Identification of Biovars and Races of *Pseudomonas solanacearum* and Sources of Resistance in Tomato in Nepal

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ABSTRACT

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Twenty-five strains of *Pseudomonas solanacearum* isolated from plants in Nepal were characterized by physiological and biochemical tests and by pathogenicity on seven hosts. Based on Hayward's classification scheme, eight strains were biovar II and 17 strains were biovar III. Results of pathogenicity tests showed that eight strains were race 3 and 17 were race 1. This is the first report of biovar III and race 1 in Nepal. Fifteen tomato cultivars were screened for resistance to *P. solanacearum* (race 1) in a greenhouse test. Incubation period, number of days required to reach 10% wilting, and average percentage of wilting were used as components of disease resistance. Four of the cultivars tested were susceptible, six were moderately susceptible, three were moderately resistant, and two were resistant. The cultivars CL1131 and Rampur small had longer incubation periods, a longer time to reach 10% wilting, and a lower average percentage of wilting than the other cultivars. These two cultivars may be sources of resistance to *P. solanacearum* in tomato in Nepal.

Bacterial wilt, caused by *Pseudomonas solanacearum* (Smith) Smith, is one of the most important and widespread bacterial diseases of crop plants in the world (8,9). *P. solanacearum* has been divided into three races on the basis of host range (2): race 1 affects tobacco, tomato, many solanaceous plants and other weeds, and certain diploid bananas; race 2 causes wilt of triploid banana and *Heliconia* spp.; and race 3 affects potatoes and tomatoes but is only weakly virulent on other solanaceous crops. Hayward (7) differentiated strains of *P. solanacearum* into four biovars according to their ability to oxidize three disaccharides and three hexose alcohols: strains of biovar I oxidize none of the carbohydrates; strains of biovar II oxidize only disaccharides; strains of biovar III oxidize both disaccharides and hexose alcohols; and strains of biovar IV oxidize hexose alcohols only.

In Nepal, Shrestha (20–22) conducted early surveys for bacterial wilt of potato in Kathmandu, Sindhupalchok, Dolkha, Makwanpur, Kaski, and Palpa districts. He found that the disease was a serious problem in some potato-growing areas in the high hills (900–1,500 m) of Nepal. Recently, bacterial wilt has also been observed near Ulleri, Sabet, and Ghandruk villages (1,800 m), and disease incidence in potato fields has approached 70% (17). In previous studies, strains of *P. solanacearum* isolated from potato fields were classified as biovar II and race 3 (17,20). Little is known, however, about strains of *P. solanacearum* from the central *Terai* (plains) regions (100–500 m) of Nepal, where tomato is grown throughout the year and the climate is characterized by hot summers and cool winters. This article reports the characterization of strains of *P. solanacearum* by biovar and race and the identification of resistant cultivars of tomato in Nepal. Preliminary results have been reported (1).

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MATERIALS AND METHODS

Disease survey. Surveys were conducted in various locations in Nepal, and samples of diseased tomato, potato, bell pepper, eggplant, tobacco, and marigold (*Tagetes* spp.) were collected (Table 1). Plants were collected from farmers' fields and kitchen gardens between May 1989 and September 1991.

Pathogen isolation. Stem sections (15–20 mm long) were cut from diseased samples and placed in test tubes containing sterile distilled water. Bacteria were allowed to flow from the vascular bundles for 5–10 min. One loopful of the bacterial suspension was streaked on tetrazolium chloride (TZC) agar medium (12), and plates were incubated for 48 hr at 28 C. After purification, single colonies of the fluidal type grown on TZC medium were selected, and cultures were maintained as suspensions in sterile distilled water in screw-cap tubes at room temperature (13).

