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High temperature effects on hypersensitive resistance to Tomato Spotted Wilt *Tospovirus* (TSWV) in pepper (*Capsicum chinense* Jacq.)

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Abstract

The tomato spotted wilt virus resistance in pepper conferred by the *Tsw* gene is less stable at 32 °C continuous temperature. Continuous high temperatures for at least nine days lead to systemic spread and necrotic symptoms in plants that are totally resistant at a lower temperature (22 °C). We show that continuous high temperatures destabilize this resistance in young plants, but older inoculated plants rarely develop systemic symptoms. Populations segregating for the *Tsw* gene showed that heterozygosity at the *Tsw* locus increased the chance of inoculated seedlings to develop systemic necrotic symptoms. Genetic background was less significantly involved in the thermosensitivity of the resistant response. As a consequence, it would be preferable to grow cultivars homozygous at the *Tsw* locus in high temperature conditions.

Introduction

Pathogen resistances in plants are often challenged by high temperatures. This phenomenon has been noted for nematode (Dropkin, 1969), fungi (Roelfs, 1988) and especially virus resistances. The interaction between the resistance processes and high temperatures has not yet been explained. The phenomenon occurs with constitutive (Fraser and Loughlin, 1982) or induced (hypersensitive) resistances. In the case of hypersensitive resistances, we do not know if the temperature interferes with the recognition event, the transduction of the signal or the defence reaction. Fraser (1983) noticed a positive correlation between tobacco mosaic virus (TMV) mutants inducing large lesions on *N′* tobacco plants at 30 °C and the thermal instability of their coat proteins. The coat protein is considered to be the elicitor of this resistance gene (Culver et al., 1991) and temperature may affect the interactions between the subunits of the TMV coat protein that would be an essential feature for the induction of the resistance. The mechanism of temperature effect on resistance suppression in other interactions remains unclear.

Several factors can modulate the disruption of the resistance at high temperatures: the viral strain (Pelham, 1972), the inoculum pressure and the stage of development of the inoculated plant (Pilowsky, 1975; Kopliovitch et al., 1978), the genetic background (Pelham, 1972; Laterrot, 1973; Kopliovitch et al., 1978; Daubèze et al., 1990; Chaîne-Dogimont, 1993; Seifers et al., 1995) and allele dosage effects (Schroeder et al., 1967).

Tomato spotted wilt virus (TSWV), the type-member of the tospovirus genus, has become a threat in many vegetable and ornamental production areas following the wide geographic distribution of *Frankliniella occidentalis* Pergande, a very efficient thrips vector of the virus (German et al., 1992). In pepper, a major gene, *Tsw*, identified in different accessions of *Capsicum chinense* Jacq., can control systemic spread of the virus by a hypersensitive response (Black et al., 1991; Boiteux, 1995; Moury et al., 1997). Reports have indicated that this resistance

was sporadically inefficient. Nuez et al. (1994) noticed that 'Young plants grown at about 30 °C show a slow hypersensitivity reaction. Small necrotic lesions progress, reach and injure veins, leaves, stem and bud, giving rise to the death of the plant'. Black et al. (1991) and Gil-Ortega and Luis (1994) observed the same phenomenon and suspected that plant age, climatic conditions and/or inoculum concentration could influence the effect. Recently, Roggero et al. (1996) studied the influence of high temperature on *Capsicum chinense* 'PI 152225'. They noticed a destabilization in the resistance of 16 and 30-day-old plants held under continuous 33 °C temperatures for 20 days and suspected the influence of epistatic effects. Since the *Tsw* gene is to be introgressed in a *C. annuum* genetic background and to breed heterozygous F_1 hybrid cultivars, it is essential to evaluate its stability in such conditions. In this report, we analyze the expression of the *Tsw* resistance gene under different temperature treatments in different genetic backgrounds and in a heterozygous or homozygous status.

Materials and methods

Plant material

Three *C. chinense* accessions resistant to TSWV were studied: 'PI 152225', PI 159236' (Black et al., 1991) and '7204' (Nuez et al., 1994) that were shown to possess the same resistance gene *Tsw* (Moury et al., 1997). The susceptible lines were *C. frutescens* 'PI 195301' and two *C. annuum* bell pepper lines 'Vania' and 'Milord' from INRA Montfavet. Segregating progenies from these lines were obtained from controlled cross-pollinations or self-pollinations of plants grown in greenhouses. Progenies involving 'Vania' or 'Milord' lead to very similar results. The results were grouped in '*C. annuum*' derived populations.

