Identification and Characterization of Resistance to Tobacco Etch Virus in *Lycopersicon* Species

R. Légnani, P. Gognalons, K. Gébré Sélassié, and **G. Marchoux,** Plant Pathology Station, and **A. Moretti** and **H. Laterrot,** Vegetable Plant Breeding Station, INRA, Avignon, BP 94, 84143 Montfavet Cedex, France

ABSTRACT

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Tomato and wild related species with or without described resistance to potyviruses were screened for resistance to tobacco etch virus (TEV). *Lycopersicon pennellii* LA 716 and *L. pimpinellifolium* LA 1478 were found to be tolerant to TEV. *L. hirsutum* PI 247087, previously reported resistant to potato virus Y (PVY), was found resistant to TEV. The virus was not detected by enzyme-linked immunosorbent assay (ELISA) in the inoculated leaves of *L. hirsutum* PI 247087 but could be recovered by back-inoculations to tobacco plants. TEV was not detected in the uninoculated leaves of *L. hirsutum* PI 247087. Virus multiplication and/or virus migration from cell to cell appeared to be impaired, preventing systemic spread of TEV in *L. hirsutum* PI 247087. The resistance of *L. hirsutum* PI 247087 to TEV is expressed at the cotyledonary stage and is efficient against four different geographical isolates. Inheritance of the resistance appears to be controlled by one recessive gene.

Tobacco etch virus (TEV) causes serious economic losses in tobacco, pepper, and tomato crops. The incidence of this potyvirus has been widely investigated in several regions of the United States. TEV has been detected in 7.1% of burley tobacco in North Carolina (11). Yield of some susceptible varieties of flue-cured tobacco can be reduced by 6 to 18% (10). Data from surveys conducted during 1984 to 1985 on pepper in California indicated that TEV was the most frequent virus (1). TEV is also commonly found on tomato (*Lycopersicon esculentum* Mill.) on the east coast of Florida (29). This virus is also present in South America (19), Cuba (8), the Philippines (24), Taiwan (26), Thailand (26), and Turkey (25).

TEV causes intense mottling on tomato leaves and fruits (27). Following artificial inoculation, the yield of tomato can be reduced by 55.5%, depending on the plant age at the time of inoculation (9). In conditions of natural infection, the crop loss may be total if seedlings are infected just after transplanting (29).

Cultural practices only delay infection and reduce losses. The most successful way to prevent disease incidence is to use resistant or tolerant varieties.

Sources of resistance or tolerance to TEV have been reported in *L. esculentum* PI 183692 (23) and PI 166989 (2), and in *L. hirsutum* PI 134417, PI 127827 (3), and PI 247087 (13). However, no commercial variety resistant or tolerant to TEV is available (27). On the other hand, there are reports of multiresistance to potyviruses in solanaceous plants (6,12,14).

The purpose of this study was to evaluate and describe resistance to TEV in some tomato cultivars and related wild species described as resistant or tolerant to potyviruses. Inheritance of resistance of a resistant accession was also studied.

Corresponding author: R. Légnani

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MATERIALS AND METHODS

Plant material. Eleven genotypes of *Lycopersicon* were tested for resistance to TEV: *L. esculentum* cv. Angela 18-1 and PI 126410, both resistant to potato virus Y (PVY) (17); three commercial hybrids of cherry tomato (Sweet 100, Sweet Million, and Micro Tom), all resistant to PVY^N (20); two *L. hirsutum* (PI 134417, resistant to TEV [3], and PI 247087, tolerant to TEV and to Peru tomato virus (PTV) [13] and resistant to PVY [15,21]); *L. peruvianum* PI 128660, resistant to PVY (17); and *L. pennellii* LA 716, *L. pimpinellifolium* LA 1478, and *L. esculentum* cv. Momor (no report of resistance to any potyvirus).

In a second step, plants of the F_1 (PI 134417 × PI 247087) obtained by crossing three plants of PI 134417 with three plants of PI 247087, the F_2 (PI 134417 × PI 247087) obtained by intercrossing six F_1 plants, and the BC_1 obtained by crossing four F_1 (PI 134417 × PI 247087) plants with three PI 247087 plants were tested to investigate the inheritance of the resistance of PI 247087 to TEV. These intraspecific crosses were used to avoid segregation distortion often observed in interspecific crosses.

