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Inheritance of a resistance specific to tomato spotted wilt *tospovirus* in *Capsicum chinense* 'PI 159236'

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Summary

Inheritance studies were conducted to determine the genetic basis of resistance in pepper against one *Tospovirus* isolate classified as tomato spotted wilt virus (TSWV). F_1, backcrosses and F_2 populations were developed using the resistant parent *Capsicum chinense* 'PI 159236' (CNPH 679) and the susceptible parent *C. annuum* 'Magda' (CNPH 192). Segregation ratios strongly indicated that the resistant response (a localization, hypersensitive-like reaction) to TSWV fits a single-dominant gene model. Under our experimental conditions, the penetrance of this gene was very high. This gene (tentatively named *Tsw*) is highly effective only against TSWV isolates. The resistance governed by the *Tsw* gene was not effective against isolates belonging to tomato chlorotic spot virus (TCSV) and groundnut ring spot virus (GRSV), two other previously described *Tospovirus* species.

Introduction

Tomato spotted wilt virus (TSWV) is a cosmopolitan plant virus which is transmitted in nature by several species of thrips (*Thripidae : Thysanoptera*) (Resende, 1993). TSWV has a wide host range and can cause important economic losses in pepper (*Capsicum annuum* L.) and in a large number of vegetable crops mainly in tropical regions of the world (de Ávila, 1992; Sether & De Angelis, 1992). The devasting effects of this virus have been recently reported also in the Northern hemisphere after introduction of the vector *Frankliniella occidentalis* Pergande (de Ávila et al., 1990).

In pepper, symptoms of TSWV infection vary depending on host genotype and can include chlorosis and necrosis of the new growth, apical downward leaf curl, necrotic (usually concentric) lesions on leaves, stems and fruits, and overall plant stunting (Boiteux et al., 1993c). Host tissue is infected systemically throughout all organs, via vascular bundles (Best, 1968). An early-infected pepper plant usually bears a very low marketable yield. Production losses ranging between 49–69% have been reported in sweet-pepper due to TSWV infection (Cupertino et al., 1984).

TSWV has been considered for years as a monotypic plant virus group, not related to any other taxonomic group of plant viruses. At the meeting of the International Committee on Taxonomy of Viruses (ICTV) held 1990 in Germany, a new genus denoted as *Tospovirus* was created within the family *Bunyaviridae*. TSWV has been placed as the prototype of this genus (Francki et al., 1991). Recently, a comparative study using serological analysis split a group of 20 TSWV-like isolates from various geographical areas across the world into three distinct serogroups (de Ávila et al., 1990, 1993a). Based on nucleotide and amino acid sequence homologies of the nucleoprotein gene from members of those three serogroups, de Ávila et al. (1993b) proposed a classification of those distinct serogroups in four different species within the genus *Tospovirus* viz. tomato chlorotic spot virus (TCSV), groundnut ring spot virus (GRSV), *Impatiens* necrotic spot virus (INSV) and TSWV. Natural infection of pepper by TSWV and TCSV has been recently reported (Boiteux et al., 1993b). So far, GRSV has been found occurring only on tomatoes in Brazil (de Ávila, 1992). However, Brazilian isolates of GRSV were found to be highly

virulent to pepper genotypes after mechanical inoculation (Boiteux & de Ávila, 1993).

Cupertino et al. (1988) were the first to report *Capsicum chinense* Jacquin 'PI 159236' (syn. CNPH 679) as a source of resistance to Brazilian isolates of TSWV and later Black et al. (1991), Boiteux & Nagata (1993); and Boiteux et al. (1993c) confirmed this resistance against other TSWV isolates. Boiteux & Nagata (1993) and Duval & Cupertino (1993) simultaneously reported the occurrence of several TSWV-like 'PI 159236'-resistance breaking isolates. So far, serological analysis of all these reported resistance breaking tospovirus isolates resulted in their classification as TCSV or GRSV (Boiteux & de Ávila, 1993; Nagata et al., 1993b). Additional screening trials against 101 serologically characterized Brazilian isolates of tospoviruses strongly supported the notion that the resistance in 'PI 159236' is not isolate-specific but apparently species-specific (Nagata et al., 1992; Boiteux & de Ávila, 1993; Nagata et al., 1993b). *C. chinense* 'PI 159236' was found to be highly resistant against all isolates belonging to TSWV but susceptible to all isolates classified as TCSV and GRSV (Boiteux & de Ávila, 1993; Nagata et al., 1993b).