TSWV strain and inoculation procedure

The TSWV strain used for inoculation tests was cloned from the tomato isolate LYE 51 originating from France (Moury et al., 1997). This strain was multiplied on the susceptible tomato cultivar 'Momor', ensuring a high inoculum concentration (this concentration was not evaluated quantitatively, but ELISA tests on LYE 51 dilution ranges showed positive results up to the 10^{-7} dilution). The strain was not multiplied on tomato more than four times successively to avoid the appearance of defective variants. LYE 51 was never previously multiplied in pepper plants because of the frequent virulence evolution of TSWV strains in *C. chinense* resistant plants (Moury et al., 1997). The inoculum preparation and inoculation procedure were as described by Moury et al. (1997). Each plant was inoculated once on both cotyledons.

Test conditions

Temperature treatments

Plants were grown initially in a greenhouse and then transferred to a growth chamber (32 °C or 22 °C, 12 h day/12 h night, 65% relative humidity) for inoculations and post-inoculation observations. Plants at the cotyledonary stage were mechanically inoculated on both cotyledons and then observed for about one month after inoculation.

In a first experiment, *C. chinense* plants belonging to the three resistant accessions and the susceptible line 'Vania' were subjected to the following temperature regimes:

T0: 22 °C continuously,
T1: 5 days at 32 °C after inoculation followed by a transfer to 22 °C,
T2: 9 days at 32 °C after inoculation followed by a transfer to 22 °C,
T3: 12 days at 32 °C after inoculation followed by a transfer to 22 °C,
T4: 3 days at 32 °C before inoculation and 22 °C continuously afterwards,
T5: 32 °C from 3 days before to 9 days after inoculation followed by a transfer to 22 °C,
T6: 32 °C for 10 h (light) alternating with 22 °C for 14 h during 12 days after inoculation.

Treatments T0, T2, and T3 were repeated twice, other treatments were performed once.

Plant age

To look for plant stage effects, plants of '7204', 'PI 152225', 'PI 159236' and 'F_1 (Vania $\times$ PI 159236)' at the following growth stages were inoculated on cotyledons and submitted to T3:

stage 1: 15 days after sowing; cotyledonary stage,
stage 2: 24 days after sowing; 1 to 2 expanded leaves,
stage 3: 31 days after sowing; 3 to 4 expanded leaves,
stage 4: 38 days after sowing; 4 to 5 expanded leaves.

Influence of temperature on segregating progenies

In further experiments, several progenies segregating for the *Tsw* locus were tested for the occurrence of systemic necrosis when maintained at 32 °C for 7, 10

or 12 days after inoculation. The *Tsw*$^+$/*Tsw*$^+$ plants were detected in the tests thanks to their systemic mosaic symptoms (before the transfer to 22 °C) and were discarded. The other plants, that never showed any mosaic symptom but often abscission of the inoculated cotyledons, were considered to possess the *Tsw* resistance gene to TSWV. The final proportion of plants with systemic necrotic symptoms, noted as susceptible, relative to the total number of *Tsw* plants, was determined. Progenies varying in the proportion of susceptible genome background or in the heterozygosity ratio at the *Tsw* locus were tested. These progenies were F_2, reciprocal BC1 and BC2 generations and selfed progenies from resistant backcross plants. The three resistant genitors and two susceptible parents (*C. annuum* and 'PI 195301') were involved, allowing to test the effects of resistant and susceptible parents, temperature treatment and population type. The tests were repeated twice except for the 'Milord × 7204' crosses because of lack of seeds due to insufficient fertility.

For BC1 and BC2 to the susceptible lines, F_1 hybrid plants and BC1 plants that were previously tested TSWV resistant (under 22 °C), were crossed to the susceptible parents. These populations were grouped in the same population type 1 for statistical analyses since they share 100% heterozygous (*Tsw/Tsw*$^+$) plants among total plants bearing the *Tsw* allele.

