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Sources of resistance to tomato spotted wilt virus (TSWV) in cultivated and wild species of *Capsicum*

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Summary

A germplasm collection of 70 cultivars and wild species of *Capsicum* was evaluated for resistance to tomato spotted wilt virus (TSWV) under field (natural inoculum) conditions. Different levels of resistance to the disease caused by this virus were observed among the tested lines. High degree of field resistance was detected in two *Capsicum baccatum* var. *pendulum,* two *C. chinense,* and three *C. annuum* lines. Controlled greenhouse tests were also carried out to confirm the resistant reaction of these seven field selected lines. These lines were mechanically inoculated with two serologically distinct isolates of TSWV obtained from different geographic regions of Brazil. The two *C. chinense* lines ('CNPH 275' and 'PI 159236') were virtually immune against one specific (TSWV-BsB) isolate but were susceptible to another isolate (TSWV-SP) used in this assay. Sources of resistance for both isolates were not found. Our experimental results strongly indicate there exists a number of genetic mechanisms (probably including action of vertical and horizontal genes) to TSWV resistance in *Capsicum* spp.

Introduction

Tomato spotted wilt virus (TSWV) infection is one of the most economically destructive diseases of pepper (*Capsicum annuum* L.) in Brazil. Characteristic symptoms of *Capsicum* plants infected with TSWV are chlorosis and necrosis of the new growth, necrotic (usually concentric) lesions on leaves, stems and fruits; and an overall plant stunting. Production losses ranging between 49–69% have been reported in sweet pepper due to TSWV infection (Cupertino et al., 1984). TSWV can cause severe epidemics in a large number of crops mainly in tropical and subtropical climatic zones. However, the devasting effects of this virus to sweet-pepper and to several other host crops have been recently reaching temperate zones of the world including Western Europe (de Ávila et al., 1991).

Control of TSWV is difficult for several reasons such as: (1) its large host range (which includes several common weeds); (2) its transmissibility by several thrips species; (3) the peculiar nature of the TSWV-thrips interaction defined as persistent and assumedly circulative; (4) the large number of viruliferous thrips present in TSWV-infected crop residues even after the crop has been plowed and rototilled; (5) adult thrips can disperse readily and are able to inoculate healthy host plants almost contin-

uously during their life span; and (6) the low efficiency of the chemical control and the rapid acquisition of resistance to insecticides by thrips (Ie, 1970; Cho et al., 1989; Rice et al., 1990). The development of resistant cultivars is one of the best alternatives in reducing crop losses from this virus infection.

Plant resistance to TSWV has been intensively studied in tomato (Cho et al., 1989; Paterson et al., 1989; Boiteux & Giordano, 1992; Stevens et al., 1992) and lettuce (O'Malley & Hartmann, 1989; Wang & Cho, 1992). However, there are few formal published works on identification of sources of resistance to TSWV in pepper (Cupertino et al., 1988; Black et al., 1991). The main objective of this investigation was to screen *Capsicum* accessions hoping to identify resistance to TSWV proper to be used in disease resistance breeding programmes.

Materials and methods

Field screening

A total of 70 pepper (*Capsicum* spp.) accessions were evaluated for their reaction to TSWV under field (natural inoculum) conditions at Centro Nacional de Pesquisa de Hortaliças (CNPH)/EMBRAPA in Brasília (DF), Brazil. On June 5, 1991, seedlings with eight true leaves fully expanded were transplanted to plots in a distrofic oxisol soil (pH 5.9 and 3.4% of organic matter). The period between April-August corresponds to the highest incidence of TSWV under Brazilian conditions (Yuki & Costa, 1985). The experimental site was surrounded by an old naturally infected tomato field as a means to ensure high viruliferous thrips pressure. The experiment was conducted in a complete randomized design with two replications. Plots were a single 4.0 m row (with 10 plants each) with a row-to-row distance of 1.0 m. A plot of the susceptible standard *C. annuum* 'Agronômico 10-G' was used after each set of seven plots as a means of monitoring uniformity of infection within the experimental area. Disease ratings were made on November 13, 1991, by two trained observers. An overall score was given to each plot, based on the following qualitative scale: 1 = no disease; 2 = weak apical leaf distortion; 3 = top distortion and weak mosaic in the older leaves; 4 = strong top leaf distortion, apical necrosis and very clear mosaic symptoms in the older leaves and 5 = severe stunting; top distortion and general necrosis. Plot means were used in all statistical analyses. Even though the recorded data from disease assessments were qualitative, they were treated as being quantitative in order to facilitate a statistical data analysis. Yields were not recorded.