All these plants were transplanted at the cotyledon stage into 10-cm-diameter pots and kept in greenhouses. After inoculation, they were transferred and kept under constant conditions in growth chambers at 25°C for 16-h days and 23°C for 8-h nights during the tests. Unless otherwise mentioned, the first three leaves of 21-day-old plants were mechanically inoculated as described in the following section, "Virus isolates." In the study of the effect of plant age on the expression of resistance, the cotyledons of 16-day-old plantlets and the first two leaves of 21-, 30-, and 40-day-old plants were inoculated.

Virus isolates. One strain, CAA 10, obtained from five subsequent local lesion passages on *Chenopodium amaranticolor* from a Californian isolate, and three isolates (CAA 1, CAA 4, and CAA 103) of TEV isolated from *Capsicum annuum* respectively in Cuba and Turkey were inoculated to *Datura stramonium* 15 days before inoculation to *Lycopersicon* genotypes. The Californian strain, CAA 10, was used in all the trials. The other isolates were only used to evaluate the spectrum of resistance of *L. hirsutum* PI 247087 to TEV. Virus inoculum was prepared by grinding 1 g of young leaves of *Datura* in 4 ml of inoculation solution (0.03 M Na_2HPO_4 containing 0.2% sodium diethyldithiocarbamate). Prior to inoculation, Carborundum at 75 mg/ml and activated charcoal at 75 mg/ml were added to the sap extract (16). Mechanical inoculations were performed by rubbing the leaves with the inoculum.

Serological and biological tests. Inoculated and noninoculated leaves were tested for the presence of TEV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Samples were prepared by grinding with a stainless steel motor driver roller at the rate of approximately 1 g of tissue in 4 ml of inoculation buffer. The polyclonal antiserum against strain 10 of TEV was prepared in our laboratory. Polystyrene plates were incubated with IgG (0.5 µg/ml) in coating buffer for 4 h at 37°C. After three rinsings, 200 µl of sap extract were added to each well. Two wells were used for each sample. Plates were incubated overnight at 4°C. After three rinsings, alkaline phosphatase conjugated IgGs were added and plates were incubated 3 h at 37°C. After three rinsings, paranitrophenyl phosphate (substrate) added at 1 mg/ml was allowed

to react at room temperature. Plates were read with a Titerteck Multiscan Plus photometer at 405 nm 1 h after adding the substrate. Extracts from noninoculated tomatoes served as the control. Samples with absorbance values greater than three times the absorbance value of controls (means of four wells) were considered positive. To confirm the serological tests, some samples were back-inoculated to indicator plants, *Nicotiana tabacum* cv. Xanthi NC.

RESULTS AND DISCUSSION

Symptoms and ELISA controls, 15 and 45 days after inoculation on 20 plants of different *Lycopersicon* genotypes inoculated with TEV strain CAA 10, are reported in Table 1. TEV caused a yellow mosaic and stunting on *L. esculentum* cv. Momor. Symptoms of TEV on *L. esculentum* Sweet 100, Sweet Million, and Micro Tom, and on *L. peruvianum* PI 128660 plants, all previously described as resistant to PVY (17,20), were as severe as those on Momor plants. Symptoms on *L. esculentum* Angela 18-1 and PI 126410 plants, both reported resistant to PVY (17), were delayed, and 45 days after inoculation the symptoms on these genotypes were as severe as those on Momor plants. *L. hirsutum* PI 134417, previously reported resistant to TEV (3), was found susceptible and showed intense dark green mosaic and growth reduction in our tests. There was a good relation between symptom expression and TEV detection by ELISA 45 days after inoculation in the above-mentioned genotypes.

No symptoms were observed on *L. pennellii* LA 716 and *L. pimpinellifolium* LA 1478 plants, but virus was detected by ELISA in noninoculated apical leaves. These two accessions could be considered tolerant to TEV. There were no symptoms on *L. hirsutum* PI 247087 plants. Young, fully expanded leaves of these plants, which were tested at 2-week intervals over a period of 3 months, remained negative by ELISA. The PI 247087 previously described as tolerant to TEV (13) was found resistant to TEV in our experiments.