F_2 populations and selfed progenies from backcross plants were obtained by self-pollinating F_1 hybrid plants or BC1 plants tested TSWV resistant. These populations share a theoretical proportion of 66.7% heterozygous (*Tsw/Tsw*$^+$) plants among total plants bearing the *Tsw* allele. They were classified in population type 2.

For backcrosses to the resistant lines ('PI 152225', 'PI 15936' and '7204'), cross-pollinations were carried out with F_1 hybrid plants or *Tsw/Tsw*$^+$ plants from type 1 populations (previously tested TSWV resistant). In these populations, a frequency of 50% *Tsw/Tsw*$^+$ plants is expected among total plants possessing the *Tsw* allele. They were classified in population type 3.

The last experiment was conducted with temperature treatment T3 on 'PI 159236' and on several progenies derived from the F_1 hybrid 'Vania × PI 159236'. These populations were:

- successive backcrosses (BC1 to BC3) of F_1 hybrid plants to the susceptible parent 'Vania',
- self-pollinations of *Tsw/Tsw*$^+$ plants selected in above BC1 to BC3 progenies,
- populations derived from *Tsw/Tsw*$^+$ plants in the BC1 to the resistant parent 'PI 159236' crossed to 'Vania'.

This last experiment was repeated twice.

ELISA tests

At the end of each test, the presence of the virus in symptomless plants was assessed in non-inoculated leaves using a double antibody sandwich ELISA test with a polyclonal antiserum prepared against the nucleoprotein of the Brazilian isolate BR-01 (de Ávila et al., 1993). The samples were considered as positive when the optical density (OD) values at 405 nm were over three times the mean of the healthy controls. For ELISA tests, mock-inoculated plants served as the negative control and infected 'Vania' or 'PI 195301' plants as the positive control. The standard deviation of the OD of three healthy controls was below 0.05 at one hour. One ELISA test was performed for each sample without replicates.

Statistical analysis

Analyses of variance and linear regression models of the percentage of plants developing systemic necrosis among plants possessing the *Tsw* gene were conducted with the PROC GLM procedure of the Statistical Analysis System (SAS Institute Inc., 1988) software. Analyses of variance were conducted in two steps. Only additive effects were first evaluated. Significant factors ($P < 5\%$) were retained and were then evaluated together with two-factor interactions through a second analysis of variance. Only significant interactions were presented with additive effects in the result tables.

Results

Influence of the temperature treatment on the C. chinense *hypersensitive resistance*

The susceptible line in the T1 to T6 treatments did not show any major difference as compared to the control treatment T0, but the symptoms appeared slightly earlier in plants held at 32 °C after inoculation. Symptoms were similar in all treatments with systemic mosaic associated with chlorotic ringspots. More severe symptoms were observed if plants were held at 32 °C for a longer period after inoculation. The transfer of these plants to 22 °C did not modify

Table 1. Effect of temperature treatments on resistance to tomato spotted wilt virus in *C. chinense* lines

32 °C treatment (code)	Proportion of susceptible *C. chinense* plants after 32 °C treatments								
	'7204'			'PI 152225'			'PI 159236'		
	N[1]	s[2]	%s[3]	N	s	%s	N	s	%s
22 °C continuously (T0)	53	0	0	45	0	0	48	0	0
from 0 to 5 dpi[4] (T1)	22	5	23	20	0	0	18	2	11
from 0 to 9 dpi (T2)	45	10	22	37	10	27	48	22	46
from 0 to 12 dpi (T3)	52	27	52	36	22	61	50	26	52
from - 3 to 0 dpi (T4)	19	0	0	30	0	0	25	0	0
from - 3 to + 9 dpi (T5)	22	4	18	20	2	10	18	10	56
10 h/day from 0 to 12 dpi (T6)	24	0	0	24	1	4	18	1	6

[1] N: total number of inoculated plants.
[2] s: number of susceptible plants (i.e plants showing general necrosis, systemic necrotic lesions and/or necrotic streaks on stems after TSWV inoculation).
[3] %s: percentage of susceptible plants.
[4] dpi: days post inoculation (negative values correspond to days before inoculation).

their symptoms once they were developed. Necrosis rarely occurred and in those cases 25 days or more after inoculation. All 183 of these inoculated plants developed susceptibility symptoms validating the inoculation procedure.

