Strain Identification and Cross-Protection of Potato Virus Y Affecting Tobacco in Chile

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ABSTRACT

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Necrosis severa, caused by potato virus Y (PVY), severely affects flue-cured, burley, and oriental tobaccos in Chile. PVY populations consisted of necrotic and nonnecrotic strains distinguished on the basis of the symptoms produced. Most Chilean isolates were identified as race 1 (M^sM^r) or race 3 (N^sN^r). However, a few were identified as race 2 (M^sN^r) on the basis of reactions they induced on tobacco cultivars susceptible and resistant to the root-knot nematode. PVY isolates were also distinguishable by their reactions on tobacco Virgin A Mutant, a noncompatible (apparently immune) reaction or compatible reactions that were either necrotic or nonnecrotic symptoms incited on veins or stems. The presence of necrosis did not necessarily appear to be associated with symptom severity. Most nonnecrotic PVY isolates exerted partial protection against a challenge inoculation with a necrotic strain, demonstrating the occurrence of this phenomenon among PVY isolates and suggesting cross-protection as a possible mechanism for PVY control. Cross-protection may also play a significant role in the epidemiology of PVY diseases.

Additional key words: *Nicotiana tabacum*, preimmunization, virus control

Plants inoculated with a mild strain of a virus often develop resistance to later superinfections by severe strains of the same or closely related viruses (8,18). This antagonism between virus strains is usually called cross-protection. Historically, cross-protection has been used in plant virus identification as a criterion for relatedness, but it also offers a potential for virus disease control. Some important viral diseases, e.g., citrus tristeza and tomato mosaic, have been controlled successfully under commercial conditions in a number of countries by preimmunization with mild strains of the respective viruses (1,2,5,7,10). In Chile, potato virus Y (PVY) incites a disease of tobacco locally called *necrosis severa*. It is the most important disease of tobacco in Chile (16,17,19), and it represents a real threat to other countries (11,12,14).

Three strains of PVY have been identified (12) according to their reactions on tobacco cultivars resistant and susceptible to the root-knot nematode (RKN) (*Meloidogyne incognita* (Kofoid & White) Chitwood). Strain M^sM^r only produces mild symptoms characterized by veinbanding and chlorotic mottle; strain N^sN^r causes severe symptoms characterized by necrosis of veins and stalks, leaf distortion, and severe stunting on tobacco cultivars both susceptible and resistant to RKN; and strain M^sN^r incites mild symptoms on cultivars susceptible to RKN but severe symptoms on tobacco cultivars resistant to RKN (20,21). The objectives of this research were to identify the strains of PVY affecting tobacco and evaluate the feasibility of cross-protection as an alternative for controlling *necrosis severa* of tobacco under Chilean conditions.

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MATERIALS AND METHODS

PVY isolates. During the growing seasons of 1981, 1982, and 1983, more than 100 samples of field-grown tobacco were collected, transported in an ice chest to the laboratory, triturated in 0.05 M potassium phosphate buffer with a mortar and pestle, and mechanically inoculated to tobacco cultivar Coker 86. Samples were taken from diseased plants with symptoms that varied from chlorotic mottle and veinbanding to severe leaf distortion and vein necrosis. Inoculated plants were maintained in a screened greenhouse for 20–25 days.

Sixty isolates that appeared serologically identical using PVY antisera in agar double-diffusion tests (9) were further characterized according to their reactions on the following: 1) flue-cured tobacco cultivars McNair 944 and NC-744 (susceptible to RKN) and NC-95 and Speight G-28 (resistant to RKN) (breeding line NC-744 possesses resistance to PVY originally derived from Virgin A Mutant [VAM] [3,4,6]); 2) Burley 21 and Burley 49; and 3) VAM (= Ti 1406). VAM is resistant to most PVY strains (3,4,6,13,15). Inoculations were performed on seedling plants at the two- to three-leaf stage, and they were maintained for 20–25 days under greenhouse conditions.

The inoculum was prepared by triturating recently infected Coker 86 leaves 1:4 (w/v) with 0.05 M potassium phosphate buffer, pH 7.2, containing about 1% Carborundum (600-mesh). Inoculations were always performed by rubbing the youngest two or three leaves with a cotton swab previously dipped in the inoculum suspension.

