Reyn. or *C. quinoa* Willd. or by electron microscopy.

Electron microscopy. Extracts from infected plants of both PaMMV and PaMoV were negatively stained with 2% phosphotungstic acid (pH 6.0) and observed with a transmission electron microscope (JEM-100CX or 100SX). Ultrastructural studies were done with the infected leaf tissues fixed in 2% glutaraldehyde, followed by 2% osmium tetroxide, and embedded in EPON 812. Immunoserological electron microscopy