To learn more about the mechanisms involved in the resistance of PI 247087, the fate of the virus in inoculated and noninoculated leaves of PI 247087 and PI 134417, both *L. hirsutum* accessions, was studied. Twenty plants of each genotype were tested by ELISA and by back-inoculations 2, 5, 7, 10, 13, 16, and 30 days after inoculation (Table 2). TEV was detected by ELISA in the inoculated leaves of PI 134417 starting 5 days after inoculation. In PI 247087 plants, the virus was never detected by ELISA from the inoculated leaves. Biological tests allowed recovery of TEV from inoculated leaves of PI 134417 plants starting 2 days after inoculation. TEV was recovered from 4/20 inoculated leaves of PI 247087 plants back-inoculated 5 days after inoculation. Seven or ten days after inoculation, TEV was recovered from 18/20 inoculated leaves of PI 247087 plants. The proportion of inoculated leaves of PI 247087 in which TEV was recovered decreased to 8/20 at 13 days. So TEV was able to infect PI 247087, but virus multiplication and/or virus migration from cell to cell were so seriously impaired that only biological tests allowed the detection of the virus in the inoculated leaves. Reduction in the number of initial infection sites may also play a role. TEV was not detected either by ELISA or by back-inoculations in the noninoculated leaves of PI 247087. These data suggest that TEV can infect PI 247087, but that systemic spread does not occur.

PVY could not be detected in the inoculated leaves of PI 247087 by ELISA (15). The mechanism of resistance of PI 247087 to PVY and TEV may be the same.

Effect of plant age on infection was studied by inoculation of 20 plants of each genotype 12, 21, 30, and 40 days after

Table 1. Symptoms and enzyme-linked immunosorbent assay (ELISA) data from *Lycopersicon* species inoculated with the strain CAA 10 of tobacco etch virus (TEV)

	Days after inoculation			
	15 days		45 days	
Genotypes	Symptoms[a]	ELISA[b]	Symptoms	ELISA[b]
L. esculentum				
Momor	y. Mo	+[c]	y. Mo, S	+
Angela 18-1	mo	+	y. Mo, S	+
PI 126410	0	+	y. Mo, S	+
Sweet 100	y. Mo	+	y. Mo, S	+
Sweet Million	y. Mo	+	y. Mo, S	+
Micro Tom	y. Mo	+	y. Mo, S	+
L. hirsutum				
PI 134417	dg. Mo	+	g. Mo, S	+
PI 247087	0	–	0	–
L. pennellii				
LA 716	0	+	0	+
L. peruvianum				
PI 128660	y. Mo	+	y. Mo, S	+
L. pimpinellifolium				
LA 1478	0	+	0	+

[a] y = yellow, Mo = mosaic, mo = mottling, 0 = no symptoms, g = green, dg = dark green, and S = stunt.
[b] Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) in uninoculated leaves of 20 plants of each genotype.
[c] + = Virus detected by ELISA, – = no virus detection by ELISA.

Table 2. Tobacco etch virus (TEV) detection from inoculated and uninoculated leaves of 20 plants of PI 247087 and PI 134417 by enzyme-linked immunosorbent assay (ELISA) and by recovery test on tobacco at different intervals after inoculation

	Days after inoculation						
	Inoculated leaves					Noninoculated leaves	
	2	5	7	10	13	16	30
ELISA A_{405} values							
PI 134417	0.13	0.61	0.63	0.75	0.89	0.69	0.14
PI 247087	0.10	0.07	0.10	0.05	0.04	0.02	0.02
Healthy	0.05	0.05	0.04	0.04	0.05	0.05	0.03
Biological test							
PI 134417	20[a]	20	20	20	20	20	20
PI 247087	0	4	18	18	8	0	0

[a] Number of infected plants/20 back-inoculated tobacco plants.

Table 3. Enzyme-linked immunosorbent assays (ELISAs) 15 days after inoculation in noninoculated leaves of the three *Lycopersicon* lines inoculated with four isolates of tobacco etch virus (TEV)

Isolates and strain		Genotypes		
Names	Countries	Momor	PI 134417	PI 247087
CAA[a] 10	California	10/10[b]	10/10	0/10
CAA 1	Cuba	10/10	10/10	0/10
CAA 4	Cuba	10/10	10/10	0/10
CAA 103	Turkey	10/10	10/10	0/10

[a] Isolated from *Capsicum annuum.*
[b] Number of positive plants/number of inoculated plants.

sowing. ELISAs were performed 15 and 30 days after inoculation for each plant age. PI 134417 and Momor were susceptible at each inoculated age. Resistance of PI 247087 to TEV was expressed in young plants only 12 days after sowing, at cotyledonary stage. This early expression of resistance of PI 247087 was previously described for PVY (15).