Biochemical and physiological tests. Strains were identified by the following tests: Gram stain (6); fluorescent pigment production on King's medium B (14); slime production from sucrose, temperature relationships, oxidation/fermentation tests, starch hydrolysis, acetoin production, hydrogen sulfide (H_2S) production, indole production, action on litmus milk, and nitrate (NO_3) reduction (4,7, 18); gelatin liquefaction (5); Tween 20 hydrolysis (16); catalase production (3); oxidase reaction (15); glucose metabolism (11); and 1, 3, and 5% sodium chloride (NaCl) tolerance (7). Sensitivity to six antibiotics—chloramphenicol (30 μg/ml), ciprofloxacin (5 μg/ml), tetracycline (30 μg/ml), gentamicin (10 μg/ml), norfloxacin (10 μg/ml), and penicillin (10 μg/ml)—was tested with antibiotic sensitivity disks. The strains were grown on peptone-sucrose agar (PSA) medium, and inhibition zones were recorded after 5 days at 28 C (10).

Biovar identification was based on the ability of strains to oxidize cellobiose, lactose, maltose, dulcitol, mannitol, and sorbitol. Carbohydrates were sterilized as described by He et al (10) and tested as sole carbon source with the medium of Hayward (7). Tests were performed at 28 C, and cultures were observed for acid production for 28 days. All tests were repeated three times.

Pathogenicity tests. Strains were tested for pathogenicity on tomato (*Lycopersicon esculentum* Mill. 'Pusa Ruby'), eggplant (*Solanum melongena* L. 'Norkee'), bell pepper (*Capsicum annuum* L. 'California Wonder'), peanut (*Arachis hypogaea* L. 'Janak'), tobacco (*Nicotiana tabacum* L. 'Belachhapi'), potato (*Solanum tuberosum* L. 'Khufri Jyoti'), and sesame (*Sesamum indicum* L. 'Rampur local'). Tomato, pepper, eggplant, and tobacco seedlings were transplanted into 30-cm-diameter plastic buckets 3 wk after sowing. Peanut and sesame seeds and potato tubers were planted directly into plastic buckets. Plants were allowed to grow to a height of 20–25 cm before they were inoculated with *P. solanacearum*.

Stock cultures of the bacterium were streaked on TZC medium and incubated for 2 days at 28 C. A single fluidal colony of each strain was transferred to individual PSA slants. After 48 hr, bacterial cells were harvested in sterile distilled water, and suspensions were adjusted to $A_{600nm} = 0.1$ (about 10^8 cells per milliliter).

Inoculum was poured around the base of each plant, and an alcohol-flamed knife was inserted 4–5 cm into the soil to cut the roots along one side (23). Ten plants of each host species were inocu-

lated with each strain. Inoculated plants were placed in a greenhouse at 28 ± 4 C and watered daily with tap water. Disease severity was assessed on the scale of He et al (10), where 1 = no symptoms, 2 = two leaves wilted, 3 = three leaves wilted, 4 = four or more leaves wilted, and 5 = plant dead. The experiment was performed twice, and means were averaged to obtain one measurement per treatment unit.

Evaluation of tomato cultivars. Fifteen tomato cultivars from different sources were evaluated for resistance to *P. solanacearum* (Table 2). Two-week-old seedlings were transplanted into clay pots. Inoculum was prepared from strain BW143 (race 1, collected from Rampur) and adjusted to 10^8 cells per milliliter as before. Plants were inoculated 3 wk after transplanting as described by Winstead and Kelman (23).

The number of days before plants developed visible symptoms (incubation period [IP]) and the number of days before wilting reached 10% (LT_{10}) were recorded for each cultivar. The percentage of infected leaves was estimated for 10 plants per treatment weekly for 5 wk. Severity values were averaged to obtain an average percentage of wilting (APW) for each cultivar. Disease reaction was rated on the basis of the APW: R = resistant (<20% of leaves wilted), MR = moderately resistant (20–40% of leaves wilted), MS = moderately susceptible (41–60% of leaves wilted), and S = susceptible (>60% of leaves wilted). IP, LT_{10}, and APW were used as measures of host resistance.

