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Genetics of tomato spotted wilt virus resistance coming from *Lycopersicon peruvianum*

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Abstract

New resistance sources coming from *Lycopersicon peruvianum,* especially those introgressed in UPV 32 line, are studied. UPV 32 resistance is controlled by a single gene. Resistance and dominance levels of this gene are conditioned by thrips transmission and isolate aggressiveness. A partial overcoming of resistance occurs due to the incomplete penetrance of the gene. Incomplete dominance of resistance also happens, which suggests gene dosage dependence. UPV 32 gene segregates independently of both *Sw-5* gene and UPV 1 resistance gene, also coming from *Lycopersicon peruvianum*. It is proposed to name *Sw-6* this new locus from UPV 32. *Sw-5* gene and UPV 1 resistance gene show higher resistance than *Sw-6*. Heterozygotes for UPV 1 resistance gene were more resistant than heterozygotes for *Sw-5*. The lower dependence of UPV 1 resistance gene on the gene dosage effect makes it very useful for the development of commercial hybrids.

Abbreviations: HA – HA-931100 Spanish isolate of TSWV; T – T-941117 Spanish isolate of TSWV.

Introduction

The Tomato Spotted Wilt Virus (TSWV) is one of the viruses causing the greatest economic losses in tomato crops. Total losses of harvest frequently happen (Roselló et al., 1996). Diverse factors make control of the disease difficult: the large amount of inoculum that is present in the field; the variability of the isolates (de Ávila, 1992); the short virus acquisition period (Sakimura, 1962), and its multiplication in the vector (Ullman et al., 1993; Wijkamp et al., 1993); the high transmission efficiency, especially for *Frankliniella occidentalis* (Wijkamp et al., 1995); the wide distribution of the thrips and their resistance to many insecticides. For this reason, the use of genetic resistance could be the most efficient long-term strategy for TSWV management.

In tomato, the first resistance was found in *Lycopersicon pimpinellifolium* (Samuel et al., 1930) and *L. esculentum* (Holmes, 1948). Finlay (1953) determined that those resistances were controlled by 5 genes, 2 dominant and allelic (*Sw-1*^{a} and *Sw-1*^{b}), and 3 recessive (*sw-2*, *sw-3* and *sw-4*). This resistance proved to be strain-specific and was quickly overcome, so these genes have scarcely been used in breeding programmes.

Germplasm screening has been made in different *Lycopersicon* species in order to find higher, and non-isolate specific resistance. Some field resistance was detected in accessions of *L. hirsutum* (PI-127826 and LA-1353) and in *L. hirsutum* var. glabratum (PI-134417 and LA-1223) (Maluf et al., 1991; Kumar and Irulappan, 1992). In field trials, it was found that some of these resistances were controlled by polygenic systems. Resistance of PI-127826 and LA-1223 have been introgressed in *L. esculentum* and a loss of resistance has been observed, compared with donor species.

LA-2931 accession of *L. chilense* was immune to mechanical and thrips inoculation (Krishna Kumar et al., 1993), but the genetic control and its behaviour in *L. esculentum* genetic background remains unknown.

L. peruvianum appears to be the best resistance source to TSWV. CNPH accessions 201 and 374, LA accessions 111, 372, 385, 441-1 and 1113-1, PI accessions 126928, 126930, 126944, 126946, 128657, 128659, 128660 and 129146 have been resistant or immune in several screening trials (Paterson et al., 1989; Maluf et al., 1991; Boiteux and Giordano, 1992; Kumar and Irulappan, 1992; Iizuka et al., 1993; Krishna Kumar et al., 1993). Notwithstanding, the expression of this resistance introgressed in *L. esculentum* depends on the accession used. The resistance derived from PI-128657 introgressed in Anahu cultivar disappeared partially during the breeding process (Paterson et al., 1989; Krishna Kumar et al., 1993). Contrarily, the resistance conferred by *Sw-5* gene (Stevens et al., 1992) introgressed in Stevens cultivar has been the preferably used by breeders. This resistance is isolate unspecific, easy to manage (dominant monogenic control) and shows good expression in *L. esculentum* background. Notwithstanding, in spite of its high protection level it does not offer immunity. Under high inoculation pressure in thrips transmission level it does not offer immunity. Under high inoculation pressure in thrips transmission, partial overcoming of this resistance has been found (Díez et al., 1995; Roselló et al., 1997).

Due to the virus variability and the complex host-TSWV interactions, it is necessary to characterize as widely as possible the genetic control and the protection level of the resistance sources found in order to use them effectively. The aim of the present work is to determine the genetic control of the TSWV resistance introgressed from *L. peruvianum* in UPV 32 and its relationship with other resistance genes also coming from *L. peruvianum*, in particular, the *Sw-5* gene.