Momor and PI 134417 were susceptible to the three isolates and the strain tested in this study (Table 3). The fact that PI 134417 was previously described as resistant to TEV (3) suggests that its resistance may be strain specific. By contrast, the resistance of PI 247087 was efficient against three isolates and one strain obtained from three different countries where TEV is considered a major disease of solanaceous crops. The spectrum of resistance to TEV seems to be relatively large. Moreover, PI 247087 was also found resistant to many PVY isolates coming from various hosts and countries (15,21).

The inheritance of resistance of PI 247087 was determined with strain CAA 10 of TEV (Table 4) in intraspecific crosses. All the F_1 (PI 134417 × PI 247087) plants were susceptible. The number of F_2 plants without virus in apical uninoculated leaves decreased between 15 and 30 days after inoculation (data not shown). It remained the same in the BC_1. Forty-five days after inoculation, all the plants found resistant at 30 days were still resistant (data not shown). Thirty days after inoculation, segregation ratios in the F_2 (PI 134417 × PI 247087) and the BC_1 (PI 134417 × PI 247087) × PI 247087 fitted with the hypothesis of one recessive gene controlling the resistance to TEV in PI 247087 (Table 4).

Resistance to PVY in the same genotype was found to be controlled by one or two recessive genes (15,22), depending on the strain used. Inheritance of PI 247087 resistance to TEV may also be strain specific. More strains need to be tested to evaluate the inheritance(s) of PI 247087 resistance to TEV. Propagation by cuttings from F_2, BC_1, and more advanced generations will determine if resistance to PVY and TEV are inherited together. In this case, a breeding program for one virus could allow combined resistance to these two potyviruses to be obtained.

L. hirsutum PI 247087, which is resistant to PVY (15,22) and tolerant to PTV (13), was found to be resistant to TEV in this study. Nonspecific resistance to potyviruses has been previously reported in tobacco and pepper species. Tobacco lines V20, Virginia mutant (VAM), and Havana 307, reported as resistant to TEV (5,12,14), are also resistant to other important potyviruses in tobacco crops: tobacco vein mottling virus (TVMV) and PVY. In pepper cvs. P 11 and SC 4652, resistance to TEV (6) and to PVY (7) is controlled by a single recessive gene. Resistance to the two potyviruses is assumed to result from a "spurious pleiotropism" or a close linkage of two distinct genes (6). The et^{av} gene, coming from Avelar, a pepper cultivar from Brazil, controls resistance to TEV and PVY and tolerance to pepper mottle virus (PeMV) (28). One dominant gene controls resistance to PVY and PeMV in the Mexican pepper line CM334 (4, 18). Nonspecific resistance can be a result of the recognition by multiresistant plants of conserved viral protein sequences.

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Table 4. Reaction of parents PI 247087, PI 134417, and segregating generations to inoculation with tobacco etch virus (TEV) strain CAA 10

	ELISA in noninoculated leaves 30 days after inoculation					
Genotypes	–[a]	+[b]	n[c]	Expected ratio	χ^2	*P*
Experiment 1						
P1[d]	20	0	20			
P2[e]	0	20	20			
F_1 (P2 × P1)	0	20	20			
F_2 (P2 × P1)	31	69	100	1:3	1.92	0.16
BC_1 (P2 × P1) × P1	52	48	100	1:1	0.16	0.69
Experiment 2						
P1	20	0	20			
P2	0	20	20			
F_1 (P2 × P1)	0	20	20			
F_2 (P2 × P1)	46	104	150	1:3	2.57	0.11
BC_1 (P2 × P1) × P1	68	82	150	1:1	1.31	0.25

[a] Number of negative plants with ELISA.
[b] Number of positive plants with ELISA.
[c] Number of inoculated plants.
[d] PI 247087.
[e] PI 134417.

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