A pool of apical leaves was sampled in each plot and evaluated by using a dot-ELISA-based protocol essentially as described by Hibi & Saito (1985) with some minor modifications. Test plant tissues were homogenized with 10 volumes of PBS-T buffer (0.137 M NaCl, 8 mM $Na_2HPO_4 \cdot 12H_2O$, 2.68 mM KCl, 1.47 mM KH_2PO_4 + final 0.5% Tween 20, pH 7.4) and spotted on a nitro cellulose membrane (NCM). The reaction was blocked using PBS-T buffer +1% Triton X-100 +1% non-fat dried milk, for 1 hour. After that, anti-TSWV serum (diluted 1:1000 with PBS-T) was added. The NCM was incubated by gently shaking for 2 hours. After that, NCM was washed three times with PBS-T. The next step consisted of the addition of the antibody (1:1000) alcaline phosphatase conjugated followed by gently shaking for 2 hours. After that, the NCN was again washed as forementioned. The substrate (5-bromo-4-chloro-3-indolyl phosphate, 0.35 mg/ml) and nitro blue tetrazolium (0.175 mg/ml) in TSM alcaline buffer (0.1 M Tris, 0.1 M NaCl, 5 mM $MgCl_2$, pH 9.5) was added. As soon as the reaction became adequate for visual analysis, the NCM was transferred to a 'stop reaction' solution (10 mM Tris, 1 mM EDTA, pH 7.5). The dot-ELISA reaction for each plot was arbitrarily recorded as: (−) = negative; (+) = weak; (++) = intermediate and (+++) = strong reaction against TSWV. These data were included as an additional evaluation criterion but were not statistically analyzed.

Greenhouse screening

The seven field selected *Capsicum* spp. lines ('CNPH 050', 'CNPH 051', CNPH 275', CNPH 2733', 'PI 159236', 'PI 163192' and 'PI 379163') were

grown from seed in 5 L pots containing sterilized soil (3 parts of red oxisol soil (150 L); 1 part sand (50 L); 1 part cow manure (50 L), plus 300 g 4-14-8 fertilizer + 350 g CaO) under greenhouse conditions. Minimum temperature was 17° C and maximum temperature was 30° C during the course of the experiments. Test plants were inoculated at age of 25 days after planting. The *C. annuum* 'California Wonder' was used as a susceptible control.

Two serologically distinct (Nagata et al., 1992) TSWV-like isolates (named TSWV-SP and TSWV-BsB) originated from two different geographic areas of Brazil (São Paulo State and Federal District) were used in this study. The isolates were mechani-

Table 1. Field reaction of seventy *Capsicum* spp. accessions to tomato spotted wilt virus (TSWV) – Brasilia (DF), Brazil

Accession	Origin	Severity index*	Dot-ELISA reaction**	Accession	Origin	Severity index*	Dot-ELISA reaction**
CNPH 050	Brazil	1.0 d***	–	PI 169134	Turkey	4.0 ab	+++
CNPH 051	Brazil	1.0 d	–	PI 171553	Turkey	4.5 ab	++
CNPH 275	Peru	1.5 d	+	PI 173877	India	3.0 bc	++
CNPH 2733	Brazil	1.0 d	–	PI 177301	Syria	4.0 ab	+
Avelar	Brazil	4.5 ab	+++	PI 178847	Turkey	3.0 bc	+
MC-4	Malaysia	2.5 bc	+	PI 182925	India	3.0 bc	+
MC-5	Malaysia	3.5 b	++	PI 183439	India	3.0 bc	+++
MC-10	Malaysia	3.0 bc	+++	PI 183440	India	3.5 b	+
Yolo Wonder	USA	4.0 ab	++	PI 183922	India	3.5 b	+++
Cascadura Honjo	Brazil	4.0 ab	+++	PI 187331	Guatemala	4.5 ab	+++
Cascadura Gigante	Brazil	3.5 b	++	PI 193469	Ethiopia	3.0 bc	+
Ikeda	Brazil	4.0 ab	+++	PI 194909	Ethiopia	4.0 ab	+
Fyuco	Argentina	4.5 ab	+++	PI 196775	Argentina	4.0 ab	++
Vyuco	Argentina	4.5 ab	+++	PI 201232	Mexico	3.5 b	+
Moro	Peru	4.0 ab	+++	PI 201234	Mexico	2.5 bc	+++
Keystone Giant	USA	4.5 ab	+	PI 205170	Turkey	4.5 ab	++
California Wonder	USA	4.5 ab	++	PI 222133	Spain	4.0 ab	+
Cal Wonder 300	USA	4.0 ab	+	PI 222135	Spain	4.0 ab	++
PM 702	France	4.0 ab	+++	PI 241646	Peru	4.5 ab	+
Margareth	Brazil	4.0 ab	++	PI 244670	India	4.0 ab	+
São Carlos	Brazil	4.5 ab	+++	PI 248169	USA	4.0 ab	++
10 R	USA	4.0 ab	++	PI 256056	Pakistan	4.0 ab	+
PI 135824	Afghanistan	3.5 b	+++	PI 257485	China	4.0 ab	++
PI 135873	Pakistan	4.0 ab	+	PI 264281	USA	4.0 ab	+++
PI 159236	USA	1.0 d	–	PI 271322	India	5.0 a	+++
PI 159266	USA	4.0 ab	++	PI 281341	El Salvador	4.0 ab	++
PI 163189	India	4.0 ab	+++	PI 281383	Mexico	2.5 bc	+
PI 163192	India	1.0 d	–	PI 295284	Spain	4.0 ab	+++
PI 163201	India	3.5 b	+	PI 322719	USA	2.0 cd	+
PI 164557	Spain	4.0 ab	+	PI 339058	Turkey	5.0 a	+++
PI 164847	India	4.0 ab	+++	PI 368395	Yugoslavia	4.5 ab	++
PI 166990	Turkey	4.0 ab	++	PI 368438	Yugoslavia	4.0 ab	+++
PI 169110	Turkey	4.0 ab	+++	PI 370369	Yugoslavia	4.0 ab	+
PI 169122	Turkey	3.0 bc	+++	PI 379163	Yugoslavia	1.5 d	–
PI 169133	Turkey	4.0 ab	+	Agronomico 10G****	Brazil	4.5 ab	+++