Cross-protection assays. The ability of 12 mild (nonnecrotic) PVY isolates to cross-protect against severe (necrotic) strains was tested on tobacco cultivar NC-95 under greenhouse conditions. Isolates were classified as mild or severe on the basis of symptoms produced on tobacco cultivar Coker 86. Groups of four uniform plants were selected and each plant was subjected to one of the following treatments: 1) test plant preimmunized with a nonnecrotic strain and challenged 7 days later with necrotic PVY strain N-2 (preliminary experiments had shown that 7 days was the shortest period between the first and the second inoculation that produced a protective effect); 2) control plants inoculated with each of the nonnecrotic PVY isolates only; 3) control plants inoculated with necrotic PVY strain N-2 only; and 4) control plants not inoculated. The presence or absence of veinal necrosis and/or necrotic lesions on stems and the fresh weights of the aerial plant parts were recorded 25 days after the challenge inoculation. Results were subjected to analysis of variance, and mean differences of fresh weights were separated according to Duncan's multiple range comparison.

RESULTS

Strain identification. Isolates of PVY collected from commercially grown tobacco in 1981, 1982, and 1983 were initially grouped into two categories (necrotic and nonnecrotic strains) according to their reactions on tobacco cultivar Coker 86. Nonnecrotic strains produced chlorotic mottle, veinbanding, or veinclearing only. Necrotic strains produced necrotic lesions along the stems, midribs, or secondary and tertiary veins; leaf distortion; leaf-blade necrosis; and/or moderate to severe stunting.

The degree of stunting induced by the necrotic and nonnecrotic isolates was dependent on the tobacco cultivar tested (Table 1). When inoculated with necrotic PVY isolates, RKN-susceptible flue-

cured type cultivars were stunted the least and RKN-resistant cultivars showed severe stunting (Table 1). Burley 21 and 49 were also severely stunted by necrotic PVY isolates. Similarly, stunting induced by nonnecrotic PVY isolates varied according to cultivar, from almost undetectable to moderate on McNair 944 or Coker 86 (Table 1).

None of the 22 nonnecrotic isolates induced necrosis on flue-cured or burley cultivars. All necrotic isolates produced vein necrosis on Coker 86, McNair 944, NC-95, Speight G-28, and Burley 21, but only 11 of 24, 8 of 24, and 14 of 16 of necrotic PVY isolates induced necrotic symptoms on NC-744, VAM, and Burley 49, respectively (Table 1). VAM was apparently immune to 15 of 24 necrotic PVY isolates tested, and NC-744 was apparently immune to 4 of 24 necrotic isolates.

Serology. All PVY isolates tested appeared to be serologically identical in the Ouchterlony agar double-diffusion test (9). There were no serological differences between necrotic and nonnecrotic Chilean isolates or between Chilean and reference isolates 78 and 179 obtained from North Carolina.

Cross-protection trials. The cross-protection effect of nonnecrotic PVY isolates was recorded about 25 days after the challenge inoculation when well-developed necrotic lesions appeared on plants inoculated with the necrotic isolate N-2. On the basis of fresh-weight results, 1 of 12 nonnecrotic PVY isolates gave complete protection, 8 of 12 gave partial protection, and 3 of 12 gave no protection against the necrotic isolate N-2. Complete cross-protection was produced with isolate M-32, followed by 80% protection with isolate M-2 (Table 2). Ten of 12 nonnecrotic PVY isolates completely suppressed necrosis development on doubly inoculated plants (Table 2).

The degree of cross-protection produced with isolates M-2 and M-32 was assessed on other cultivars (Table 3). On the basis of yield, protection varied from complete on tobacco NC-628 and NC-95 to only slight protection obtained on Burley 49 with isolate M-2. Both isolates completely inhibited necrosis development, except on Burley 49 tobacco (Table 3).

DISCUSSION

PVY isolates from tobacco in Chile fell into two distinctive groups based on severity of the symptoms elicited on flue-cured or burley tobacco cultivars. Nonnecrotic strains only incited vein-clearing, veinbanding, and chlorotic mottling without affecting plant growth significantly. However, symptoms incited by necrotic strains were veinbanding, veinclearing, and chlorotic mottling always followed by vein or stem necrosis. Some exceptions were found on tobacco cultivars VAM and NC-744, which carry a gene for resistance to PVY (3,4,6,13) (Table 1).

According to the criteria proposed by Gooding and Tolin for strain identification (12), most Chilean PVY isolates were either race 1 (M^sM^r) or race 3 (N^sN^r). Evidence for race 2 (M^sN^r) was found only in a few instances.