The resistant *C. chinense* accessions showed a hypersensitive response (HR) as early as 3 to 4 days after inoculation under continuous 22 °C (T0). Necrotic, well-delimited, circular local lesions appeared, followed by the abscission of the inoculated cotyledons. Subsequently, none of the plants developed systemic symptoms and all were shown TSWV-free by ELISA. However, under 32 °C after inoculation, the resistant genitors did not develop distinct local lesions, but a diffuse necrosis, often followed by abscission of the inoculated cotyledons. Systemic necrotic symptoms appeared on some plants in treatments T1, T2, T3 and T5 (Table 1) from 5 days following inoculation to 4 days after transfer to 22 °C, and consisted of systemic necrotic lesions, general necrosis and/or necrotic streaks on stems.

No significant difference was observed between repeats of the tests nor between *C. chinense* genotypes (analysis of variance, results not shown). Increasing the duration of the high temperature treatment from 5 to 12 days (T1 to T3) increased the percentage of necrotic plants, whereas no systemic necrosis was observed in the control (T0) treatment. Pretreatment under high temperature (T4) did not affect the resistance response. Similarly, T2 and T5 treatments, both characterized by 9 days at 32 °C after inoculation, had a similar effect on systemic necrosis. Under alternated exposure to high and low temperatures (32 °C/22 °C) (T6), very few systemic symptoms appeared on *C. chinense* (2/66 plants). The influence of the duration of heat treatment and of interaction with the genotype was tested with the results of treatments T1, T2, T3 and T5. This analysis of variance of percentage values was possible because of the normality of the data and of quasi-normality of the residues (Wilks and Shapiro test, W = 0.31 for data and W = 0.03 for residues) and because there was no significant difference between intra-effect variances (Bartlett test, B = 0.13). Genotype effect was previously shown to be unsignificant and the major factor affecting the proportion of necrotic plants was confirmed to be the length of the 32 °C passage after inoculation (Table 2). A minor interaction effect between the temperature treatment and the *C. chinense* genotype was detected. The longer the duration at 32 °C after inoculation, the higher the proportion of plants showing systemic necrosis. Nevertheless, none of the treatments lead to 100% necrotic plants. A maximum of 61% of the plants were systemically affected in this experiment.

Long durations at 32 °C (more than 10 days) induced abnormalities in the plants. TSWV inoculated, mock-inoculated, and uninoculated plants from 'PI 152225' and 'PI 159236' were identically affected but '7204' did not. The plants showed mottling-like deformation of the leaves, chlorotic patterns and slow growth, resembling the phenomenon previously described as a 'virus-like syndrome' (Inai et al., 1993). These deformations also occurred in healthy progenies involving 'PI 152225' and 'PI 159236' (but not '7204'). No virus could be detected in these uninoculated deformed plants by DAS-ELISA tests. This

Table 2. Analysis of variance of the percentage of susceptible plants in the *C. chinense* lines after tomato spotted wilt virus inoculation and high temperature treatments

Effect	df	Sum of squares	Mean square	F value	*P*
total	14	5203			
temp	2	2883	1441	20.0	0.002
temp × genotype	6	1887	315	4.4	0.048
error	6	433	72		

Analysis of variance was performed according to the following model
$\%s_{ij} = m + temp_i + (temp \times genotype)_{ij} + \varepsilon_{ij}$.
with %s: percentage of susceptible plants among inoculated plants; m: mean susceptible plants percentage; temp: temperature treatment; genotype: *C. chinense* line; ε: residual effect.

Table 3. Effect of plant age on susceptibility of tomato spotted wilt virus in plants held at 32 °C for 12 days after inoculation and then transferred to 22 °C

Plant age at time of inoculation	Number of susceptible plants/total number of inoculated plants				
	'PI 152225'	'PI 159236'	'7204'	'F_1 (Vania × PI 159236)'	'Vania'
15 days after sowing	7^1/10	7^1/10	8^1/14	11^1/11	10^2/10
24 days after sowing	0/11	0/11	1^3/8	5^1/11	10^2/10
31 days after sowing	0/9	0/11	0/7	2^4/11	10^2/10
38 days after sowing	0/12	0/12	0/9	0/8	8^2/10

[1] general necrosis.
[2] mosaic.
[3] necrotic ringspots.
[4] necrotic streak on the stem.

syndrome was however easily distinguishable from TSWV mosaic symptoms which appeared earlier.