A complete randomized block design was used with four replications. The ex-

Table 1. Strains of *Pseudomonas solanacearum* from Nepal used in physiological and pathogenicity tests

Strain designation	Host plant	Location	Biovar	Severity of wilting[a]							Race
				Tomato	Potato	Eggplant	Pepper	Peanut	Sesame	Tobacco	
BW38	Pepper	Heatuda	III	H	M	H	H	0	0	L	1
BW80	Tomato	Janakpur	III	H	M	L	H	0	M	0	1
BW81	Tomato	Janakpur	III	H	M	H	H	L	L	L	1
BW82	Tomato	Janakpur	III	H	L	H	M	M	0	0	1
BW85	Tomato	Janakpur	III	H	M	M	M	M	0	0	1
BW89	Tomato	Janakpur	III	H	M	M	M	L	0	0	1
BW90	Tomato	Janakpur	III	H	H	H	L	M	0	0	1
BW91	Pepper	Rampur	III	M	M	L	L	L	0	0	1
BW98	Tobacco	Gunjanagar	III	H	L	M	H	M	0	M	1
BW100	Eggplant	Gunjanagar	III	H	L	L	M	L	0	0	1
BW102	Eggplant	Raijung	III	H	H	H	H	L	L	0	1
BW108	Tobacco	Junaligaon	III	H	H	H	H	L	0	H	1
BW143	Tomato	Rampur	III	H	H	H	L	0	0	L	1
BW151	Potato	Hilae	II	H	H	M	L	0	0	0	3
BW155	Potato	Hilae	II	H	H	L	L	0	0	0	3
BW164	Potato	Hilae	II	H	H	M	L	0	0	0	3
BW165	Potato	Hilae	II	H	H	L	L	0	0	0	3
BW174	Potato	Lumle	II	M	H	L	L	0	0	0	3
BW179	Tomato	Chanuali	III	H	H	H	H	0	0	L	1
BW180	Eggplant	Chanuali	III	H	H	H	H	L	0	L	1
BW181	Tomato	Rampur	III	H	H	H	M	L	0	0	1
BW182	Marigold	Rampur	III	H	L	H	M	0	0	L	1
BW183	Potato	Lumle	II	H	H	L	M	0	0	0	3
BW184	Potato	Lumle	II	H	H	M	L	0	0	0	3
BW185	Potato	Lumle	II	H	H	L	M	0	0	0	3

[a] Based on average disease indices of 10 plants 30 days after inoculation: H = high (disease index 4.1–5.0), M = medium (2.6–4.0), L = low (1.1–2.5), and 0 = none (1.0), following He et al (10).

Table 2. Responses of tomato cultivars to *Pseudomonas solanacearum* (race 1) in a greenhouse test[a] in Nepal

Cultivar or line	Seed source[b]	IP[c] (days)	LT_{10}[d] (days)	APW[e] (%)	Reaction[f]
CLN475BC$_1$F$_2$-265-4-19	AVRDC	3 d	5 g	71.04 a	S
CLN475BC$_1$F$_2$-265-12-1-20	AVRDC	3 d	5 g	63.90 c	S
CLN475BC$_1$F$_2$-274-0-15-4	AVRDC	4 cd	11 d	66.63 b	S
CLN475BC$_1$F$_2$-274-0-15-0	AVRDC	4 cd	7 fg	55.63 d	MS
CLN475BC$_1$F$_2$-285-0-20-0	AVRDC	5 c	9 ef	43.91 g	M
CLN475BC$_1$F$_2$-285-0-12-0	AVRDC	3 d	6 g	71.74 a	S
CL5915-93D4-1-0	AVRDC	5 c	12 d	43.59 g	MS
CL5915-93DR-1-0-1-2	AVRDC	3 d	8 f	49.60 e	MS
CLN5915-206D4-2-5-0	AVRDC	5 c	9 ef	45.87 ef	MS
BWR1	India	8 ab	18 b	32.53 j	MR
AC111	Nepal	7 b	16 c	33.70 i	MR
AC142	Nepal	5 c	10 de	39.90 h	MR
AC282	Nepal	3 d	7 fg	45.42 f	MS
Rampur small	Nepal	9 a	23 a	19.17 k	R
CL1131	AVRDC	9 a	22 a	18.10 l	R

[a] Means of two independent experiments were averaged to obain an overall mean for each treatment. In each column, means followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[b] AVRDC = Asian Vegetable Research and Development Center.
[c] Incubation period (IP) = number of days before visible symptoms developed.
[d] LT_{10} = number of days before wilting reached 10%.
[e] APW = average percentage of wilting.
[f] R = resistant (<20% of leaves wilted), MR = moderately resistant (20–40% of leaves wilted), MS = moderately susceptible (41–60% of leaves wilted, and S = susceptible (>60% of leaves wilted).

periment was conducted twice, and the means of the two independent experiments were averaged to obtain an overall mean for each treatment. The SAS software package (19) was used for data analysis.