* = Disease severity rating = 1 to 5, where 1 = no symptoms and 5 = plants showing severe necrosis and stunting.
** = Dot-ELISA reaction = (–) = negative; (+) = weak; (++) = intermediate; and (+++) = strong reaction against TSWV.
*** = Means not sharing a letter in common differ significantly (P = 0.01) according to Duncan's multiple range test.
**** = Susceptible control.

cally maintained in *Nicotiana rustica* plants. Inoculation was made by grinding *N. rustica* infected leaves in 0.1 M potassium phosphate buffer (pH 7.0) + Na_2SO_3 (0.01 M) and rubbing extracts on leaves of the selected *Capsicum* spp. lines, previously dusted with 600-mesh Carborundum. Ten plants of each line were inoculated with each TSWV isolate. The inoculated leaves were rinsed with distilled water immediately after inoculation. Plants were reinoculated 1-week later to ensure infection. Plants were scored visually for TSWV symptoms up to 8 weeks after the last inoculation. Inoculated plants were evaluated by using the same forementioned disease ratings and also by the same dot-ELISA-based protocol as described before.

Results

Field screening

Inoculum pressure was adequate as indicated by a severe and uniform infection observed on the susceptible standard 'Agronômico 10-G'. Symptoms of TSWV infection became clearly detectable among accessions about 20 days after transplanting. Upper leaves of the susceptible plants turned small and chlorotic. Those plants gradually showed chlorotic concentric rings that eventually became necrotic. Some highly susceptible materials showed a strong overall stunting or even became completely blighted and died.

Table 1 shows the result of Duncan's multiple-range test for the 70 tested accessions. A wide variability of responses to TSWV, under field conditions, existed in the *Capsicum* spp. germplasm. The mean disease rating observed for all tested lines was 3.43 with individual lines presenting a range of response to this disease varying from 1.0 to 5.0. As expected, a very low variability was observed within lines because the majority of them was selfed at least three times before evaluation. Five accessions were identified as having a high degree of field resistance to TSWV as demonstrated simultaneously by visual rating and dot-ELISA analysis; and two lines had low visual score and negative reaction in dot-ELISA (Table 1).

Greenhouse screening

Results of the greenhouse evaluation are shown in Table 2. All plants of the susceptible control 'California Wonder' showed severe TSWV-like symptoms for both isolates verifying their virulence. In this experiment a clear distinction was observed in relation to two *C. chinense* lines ('CNPH 275' and 'PI 159236'). These lines were immune to the TSWV-BsB isolate. They reacted initially to this isolate with necrotic local lesions followed by a pre-

Table 2. Greenhouse reaction of seven field selected lines of *Capsicum* spp. against two serologically distinct tomato spotted wilt virus (TSWV) isolates (TSWV-SP and TSWV-BsB)

Accession	Species	TSWV-BsB		TSWV-SP	
		DR*	ELISA**	DR	ELISA
CNPH 050	*C. baccatum*	5.0	+++	5.0	+++
CNPH 051	*C. baccatum*	5.0	+++	5.0	+++
CNPH 275	*C. chinense*	1.0	–	5.0	+++
CNPH 2733	*C. annuum*	3.0	+	3.0	++
PI 159236	*C. chinense*	1.0	–	5.0	+++
PI 169132	*C. annuum*	3.0	++	3.0	++
PI 379163	*C. annuum*	4.0	++	3.0	++
CNPH 296***	*C. annuum*	5.0	+++	5.0	+++

* DR (Disease rating) = 1 to 5, where 1 = no symptoms and 5 = plant showing severe necrosis and stunting.