Influence of growth stage on the reaction of TSWV-resistant plants

The occurrence of the systemic symptoms in plants after 12 days at 32 °C and transfer to 22 °C is indicated in Table 3. More than half of the *C. chinense* plants were susceptible at stage 1, with a maximum of 70% (of 10 inoculated plants). The susceptibility percentages at this stage were not statistically different from those in the first experiment (Table 1) as assessed by Chi-2 tests (result not shown). Only one '7204' plant at stage 2 showed systemic necrotic ringspots on one leaf only and older plants did not exhibit any systemic symptoms. The F_1 hybrid between 'Vania' and 'PI 159236' remains partly affected even at later stages (2 and 3). This higher susceptibility of hybrid plants corroborates following results obtained with populations segregating at the *Tsw* locus. The susceptible control was fully infected whatever the treatment, except two plants (of ten) at the latest stage.

Effect of a high temperature treatment on the hypersensitive response in segregating populations

Several effects were tested on the proportion of susceptible plants among *Tsw*-bearing plants through analysis of variance: repetition of the test, *C. chinense* parent, susceptible parent (*C. annuum* or 'PI 195301'), population type (categorized according to the expected proportions of *Tsw/Tsw* and *Tsw/Tsw*$^+$ plants) and temperature treatment. Repetition and *C. chinense* parent effects were not significant (results not shown). Data ranged according to significant factors are given in Table 4 and analysed in Table 5. The model taking into account only significant effects fit the hypotheses of the variance analysis (W = 0.70 (residues); B = 0.49). The major effects were the temperature treatment (length of 32 °C regime), and the population type. The interaction between these two factors was significant and a weak effect of the susceptible parent was also observed. The major effect of temperature treatment confirms the preceeding results. The effect of the population type was analysed according to the heterozygosity level at the *Tsw* locus. This parameter affects the proportion of susceptible plants: the higher the heterozygosity, the higher the proportion of sus-

Table 4. Proportions of tomato spotted wilt virus susceptible plants in segregating populations subjected to 32 °C treatments after inoculation. Only plants possessing the *Tsw* allele were considered in the experiment, Tsw^+/Tsw^+ plants were discarded

Population type	Susceptible parent	Days at 32 °C (after inoculation)	N′[a]	s[b]	%s[c]
Population type 1	*C. annuum*	7	50	14	28
		10	133	94	71
(100% *Tsw/Tsw*$^+$ plants among		12	109	108	99
plants possessing the *Tsw* allele	'PI 195301'	7	66	12	18
		10	93	72	77
		12	41	39	95
Population type 2	*C. annuum*	7	51	1	2
		10	64	30	47
(66.7% *Tsw/Tsw*$^+$ plants among		12	96	80	83
plants possessing the *Tsw* allele)	'PI 195301'	7	92	18	20
		10	89	50	56
		12	49	48	98
Population type 3	*C. annuum*	7	40	0	0
		10	77	6	8
(50% *Tsw/Tsw*$^+$ plants among		12	128	100	78
plants possessing the *Tsw* allele)	'PI 195301'	7	82	5	6
		10	89	29	33
		12	107	96	90

[a] Total number of plants possessing the *Tsw* allele in the populations.
[b] s: number of susceptible plants in the population after TSWV inoculation and 32 °C treatment among plants possessing the *Tsw* allele.
[c] %s: percentage of susceptible plants among plants possessing the *Tsw* allele relative to total plants possessing the *Tsw* allele.