For an effective breeding programme, better information concerning TSWV-resistance inheritance is required. The main purpose of this work was to study the genetic basis of this apparent species-specific resistance to TSWV present in *Capsicum chinense* 'PI 159236' line.

Materials and methods

This work was conducted in a partially shaded wire-mesh screenhouse at the Centro Nacional de Pesquisa de Hortaliças (National Research Center for Vegetable Crops) CNPH/EMBRAPA in Brasilia (Federal District), Brazil.

Plant material

The pepper populations evaluated consisted of ca. 40 plants each of the resistant parent *C. chinense* 'PI 159236' (CNPH 679) and susceptible bell type cultivar Magda (CNPH 192); about 70 plants of F_1 between the resistant and susceptible parents, about 80 and 100 backcross progeny with 'PI 159236' and with 'CNPH 192' as the recurrent parents, respectively; and about 110 F_2 plants derived from selfed F_1 plants. Due to unilateral low compatibility, the resistant parent ('PI 159236') was used only as staminate parent. Thus, reciprocal (maternal) differences for disease reaction were not evaluated. Plants were maintained in plastic 5 L pots containing sterile soil (oxisol) fertilized with cow manure and a NPK 10-10-10 formulation. Temperature range during the course of the experiment was 19°–31° C.

Tospovirus isolates

Three isolates were used in this present assay. These isolates were classified as TSWV (isolate BR-01), TCSV (isolate BR-03) and GRSV (isolate BR-08) based upon serological analysis and also by the phylogeny of their nucleoprotein gene sequences (de Ávila et al., 1993b). All isolates were obtained from naturally infected tomato plants in Brazil. The viruses were maintained separately in *Nicotiana rustica* plants. Three sets of leaves infected with each virus were stored in a freezer at - 80° C prior to utilization.

Inoculation

Test plants were mechanically inoculated using leaf extracts from infected *N. rustica* plants. The inoculation protocol was essentially that described by Boiteux & 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% sodium sulphite. Leaves of the tested plants were dusted with 600 mesh carborundum before inoculation. Plants were inoculated three times (18, 21, and 25 days after planting).

Evaluation

Plants were assessed at intervals of 3–5 days up to 8 weeks after the last inoculation. Plants without obvious symptoms were assayed for TSWV, TCSV and GRSV using a DAS-ELISA protocol similar to that described by de Ávila et al. (1990) to confirm infection. Only non-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 some hypersensitive reaction to the virus (Fonseca & Boiteux, 1992). Thus, only systemically infected plants were considered as susceptible. Data were analysed by the chi-square goodness-of-fit test (Snedecor & Cochran, 1980).

Table 1. Segregation data for disease reaction of parental (P), F_1, backcrosses (BC) and F_2 populations of pepper after inoculation with tomato spotted wilt virus isolate BR-01

Crosses and generations		Number of plants		Ratio* (R : S)	χ^2	P
		T**	R			
'Magda'	(P_1)	40	0	(0 : 1)	–	1.0
'PI 159236'	(P_2)	42	42	(1 : 0)	–	1.0
$P_1 \times P_2$	(F_1)	71	68	(1 : 0)	0.13	(0.50–0.75)
$F_1 \times P_1$	(BCP_1)	103	57	(1 : 1)	1.17	(0.25–0.50)
$F_1 \times P_2$	(BCP_2)	80	80	(1 : 0)	–	1.0
$F_1 \times F_1$	(F_2)	109	86	(3 : 1)	0.88	(0.25–0.50)

* Hypothesized segregation ratio of resistance (R) to susceptible (S) plants.