** Dot-ELISA reaction = (–) = negative; (+) = weak; (++) = intermediate; (+++) = strong reaction against TSWV.

*** Susceptible control (bell pepper 'California Wonder').

mature 'leaf dropping', closely resembling a hypersensitive response to the virus, observed only in the inoculated leaves. Systemic infection was not confirmed by dot-ELISA. A similar result was obtained for 'PI 159236' after mechanical inoculation with seven TSWV isolates from the U.S.A. (Black et al., 1991). On the other hand, the isolate TSWV-SP was highly virulent and became systemic in all 20 inoculated plants of both *C. chinense* accessions. The two *C. baccatum* and the three *C. annuum* lines tested were susceptible for both TSWV isolates used in this experiment.

Discussion

Field resistance, horizontal resistance (Robinson, 1976) or rate-reducing resistance (Nelson, 1973) is extremely desirable due to its capacity to restrict epidemic development of certain diseases. However, the assessment of horizontal resistance is usually over-looked in plant breeding programmes because horizontal resistance is known to be affected by several environmental factors. This kind of resistance may also represent, sometimes, not a true horizontal resistance but a mixture of vertical resistance genes in the same genotypic background (Robinson, 1976). A high level of field resistance was identified in at least ten accessions of *Capsicum* spp. The field resistance was more characteristically expressed in two *C. baccatum* lines ('CNPH 050' and 'CNPH 051'). These lines showed a 'field immunity' as demonstrated by visual analysis and dot-ELISA (Table 1). However, these same lines were highly infected after mechanical inoculation with two TSWV-like isolates under greenhouse conditions (Table 2).

Vector transmission of viruses is important in determining the development of an epidemic. Virus transmissibility can be modified by host genes that affect plant preference and feeding behavior by vectors (Fraser, 1990). The distinct response showed by the *C. baccatum* lines ('CNPH 050' and 'CNPH 051') after field exposure and mechanical inoculation may be partially explained by the presence of a mechanism of non-preference (or less attractiveness) to the TSWV-thrips vectors.

The presence of isolate-specific resistance was confirmed for two *C. chinense* lines ('CNPH 275' and 'PI 159236'). These lines showed an immunity-like response to the TSWV-BsB isolate. On the other hand, as previously reported (Boiteux & Nagata, 1993), these lines were highly susceptible to members of a second 'serological group' of isolates in which TSWV-SP is included (Nagata et al., 1992). A similar response was also observed for the other *C. chinense* line 'PI 152225' (data not shown); a previously reported source of resistance to seven TSWV isolates from the U.S.A. (Black et al., 1991). Additional studies including screening of new genotypes against a greater and more representative number of TSWV-like isolates will be necessary to identify sources of large-spectrum resistance genes.

The data obtained from greenhouse and field evaluations strongly suggest: (1) there is an absence of horizontal resistance genes in the 'CNPH 275' and 'PI 159236' becauce they were highly susceptible to the TSWV-SP isolate infection (disease score = 5.0); (2) the resistance (a hypersensitive-like response) shown by 'CNPH 275' and 'PI 159236' to the TSWV-BsB isolate is probably due to a vertical-like resistance gene (or genes). No general symptoms (disease score = 1.0) and no systemic infection (as demonstrated by dot-ELISA) were observed in these lines in response to inoculation with TSWV-BsB; and (3) the presence of distinct mechanisms of field resistance acting in *C. chinense* and *C. baccatum* lines.

The main concern of several breeding programmes throughout the world is to incorporate stable and durable forms of resistance to *Capsicum* diseases into commercial pepper cultivars (Reifschneider et al., 1992). The presence of isolate-specific resistance gene combined with low horizontal resistance makes sources such as 'CNPH 275' and 'PI 159236' highly vulnerable. Thus, it is likely that the resistance introgressed from these lines into commercial *C. annuum* cultivars can be less effective after growing these materials on a large scale in different geographic regions of Brazil. A recurrent selection-based breeding programme could be a recommended methodology for an effective exploitation and incorporation into improved pepper cultivars

of genetic variability for TSWV resistance present in *Capsicum* spp.

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