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Allelic relationships between genes for resistance to tomato spotted wilt tospovirus in *Capsicum chinense*

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Abstract Pepper (*Capsicum chinense* Jacq.) has been reported to be an important reservoir of resistance genes to tomato spotted wilt virus (TSWV). The genes for TSWV resistance present in three *C. chinense* lines ('PI 152225', 'PI 159236' and 'Panca') were investigated for allelism. All resistant lines were crossed with each other. Parents, F_1, backcrosses and F_2 populations (including reciprocals) developed from those crosses were mechanically inoculated with a highly virulent TSWV isolate. Susceptible *C. annuum* cv 'Magda' was used to check inoculum virulence. Fifty plants of the F_1 hybrids; 'Magda' x 'PI 152225', 'Magda' x 'PI 159236', and 'Magda' x 'Panca, were also inoculated with the TSWV isolate. The resistance response in all *C. chinense* sources was associated with a localized, hypersensitive-like reaction that was phenotypically expressed as a prompt formation of large local lesions accompanied by premature leaf abscission. All F_1 generations presented a final score of resistant; indicating that the expression of resistance to TSWV is conditioned by a dominant gene regardless of the source. The absence of segregation for resistance to TSWV that was observed in all generations of the crosses between *C. chinense* lines indicated that either a tightly linked group of genes exists or that the resistance is governed by the same single major gene (probably the already described *Tsw* gene). Previous reports have indicated that the *Tsw* gene is not effective against tospovirus members of serogroup II, i.e. tomato chlorotic spot virus (TCSV) and groundnut ring spot virus (GRSV). In the assay described here, all of the *C. chinense* lines showed, after mechanical inoculation, an identical susceptibility response to the TCSV and GRSV isolates.

Key words *Capsicum chinense* · Resistance gene · Genetics · Pepper · Tomato spotted wilt virus · Tospoviruses

Introduction

Tomato spotted wilt virus (TSWV) is the type member of the recently created plant virus genus *Tospovirus* within Family *Bunyaviridae* (Francki et al. 1991). TSWV is a cosmopolitan virus that is almost exclusively transmitted in nature by thrips (*Thripidae: Thysanoptera*). Recent studies based on serological analysis and phylogeny of the nucleoprotein gene sequences of several TSWV-like isolates have indicated that the *Tospovirus* is not a monotypic genus as initially supposed but comprises a complex of genetically and biologically distinct subgeneric taxa (de Avila et al. 1993). So far, the *Tospovirus* genus consists of four viral species: TSWV (member of serogroup I), tomato chlorotic spot virus (TCSV) and groundnut ring spot virus (GRSV) all members of the serogroup II, and the sole member of the serogroup III, *Impatiens* necrotic spot virus (INSV) (de Avila et al. 1993).

Infection by tospoviruses has been responsible for important economic constraints in sweet pepper (*Capsicum annuum* L.) and in a large number of vegetable and ornamental crops especially those found in the tropical and subtropical regions of the world). Production losses as high as 69% have been reported in sweet pepper cultivated under open-field conditions (Cupertino et al. 1984). Natural infection of pepper (*Capsicum* spp.) by TSWV, TCSV and GRSV has been already reported in Brazil (Boiteux et al. 1993b; 1994). The use of resistant cultivars is one of the most interesting approaches toward control of the diseases caused by tospoviruses in pepper due to their ready-to-use characteristics and their inherent benefit in reducing environmental damages such as those associated with insecticide overspraying abuses.

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Sources of host resistance to TSWV have been found in *Capsicum* spp. (Cupertino et al. 1988; Black et al. 1991; Boiteux et al. 1993c). According to the recent literature, *C. chinense* Jacquin is the most important reservoir of TSWV resistance alleles. So far, three distinct resistant sources to TSWV, 'PI 152225', 'PI 159236' (syn. 'CNPH 679') and the Peruvian cv 'Panca' (syn. 'CNPH 275') have been identified inside that host species (Cupertino et al. 1988; Black et al. 1991; Boiteux et al. 1993c). Studies concentrated on 'PI 159236' have strongly supported the notion that the resistance in this line is not isolate-specific (Boiteux and Nagata 1993; Duval and Cupertino 1993) but apparently species-specific (Nagata et al. 1992, 1993a; Boiteux and de Avila 1993). *C. chinense* 'PI 159236' was found to be highly resistant to all isolates of TSWV but susceptible to all isolates classified as TCSV and GRSV (Boiteux and de Avila 1993; Nagata et al. 1993b). Boiteux and de Avila (1994) found that the apparently TSWV-specific resistance of 'PI 159236' is inherited as a dominant, highly penetrative, single-gene character, and they tentatively denoted the novel resistant gene *Tsw*. This *Tsw* gene exhibits as its main phenotypic characteristic, the presence of a localized (Bos 1978; White and Antoniw 1991) or hypersensitive-like (Fraser 1990) reaction to TSWV isolates. A similar host response has also been observed in 'Panca' and 'PI 152225' (Boiteux et al. 1993c).