Chilean PVY isolates, typed as race 3, were more severe on RKN-resistant than on RKN-susceptible tobacco cultivars and produced veinal necrosis on Burley 21, a cultivar normally not affected with necrotic lesions when inoculated with PVY race 3 in the United States (4). Additional differences among severe PVY isolates appeared on NC-744, VAM, and Burley 21 and 49 (Table 1). For instance, three strains may be differentiated on the basis of the symptoms incited on tobacco cultivar VAM, which carried a single recessive factor for resistance to PVY (4,13). Severe PVY isolates fall into one of the following categories according to the reaction observed on VAM tobacco plants: 1) apparently immune or noncompatible (no symptoms were observed after inoculation with 15 of 24

Table 1. Reactions of flue-cured and burley tobacco cultivars to infection by necrotic and nonnecrotic strains on potato virus Y from tobacco

Tobacco cultivar	Reaction to RKN[a]	Nonnecrotic isolates[b]				Necrotic isolates[b]			
		Foliar symptoms[c]		Stunting[d]		Foliar symptoms[c]		Stunting[d]	
		M	N	Mean	SD	M	N	Mean	SD
Flue-cured									
McNair 944	S	21/22[e]	0/22	2.6	1.6	24/24	24/24	1.6	1.5
NC-744	S	13/14	6/14	1.6	0.7	20/24	11/24	1.0	1.5
NC-95	R	22/21	0/21	1.1	0.9	24/24	24/24	3.3	1.0
Coker-86	R	22/22	0/22	2.6	1.9	24/24	24/24	3.9	0.9
Speight G-28	R	22/22	0/22	2.5	1.6	24/24	24/24	3.7	0.9
VAM	?	9/21	0/21	0.1	0.3	9/24	8/24	1.2	1.5
Burley									
Burley 21	S	22/22	0/22	2.5	1.7	24/24	24/24	3.5	1.1
Burley 49	?	21/21	0/21	1.9	0.6	16/16	14/16	3.7	1.1

[a] RKN = root-knot nematode (*Meloidogyne incognita*). S = susceptible to RKN, R = resistant to RKN, ? = RKN susceptibility unknown.
[b] Nonnecrotic and necrotic strains were selected initially according to the absence or presence of vein necrosis on *Nicotiana tabacum* 'Coker 86.'
[c] M = chlorotic mottle and veinbanding, N = vein necrosis, and SD = standard deviation.
[d] Stunting category: 0 = no stunting, 1 = 1–20% height reduction, 2 = 21–40% height reduction, 3 = 41–60% height reduction, 4 = 61–80% height reduction, and 5 = more than 80% height reduction, relative to healthy controls, 20–25 days after the inoculation.
[e] No. of isolates expressing indicated symptoms/no. of isolates tested.

Table 2. Evaluation of the protective effects on tobacco cultivar NC-95 of nonnecrotic (mild) isolates of potato virus Y (PVY) from tobacco against a challenge inoculation with a necrotic (severe) PVY isolate

Treatment	Necrosis[y]	Fresh weight[z] (%)
	Trial 1	
Uninoculated	0.0	100.0 a
Inoculated		
Mild		
M-2	0.0	75.0 ab
M-10	0.0	68.3 ab
M-16	0.0	68.3 ab
Severe (S)	3.3	51.7 b
M-2 + S	0.0	80.0 a
M-10 + S	0.0	70.0 ab
M-16 + S	3.5	70.0 ab
	Trial 2	
Uninoculated	0.0	100.0 ab
Inoculated		
Mild		
M-21	0.0	75.1 bc
M-25	0.0	52.7 c
M-32	0.0	90.7 ab
Severe (S)	5.0	61.2 bc
M-21 + S	0.0	69.2 bc
M-25 + S	0.0	40.2 c
M-32 + S	0.0	100.0 a
	Trial 3	
Uninoculated	0.0	100.0 a
Inoculated		
Mild		
M-8	0.0	46.1 b
M-11	0.0	50.2 b
Severe (S)	4.0	41.4 b
M-8 + S	0.0	53.4 b
M-11 + S	0.0	38.7 b
	Trial 4	
Uninoculated	0.0	100.0 ab
Inoculated		
Mild		
M-23	0.0	37.6 cd
M-29	0.0	53.4 abcd
M-33	0.0	100.0 a
M-34	0.0	67.8 abc
Severe (S)	3.7	39.0 cd
M-23 + S	0.5	19.0 d
M-29 + S	0.0	41.9 cd
M-33 + S	0.0	56.1 abcd
M-34 + S	0.0	49.6 bcd

[y] Degree of necrosis observed on veins or stems 25 days after plants were challenged with severe PVY strain N-2 and categorized as: 0 = absence of necrosis, and 1–5 = slight to severe vein necrosis.
[z] Percentage of fresh weight of the aerial plant parts 25 days after challenge inoculation with severe PVY strain N-2 (100% = healthy). Numbers followed by the same letter do not differ significantly at $P = 0.05$ according to Duncan's multiple range comparison.