** Total (T) of evaluated plants to resistant (R) plants.

Results

Preliminary screening tests indicated that *C. chinense* 'PI 159236' was resistant only to isolates classified as TSWV but susceptible to TCSV and GRSV isolates (Boiteux & de Ávila, 1993; Nagata et al., 1993b). These data were once more confirmed in this present study. Therefore, inheritance studies were only conducted using TSWV isolate BR-01. Results of this study are shown in Table 1. All plants of the susceptible parent (P_1) *C. annuum* 'Magda' (CNPH 192) showed severe symptoms following inoculation with the TSWV isolate verifying its virulence. On the other hand, the *C. chinense* 'PI 159236' (P_2) line was found to be highly resistant against the TSWV isolate used in this experiment. Segregation for resistant and susceptible disease reactions closely fit ratios of 1 : 0, 1 : 0, 1 : 1 and 3 : 1 in the F_1; backcross (BC) to the resistant parent; BC to the susceptible parent; and F_2 populations, respectively (Table 1). Chi-square goodness-of-fit test on these data supports this conclusion (Table 1). These segregation ratios strongly suggest a single-dominant gene model for resistance to TSWV.

All resistant plants in P_2, F_1, and backcrosses generations reacted initially to TSWV with necrotic local lesions followed by a premature leaf dropping, closely resembling a hypersensitive response to the virus. The final phenotypic score of those generations indicated resistance because no systemic infection was detected by visual analysis or ELISA. All plants phenotypically scored as resistant in the F_2 generation also showed local lesions and leaf dropping response to TSWV infection. Under our experimental conditions, the penetrance of the gene was very high but not complete (Table 1).

Discussion

Formation of large local lesions in response to a virus infection is generically defined as localization. Localization usually occurs at or close to the 'locus' or site where the virus was inoculated (Bos, 1978). That reaction may avoid spread of the virus from the sites of inoculation to other parts of the plant, or viral replication may be so restricted that spread does not occur (Ponz & Bruening, 1986). Virus localization is considered to be a resistant response to the virus infection (Fraser, 1990). Therefore, the local lesion plus the premature leaf dropping of the inoculated leaves presented by *C. chinense* 'PI 159236' can be considered as the main phenotypic characteristic associated with the resistant response to TSWV.

In several host-virus interactions, localization is a phenomenon associated with resistance alleles which are phenotypically dominant (Fraser, 1990). The data obtained in this present work are in agreement with this general observation. In a similar way, the resistant response of 'PI 159236' to TSWV is inherited as a dominant single-gene character. We tentatively named this gene as *Tsw* ('tomato spotted with virus localization') in accordance with the current rules for designating pepper genes (Greenleaf, 1986).

The genotypic variability present in the genus *Tospovirus* must be considered in breeding programmes throughout the world (Boiteux et al., 1993b, 1993c). Field utility of the resistance conferred by

the *Tsw* gene may be dependent upon the *Tospovirus* species present in a given geographic region. A preliminary survey in Brazil of *Tospovirus* isolates infecting vegetable crops (including *Capsicum* spp.) and weeds indicated that TSWV is predominant in the Central part of the country and TCSV is predominant in the Sao Paulo State area (Boiteux et al., 1993b; Nagata et al., 1993a, 1993b). Clearly there is a need for resistance genes effective also against TCSV, GRSV and perhaps others not yet characterized *Tospovirus* species that are able to infect *Capsicum* spp. Encouraging in this regard is that the TSWV resistance gene (*Sw-5*) in tomato (Stevens et al., 1992) has recently been shown to be effective against TCSV and GRSV isolates (Boiteux & Giordano, 1993; Boiteux et al., 1993a).

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