Table 5. Analysis of variance of the percentage of TSWV susceptible plants among plants possessing the *Tsw* allele in several populations involving *C. chinense* genitors and susceptible lines. The plants were subjected to high temperature treatments before transfer to normal growth conditions and apparition of susceptibility symptoms. The explicative model is [1]: $\%s_{ijk} = m + pop_i + temp_j + s\text{–}parent_k + (temp \times pop)_{ij} + \varepsilon_{ijk}$

Effect	df	Sum of squares	Mean squares	F value	*P*
total	34	42866			
pop	2	3521	1760	18.7	0.000
temp	2	32769	16384	173.6	0.000
s–parent	1	523	523	5.5	0.027
temp × pop	4	1610	403	4.3	0.009
error	25	2359	94		

Residual standard error = 9.7.
[1] %s: percentage of susceptible plants among plants possessing the *Tsw* allele; m: mean susceptibility percentage; pop: population type (according to the expected segregations at the *Tsw* locus: 'backcross by susceptible parent type' = 100% heterozygous plants among plants possessing the *Tsw* allele, 'F_2 type' = 66.7% heterozygous plants among plants possessing the *Tsw* allele and 'backcross by resistant parent type' = 50% heterozygous plants among plants possessing the *Tsw* allele; s–parent: susceptible parent ('Vania' or 'PI 195301'); ε: residual effect.

Table 6. Results of the regression models explaining the proportion (%s) of susceptible plants after tomato spotted wilt virus inoculation at continuous 32 °C with the variables %htz (theoretical proportion of *Tsw/Tsw*$^+$ plants among resistant ones) and % annuum (theoretical proportion of *C. annuum* genome)

Regression model	R^2	σ^2_{res}	F	P
$\%s_i = \alpha + \beta$* %htz$_i + \gamma$* % annuum$_i + \varepsilon_i$	0.60	12.3[1]	4.6	0.062
$\%s_i = \alpha + \beta$ %htz$_i + \varepsilon_i$	0.60	11.4[1]	10.6	0.014
$\%s_i = \alpha + \gamma$* % annum$_i + \varepsilon_i$	0.40	15.6[1]	4.7	0.065

[1] for comparison, $\sigma^2_{\%s} = 16.9$.

ceptible plants. However, another parameter varies in the segregating populations tested: the genetic background (percentage of genome from the susceptible parent). This parameter is correlated with the percentage of heterozygosity at the *Tsw* locus in the upper progenies. In order to distinguish between these two hypotheses, new progenies were tested, in which the percentage of heterozygosity and the percentage of susceptible genetic background were less correlated. These populations were obtained from the F_1 hybrid 'Vania × PI 159236' with a range of *C. annuum* background from 0 to 93.75%. They were held 12 days at 32 °C before being transferred to 22 °C. The putative effects of the percentage of heterozygosity and of the theoretical proportion of 'Vania' (susceptible) genome in the populations on the destabilization of the resistance were tested in regression models (Figure 1, Table 6). The most suitable regression model only involves the percentage of heterozygosity (Table 6) with a determination coefficient (R^2) of 0.6 and a F probability of 0.014. Adding the effect of the percentage of the 'Vania' genetic background to the model did not increase the R^2, but increased the F probability (0.062). We can then postulate that homozygous resistant plants are less sensitive to high temperatures than heterozygous ones.

Discussion

Conditions affecting the hypersensitive resistance of C. chinense *to TSWV*

We have observed a destabilization of the hypersensitive resistance in *C. chinense* accessions 'PI 152225', 'PI 159236' and '7204' at 32 °C similar to the report by Roggero et al. (1996) for 'PI 152225'. This destabilization was characterized by the occurrence of systemic necrotic symptoms when inoculated resistant plants were first kept at 32 °C and then transferred to 22 °C. Five, nine and twelve days at 32 °C lead to increasing proportions of systemic necrotic plants. As Roggero et al. (1996) reported, we also observed that repeated short periods of high and low temperatures greatly reduced the percentage of plants that developed systemic necrosis. The most severe conditions gave a large proportion (up to 70%) of systemic necrotic plants (12 days at 32 °C, inoculation of both cotyledons at the cotyledonary stage). Roggero et al. (1996) observed 41% (100/244) systemically affected 'PI 152225' plants during a 20-day period at 33 °C following inoculation at the two-true-leaf stage. Differences in the severity of the tests may result from the different procedures (virus isolates, early plant growth, inoculated stages and organs). Nevertheless, we rarely observed the destabilization of the resistance for plants older than the cotyledonary stage (Table 3) even though the cotyledons remained functional up to 31 days after sowing (as indicated by the susceptible 'Vania' control).