Table 3. Evaluation of the cross-protection effect elicited by nonnecrotic (mild) potato virus Y (PVY) isolates M-2 and M-32 on burley, flue-cured, and oriental tobaccos

Tobacco cultivar	Healthy		Mild (M)		Severe (S)		Protected (M + S)	
	N[a]	FW (%)[b]	N	FW (%)	N	FW (%)	N	FW (%)
Mild isolate M-2								
Burley								
Burley 21	0.0	100	0.0	85.4	5.0	46.3	0.0	69.5
Burley 49	0.0	100	1.6	26.0	2.0	20.9	1.2	38.9
Flue-cured								
NC-95	0.0	100	0.0	100.0	4.0	58.8	0.0	79.8
NC-628	0.0	100	0.0	81.3	2.6	81.9	0.0	100.0
Coker 86	0.0	100	0.0	78.4	4.6	53.3	0.0	57.9
Coker 51	0.0	100	0.0	51.6	3.4	42.8	0.0	59.9
Mild isolate M-32								
Burley								
Burley 21	0.0	100	0.0	67.6	4.2	54.6	0.0	69.0
Burley 49	0.0	100	0.0	72.7	3.8	49.0	0.0	65.0
Flue-cured								
NC-95	0.0	100	0.0	91.8	4.0	71.1	0.0	100.0
NC-628	0.0	100	0.0	85.1	2.8	68.5	0.0	100.0
Coker 86	0.0	100	0.0	67.4	2.0	48.9	0.0	73.5
Coker 51	0.0	100	0.0	94.6	4.0	52.0	0.0	79.0
Oriental								
Gavourkoy	0.0	100	0.0	82.8	2.0	78.7	0.0	85.6

[a] N = degree of necrosis observed on veins or stems 25 days after plants were challenged with severe PVY isolate N-2 and categorized as: 0 = absence of necrosis and 1–5 = slight to severe vein necrosis.
[b] Percentage of fresh weight (FW) of the aerial plant parts 25 days after challenge inoculation with severe PVY isolate N-2 (100% = healthy).

severe PVY isolates), and 2) compatible. Eight of 24 induced necrotic symptoms, and one induced only nonnecrotic symptoms. Similarly, 12 of 24 nonnecrotic isolates induced no symptoms on VAM, whereas nine induced mild symptoms. Consequently, VAM may serve as an additional differential host for typing PVY strains from tobacco. No significant differences in their response to severe PVY isolates appeared between Burley 21 and Burley 49, a black shank (*Phytophthora parasitica* (Dast.) var. *nicotianae* (B. de Haan) Tucker) susceptible and resistant burley tobacco respectively (14).

The detrimental effects of PVY strains were not necessarily associated with symptom type. All necrotic strains tested induced a necrotic response, but their effects on growth varied from slight on RKN-susceptible to severe on RKN-resistant flue-cured cultivars (Table 1). Similarly, a moderate to severe degree of stunting was obtained on flue-cured or burley tobaccos inoculated with nonnecrotic PVY strains (Table 1). For instance, 9 or 22 nonnecrotic isolates produced severe stunting effect on Coker 86, and 6 of 22 affected growth severely on Burley 21. Furthermore, most nonnecrotic PVY isolates totally inhibited the development of necrotic lesions on doubly inoculated plants (Tables 2 and 3) but did not always reduce the detrimental effect of the necrotic PVY isolate on growth. This is consistent with our previous field observations (17) regarding the effects of a necrotic PVY isolate on NC-744, which caused necrosis after inoculation but did not significantly affect yields or quality.

The protective effect of nonnecrotic PVY strains was dependent on the tobacco cultivar and the nonnecrotic PVY isolate. For instance, PVY isolate M-2 conferred a highly protective effect on NC-628 but a slightly protective effect on Burley 49 (Table 3). In conclusion, cross-protection has a potential for reducing losses caused by *necrosis severa* of tobacco, but additional research is needed before it can be employed. Cross-protection between necrotic and nonnecrotic isolates may also have a significant effect on population dynamics in nature and it may affect the epiphytic outbreaks of PVY on tobacco.

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