The main purpose of the present study was to examine allelic relationships between *C. chinense* sources of resistance to TSWV in order to determine whether these genes (present in the accessions 'PI 152225', 'PI 159236' and 'Panca') are allelic among themselves. This information is of paramount importance in the establishment of practical and economic breeding strategies for the incorporation of that genetic resistance to TSWV in commercial sweet pepper cultivars and hybrids.

Materials and Methods

This work was conducted in a partially shaded, insect-free, air-refrigerated greenhouse at the Centro Nacional de Pesquisa de Hortaliças (National Research Center for Vegetable Crops) CNPH/EMBRAPA in Brasilia (DF), Brazil.

Plant material

The pepper populations consisted of approximately 60 plants each of the TSWV-resistant materials 'PI 152225', 'PI 159236' and 'Panca' and approximately 40 plants of the susceptible control *C. annuum* 'Magda'. About 50 plants of each of the following F_1 hybrids, 'Magda' × 'PI 152225', 'Magda' × 'PI 159236' and 'Magda' × 'Panca', were also included as additional checks. Plants of each F_1, F_2 and backcross (including reciprocals) generations derived from the following crosses, 'PI 152225' × 'PI 159236', 'PI 159236' × 'Panca', 'PI 152225' × 'Panca', were used for the tests of allelism. All plant materials were maintained in plastic boxes (40 × 30 × 10 cm) containing sterile soil (oxisol) fertilized with cow manure and a NPK 4–14–8 formulation. Two rows, with 8–10 plants per row, were grown in each box. Temperature range during the course of the experiment was 17°–27 °C.

Tospoviruses

The TSWV isolate used in this present assay was obtained from a naturally infected tomato plant at the experimental field of the CNPH/EMBRAPA in Brasilia (DF), Brazil. The inoculum was multiplied in *Nicotiana rustica* plants, and the infected leaves were stored in a freezer at −80 °C prior utilization. The *C. chinense* lines were also inoculated separately with TCSV (tomato isolate from Campinas-SP, Brazil) and with a pepper isolate of GRSV obtained in Janauba-MG, Brazil (Boiteux et al. 1994).

Inoculation

Test plants were mechanically inoculated using leaf extracts from infected *N. rustica* plants. The inoculation protocol was essentially that described by Boiteux and Giordano (1992) with some minor modifications. Inoculum was prepared by grinding infected leaves in 0.01 *M* sodium phosphate buffer (pH 7.0) containing 1% Na_2SO_3. Leaves of the tested plants were dusted with 600-mesh carborundum before inoculation. The plants were inoculated three times (17, 20 and 24 days after planting).

Evaluation

Plants were scored visually for TSWV symptoms at 3- to 5-day intervals for up to 10 weeks after the last inoculation. Susceptible reactions (characterized by chlorosis or necrosis of the new growth and apical downward leaf curl) were recorded, and susceptible plants were eliminated with each evaluation. Immediately after the last disease assessment, leaf samples of plants without obvious symptoms (visually considered as resistant) were collected and checked for TSWV latent infection using an ELISA protocol as previously described (Boiteux and Giordano 1993). Only non-mechanically inoculated leaves of the apical region of the test plants were taken for analysis by ELISA. This procedure was employed to avoid a possible misinterpretation of the results due to a hypersensitive reaction to TSWV (Fonseca and Boiteux 1992). Assymptomatic plants were considered as infected (susceptible) when the reaction of their sap against the TSWV polyclonal antiserum was clearly distinct from the negative control (healthy) samples after the ELISA test. Data were analyzed by the chi-square goodness-of-fit test.

Results

All plants of the susceptible control 'Magda' showed typical and severe symptoms of infection by tospoviruses, thereby verifying the virulence of the isolates. The parental lines ('PI 152225', 'PI 159236' and 'Panca') presented a final score of resistant, after mechanical inoculation, to the TSWV isolate but not to TCSV and GRSV isolates. Therefore, allelism tests were only conducted using the TSWV isolate (Table 1).

The *C. chinense* lines reacted to the TSWV isolate with a localized hypersensitive-like response that was characterized by the formation of large necrotic local lesions (about 4–6 days after inoculation) followed by a premature leaf dropping (restricted to the inoculated leaves). All plants of the F_1 hybrids between 'Magda' × 'PI 152225', 'Magda' × 'PI 159236', 'Magda' × 'Panca' presented phenotypically identical hypersensitive reactions and were also scored as resistant to the TSWV isolate (Table 1). These data indicated the presence of dominant resistant genes to this virus in all sources. The F_1 hybrids of 'PI 152225' × 'PI 159236', 'PI 152225' × 'Panca' and 'PI 159236' × 'Panca' and

Table 1 Reaction to tomato spotted wilt virus (isolate BsB) of parental, F_1 and F_2 progenies of crosses of three pepper (*C. chinense*) resistant lines ('PI 152225', 'PI 159236' and 'Panca')