A strong plant age effect on the susceptibility to TSWV under high temperature treatment was noted (Table 3). It can either be due, indirectly, to the inoculum pressure (cotyledons only were inoculated, thus representing a decreasing relative surface of the growing plant) or to the plant age itself. Our experiments do not allow to distinguish between these hypotheses. In the second situation, the increased resistance in older plants could involve mature plant and/or mature tissue resistance (Smit and Parlevliet, 1990) or quantitative (partial) resistance (Parlevliet and Kievit, 1986). Mature plant and tissue resistance has been described by Buiel and Parlevliet (1996) in groundnut resistance to groundnut bud necrosis tospovirus. Quantitative resistance to TSWV long-distance movement in pepper also has been observed in *Tsw*$^+$/*Tsw*$^+$ plants (Moury, 1997). Its expression is highly dependent on the growth stage of the plant and it seems oligogenically determined. Further experiments will

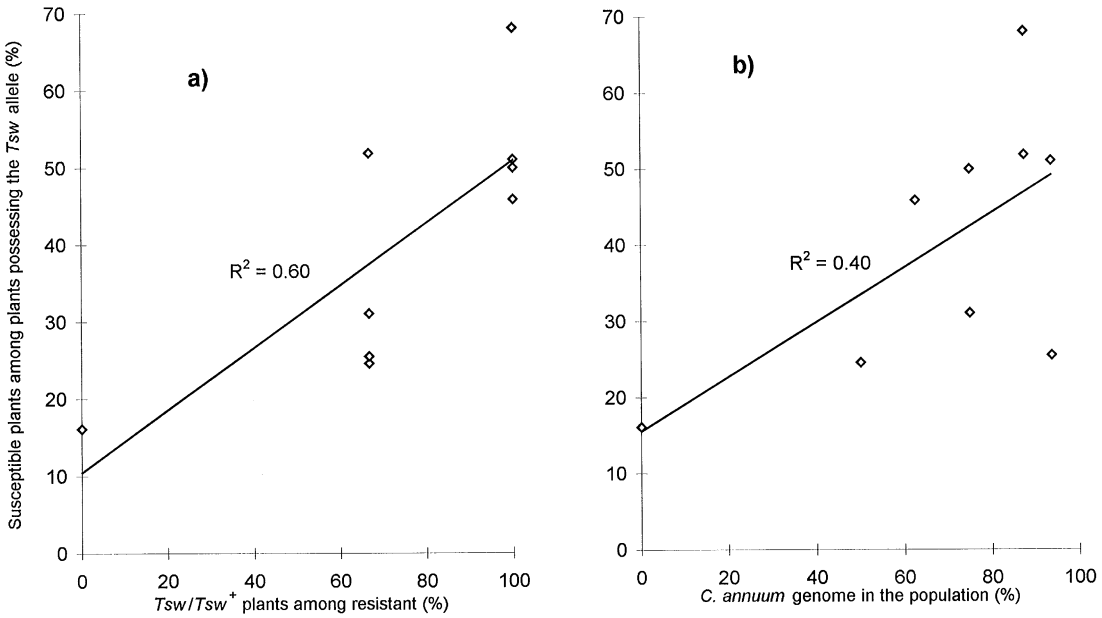

Figure 1. Repartition of progenies derived from 'Vania' and 'PI 159236' according to the frequency of susceptible plants among plants possessing the *Tsw* allele (ordinate) and: (a) the frequency of heterozygous (*Tsw/Tsw*$^+$) plants or (b) the theoretical *Capsicum annuum* genome proportion in the population. Plants were held at 32 °C for 12 days and then transferred to 22 °C. Linear regression coefficients of determination (R^2) are indicated.

be necessary to assess the influence of plant growth on TSWV susceptibility in the presence of the *Tsw* allele and under high temperature. Plant growth and/or inoculum pressure have a less accurate incidence on F_1 hybrid (Table 3) which emphasizes the higher TSWV susceptibility of heterozygous vs. homozygous (*Tsw/Tsw*) plants under high temperature.