RESULTS

Biochemical and physiological tests. All strains were rod-shaped, motile, and gram-negative. All virulent strains produced fluidal colonies with pink or light red centers after 48 hr on TZC medium. None of the strains produced fluorescent pigment on King's medium B, and all strains grew vigorously at 30 C. Strains BW91, BW98, BW100, and BW181 showed slight growth at 40 C. Strains BW183, BW184, and BW185 grew slightly at 10 C. All strains were negative for starch hydrolysis, acetoin production, gelatin liquefaction, and indole production (*data not shown*). All strains were strict aerobes and were oxidative. All strains were positive for alkaline reaction on litmus milk; production of slime, NO_3, and H_2S; presence of catalase and oxidase; hydrolysis of Tween 20; and growth in 1% but not 3 or 5% NaCl. Strains varied in susceptibility to chloramphenicol, ciprofloxacin, gentamicin, and norfloxacin (*data not shown*). All strains were resistant to penicillin and tetracycline. Of the 25 strains, eight were biovar II and 17 were biovar III (Table 1). Biovars I and IV were not detected.

Pathogenicity. All strains caused rapid wilting of tomato (Table 1). Eggplant, potato, and pepper were susceptible to most strains. Nearly 90% of the race 1 strains were pathogenic to peanut. Tobacco and sesame were relatively poor hosts to these strains of *P. solanacearum*.

Of the 25 strains, eight were race 3 and 17 were race 1. All strains collected from potato were race 3, and strains isolated from other host plants were race 1.

Evaluation of tomato cultivars. Significant differences in IP, LT_{10}, and APW were observed among the 15 inoculated tomato cultivars (Table 2). IP was longer (9 days) with CL1131 and Rampur small than with the other cultivars. Similarly, LT_{10} was considerably longer (22–23 days) in CL1131 and Rampur small than in the other cultivars (5–12 days). Differences in susceptibility were apparent among cultivars. Four of the 15 cultivars—CLN475BC$_1$F$_2$-265-4-19, CLN475BC$_1$F$_2$-265-12-1-20, CLN475-BC$_1$F$_2$-274-0-15-4, and CLN475BC$_1$F$_2$-285-0-12-0)—were particularly susceptible to bacterial wilt. CL1131 and Rampur small exhibited resistance to *P. solanacearum*.

DISCUSSION

Biovars and races of *P. solanacearum* identified in Nepal were similar to those reported from other countries (2,7,10). Most of the strains tested in this study were from lowland areas of Nepal and were biovar III. All biovar II strains were collected from potato grown at higher altitudes. Shrestha (20,21) also found that biovar II was widely distributed in the middle- and high-altitude hills of Nepal. Although bacterial wilt of potato has been known in Nepal for over 25 yr, the characteristics of the pathogen occurring in lowland areas have not previously been investigated. Based on the race classification system for *P. solanacearum* (2), two-thirds of the strains isolated for this study were race 1. The presence of race 1 in these areas of Nepal is attributable to continuous tomato monoculture and the use of susceptible tomato cultivars (such as Pusa Ruby) throughout the year.

The disease reaction of tomato cultivars was rated on the basis of percentage of wilting. Resistant cultivars were easily differentiated from susceptible cultivars on the basis of the IP, LT_{10}, and APW values. These three parameters appeared to be reliable for the evaluation of resistance to *P. solanacearum*. CL1131 and Rampur small (a local cultivar) displayed the highest level of resistance.

Further field experiments are under way to determine host specificity and the effect of environment on the stability of resistance. Because phenotypic diversity has been observed among strains of *P. solanacearum,* more strains should be collected from additional locations and hosts in Nepal in order to establish geographic distribution of the races and biovars. These data are needed to develop effective control measures for bacterial wilt in Nepal.

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