Crosses and generations Number	Number of plants		Ratio^{b} (R:S)	χ^2	P
	Total^{a}	Resistant			
Panca (P_1)	58	58	(1:0)	–	1.0
PI 152225 (P_2)	55	55	(1:0)	–	1.0
PI 159236 (P_3)	55	55	(1:0)	–	1.0
Magda (SC^{c})	40	0	(0:1)	–	1.0
Magda × P_1	48	48	(1:0)	–	1.0
Magda × P_2	46	46	(1:0)	–	1.0
Magda × P_3	43	42	(1:0)	0.023	(0.95–0.99)
$P_1 \times P_2$ (F_1 a)	112	112	(1:0)	–	1.0
$P_1 \times P_3$ (F_1 b)	81	81	(1:0)	–	1.0
$P_2 \times P_3$ (F_1 c)	129	129	(1:0)	–	1.0
F_1 a × F_1 a (F_2 a)	153	153	(1:0)	–	1.0
F_1 b × F_1 b (F_2 b)	284	281	(1:0)	0.032	(0.95–0.99)
F_1 c × F_1 c (F_2 c)	211	210	(1:0)	0.005	>0.99

[a] Data were polled for all reciprocal crosses between the sources of TSWV resistance

[b] Hypothesized ratio of resistant (R) to susceptible (S) plants according to the presence of a single allelic gene in all the *C. chinense* sources

[c] The susceptible control (SC) *C. annuum* 'Magda' was used only as maternal parent in the F_1 crosses with the *C. chinense* sources of TSWV resistance

their reciprocals presented similar hypersensitive-like reactions to TSWV. Reciprocal differences for disease reaction were not significant ($\chi^2 < \chi^2$ 0.90). Therefore, data were pooled for all F_1, backcross (BC) and F_2 populations derived from reciprocal crosses (Table 1). All F_2 populations originating from the F_1 generations did not segregate for susceptibility (Table 1). The corresponding backcrosses did not segregate either (data not shown). Under our experimental conditions, the penetrance of the resistance gene was high, but not complete, as indicated by the low number of susceptible plants observed in the F_1 and F_2 generations (Table 1).

The initial symptoms induced by the TCSV and GRSV isolates in all of the *C. chinense* lines was also characterized by the formation of large local lesions and premature leaf dropping. However, in this case, that reaction was not able to avoid the further systemic spread of TCSV and GRSV. Systemic symptoms of these tospoviruses in the three *C. chinense* lines included chlorosis and necrosis of the new growth, necrotic concentric lesions on leaves and overall plant stunting.

Discussion

All *C. chinense* resistant lines reacted to the TSWV isolate with a localized (Bos 1978; White and Antoniw 1991), hypersensitive-like reaction. This kind of resistant response against TSWV infection is essentially similar to that previously reported to be associated with the presence of a dominant major gene (tentativelly denoted as *Tsw*) identified in the line 'PI 159236' (Boiteux and de Avila 1994). The incomplete penetrance of this resistant gene or the presence of minor modifying genes may explain some non-expected fluctuations, e.g. a few plants with a susceptible reaction that were observed within segregating F_2 populations of the crosses between the TSWV-resistant lines and also in 1 interspecific F_1 hybrid (Table 1). The absence of the typical segregation ratio of 15 resistant to 1 TSWV-susceptible plant in the F_2 generations of the crosses involving the three *C. chinense* lines strongly indicates that either all three lines have the same gene or that there is a group of resistance genes that are closely linked. Since no significant segregation occurred in any of the crosses between all of the resistant *C. chinense* lines, it is suggested that these lines have functionally the same *Tsw* gene. However, a small possibility does exist that distinct multiple genes for TSWV resistance are present in the lines 'PI 152225' and 'Panca'. Additional inheritance studies using these two *C. chinense* lines will be necessary to confirm this hypothesis.

The major gene *Tsw* exhibits, which was also demonstrated here, a characteristic ineffectiveness to TCSV and GRSV isolates after mechanical transmission. However, preliminary data on the performance of these *C. chinense* lines under TCSV and GRSV field inoculum (where the virus is transmitted by thrips) indicated comparatively good levels of rate-reducing (Nelson 1973) resistance (L.S. Boiteux, unpublished results). This result was partially expected because field inoculation by thrips clearly produces less dramatic results than the mechanical inoculation used in this study. If confirmed (in different trials over the years), this phenomenon will provide evidence of a peculiar kind of rate-reducing resistance that is conditioned by the presence of a major 'quasi-defeated' gene.

The search for wide-spectrum resistance genes to tospoviruses in *Capsicum* spp. is still an important demand for breeding programmes, especially in Neotropical areas of the world where the genetic diversity of the genus *Tospovirus* appears to be higher (de Avila et al. 1993). According to this present work, the choice of parental material in sweet pepper breeding programmes (when the focus is exclusively resistance to TSWV) is independent of the resistance source because all three lines carry apparently the same gene for resistance. However, some of these *C. chinense* lines present more attractive genetic attributes for use as a pollen donor. An illustrative example is line 'PI 159236', which has been found to present good levels of partial resistance to the crown rot disease caused by the fungus *Phytophthora capsici* Leonian (Boiteux et al. 1993a); this resistance has not been identified, for instance, in 'Panca' (Reifschneider et al. 1992).

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