Effect of the heterozygosity at the Tsw *locus and of genetic background*

Early growth stage and severe conditions were chosen to analyse the effect of resistance introgression into susceptible cultivars on the destabilization due to high temperature. In *C. chinense* parental lines, systemic necrosis never affected more than 70% of the plants whereas up to 99% of the resistant plants developed systemic necrosis in the progenies, particularly in populations backcrossed by the susceptible parent. This suggests that a genetic effect controls the stability of the resistance at high temperature, either of the *Tsw* gene itself (in a homozygous or heterozygous status), or the genetic background. The first hypothesis (a gene dosage effect) has already been observed in tomato where heterozygosity also affects the expression of ToMV resistance due to *Tm-2*^{2} over 30 °C (Schroeder et al., 1967). The second hypothesis (effect of genetic background) assumes that genetic factors independent of the *Tsw* locus exist in the *C. chinense* genome that 'stabilize' the resistance and impede the spread of the virus in the uninoculated leaves at high temperatures. Such factors have already been identified in pepper for the hypersensitive resistance to tobacco mosaic virus (TMV) conferred by the *L* locus (Daubèze et al., 1990; Palloix, 1992; Chaîne-Dogimont, 1993).

In our regression models, we can assume that the theoretical percentage of heterozygosity is a suitable estimation of its real value since no segregation distortion between resistant (*Tsw/Tsw* or *Tsw/Tsw*$^+$) and susceptible plants (*Tsw*$^+$/*Tsw*$^+$) was noted (data not shown). In artificial testing, occurrence of adapted TSWV strains overcoming the *Tsw* allele was underlined by Moury et al. (1997). However, this phenomenon showed a low frequency (0.5% of the inoculated TSWV-resistant plants) and it may not affect the segregation ratio between the *Tsw/Tsw*$^+$ and *Tsw/Tsw* plants that we studied. The only deviation that may affect the analysis is the *C. annuum* genome proportion that was slightly overestimated, due to the linkage drag around the *Tsw* locus. However, only resistant plants were compared in the study and the deviation may be very low compared to the overall range of variation of the recipient genome.

Our results suggest that heterozygosity at the *Tsw* locus is responsible for the 'population type' effect on the systemic symptoms observed at high temperatures similar to what has been observed for the tomato resistance gene *Tm-2*^{2} (Schroeder et al., 1967). We did not observe any significant genetic erosion due to the loss of unlinked additional genes from *C. chinense* during the backcross process, nor any difference between the *C. chinense* sources for *Tsw*.

Implications for pepper breeding programs for TSWV resistance

A few examples illustrate that introgressing genes from related species into cultivars may result in a decrease in resistance level. This was shown in pepper for TMV resistance (A. Palloix, unpubl.). Evidence was given that secondary genes present in some accessions were stabilizing the resistance under high temperatures (Palloix, 1992). This was also suggested by Roggero et al. (1996) for resistance to TSWV in *C. chinense.* We did not observe any genetic erosion of the resistance at high temperature all along the backcrosses. However, a weak but significant effect of the susceptible parent was detected in the Table 5. *C. frutescens* genetic background seems to confer a higher sensitivity to temperature than *C. annuum* (Table 4). This indicates that a favourable genetic background may be screened in susceptible (recipient) parents, rather than in the *C. chinense* resistance sources. It would be interesting to study the influence of genes from other germplasms, for example those that stabilize the hypersensitive resistance of pepper to TMV and *Phytophthora capsici* over 30 °C (Daubèze et al., 1990). If the infection step affected is common to several viruses (for example cell-to-cell migration), the stabilizing genes detected in the pepper/TMV interaction could be efficient in the pepper/TSWV interaction. This would perhaps help to understand what the role is of temperature in the plant/virus interaction, phenotypically identical for several hypersensitive resistances.

The main genetic effect has been attributed to the heterozygosity or homozygosity at the *Tsw* locus. An F_1 hybrid heterozygous at the *Tsw* locus may be less resistant than a homozygous one when cultivated under continuous high temperature conditions (tropical climate). As a consequence, it could be preferable to grow homozygous cultivars at the *Tsw* locus.

Destabilization of the hypersensitive resistance confered by *Tsw* under high temperature could impede an accurate screening of *Tsw* plants. In breeding programs for TSWV resistance in such conditions, we would recommend to inoculate plants at the four-expanded-leaf growth stage. This growth stage seems the best compromise for the expression of resistance in *Tsw* plants and of susceptibility in *Tsw*$^+$ plants under continuous high temperatures (Table 3). Alternatively, marker-assisted selection of the *Tsw* gene could be carried out whatever the climatic conditions.

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