Materials and methods

Plant material

UPV 32 and UPV 1 breeding lines show resistance to TSWV introgressed from *L. peruvianum* accessions collected by our working group in Casma valley and Huallanca (department of Ancash, Peru) (Cuartero et al., 1984). The UPV 32 breeding programme was started with the cross between Fortuna C, *L. esculentum* line carrying the potato leaf morphological marker, and PE-28_1, using the pollen mixture technique. Once incompatibility barriers were overtaken, in the third backcross, the recurrent genitor was changed for the hybrid Vemone (Sluis & Groot, the Netherlands). UPV 32, which is the tenth generation of this breeding programme, is a tomato line for fresh market with medium size fruits (approximately 120 g) and red in colour when mature. UPV 32 was crossed as male genitor with the susceptible line NE-1 in order to study the genetic control of resistance. F1, F2, BC1 (F1 × NE-1) and BC2 (F1 × UPV 32) generations were obtained.

The UPV 1 breeding programme was similar to that described for UPV 32. The accession PE-18 of *L. peruvianum* was used as a resistance source, and Fortuna C used as the first recurrent genitor. In the third backcross, the recurrent genitor was changed for the hybrid Arletta (Royal Sluis, the Netherlands). UPV 1 is the thirteenth generation of this breeding programme. UPV 1 line shows a high level of resistance to TSWV, similar to that conferred by *Sw-5* gene (Roselló et al., 1997). UPV 1 resistance is monogenic and allelic, or highly linked to *Sw-5* gene (Roselló et al., unpubl.). In order to study the relationship between UPV 32 and UPV 1 resistances their F1 and NE-1 × (UPV 32 × UPV 1) cross were obtained. The F1 (NE-1 × UPV 1) was also made.

RDD line was derived from a line carrying *Sw-5* gene, supplied by Dr. Stevens from Brigham Young University (Utah, USA). The crosses UPV 32 × RDD, NE-1 × (UPV 32 × RDD) and NE-1 × RDD were made in order to study the relationship between UPV 32 and RDD resistance genes.

L. esculentum cv. Rutgers was used as a susceptible control.

Seeds were heated at 80 °C for 24 h to prevent possible ToMV seed transmission.

Mechanical inoculation

Mechanical inoculations were conducted in a growth chamber. The environmental conditions were: 25 °C/18 °C (day/night) temperature, 60%/95% (day/night) of relative humidity and 65 to 85 μmol s^{-1} m^{-2} of irradiance from Sylvania Grolux fluorescent tubes with a wavelength interval between 400 and 700 nm. The photoperiod was 14 light hours.

The plants were transplanted to pots (6 × 6 × 8 cm). All fully expanded leaves of plants at 4 leaves growth stage were inoculated. The upper leaves were reinoculated a week later.

Two TSWV Spanish isolates with different biological and biochemical characteristics were chosen because of their high aggressiveness. T-941117

was collected from naturally infected *L. esculentum* plants. HA-931100 comes from infected *Vicia faba* and *Cynara scolymus* plants. Abad (1996) classified these isolates in two different restrictotypes. She utilized the PCR-INIA technique (Nolasco et al., 1993) in order to amplify a 456 pb DNAc fragment of viral S RNA. This fragment includes the terminal 5′ coding region of the N protein gene and the complete 3′ noncoding region. The primers 5′-AGAGCAATTGTGTCA-3′, complementary to 3′ viral end and 5′-ATCAAGCTTCTGAAGGTCAT-3′, complementary to nucleotides 2472-2492 of the 5′ viral end of BR-01 TSWV isolate (de Haan, 1991), were used. Each of the 35 cycles of PCR amplification consisted of 45 s of denaturation at 94 °C, 1 min of annealing at 52 °C and 1 min of extension at 72 °C. Finally, a stage of 10 min at 72 °C was carried out. Amplified DNAc fragments were digested by Sau A31, Hinfl y TaqI restriction enzymes (Boehringer Mannheim GmbH, Germany) and subjected to electrophoresis in 8% polyacrilamide gel containing ethidium bromide. DNAc fragments from HA isolate had one restriction site for Sau A31, two for HinfI and none for TaqI. DNAc fragments from T isolate had two restriction sites for Sau A31, one for HinfI and one for TaqI.

These two isolates were maintained on susceptible tomato plants (NE-1, Muchamiel and Marmande) in the transmission cages in two different grow rooms, one in Valencia, an another in Murcia (Spain). Every 4 months younger susceptible plants were set in the transmission cages in order to replace the inoculum source plants once infected. All the infected tissue used in the preparation of the inoculum always came from plants infected by thrips. Therefore, the inoculum used in mechanical inoculation were always first generation inoculum.

Inoculum was prepared by mixing 2 g of infected leaf tissue with 10 ml of inoculation buffer and 0.1 g of Carborundum (600 mesh). Potassium phosphate buffer (0.1 M, pH 7.0), containing sodium sulfite (0.2% w/v) and sodium diethyldithiocarbamate trihydrate (DIECA) (0.2% w/v), was used as inoculation buffer.

Inoculation by thrips

The inoculations were carried out in Valencia (Spain). The environmental growth room conditions were: 22 to 25 °C temperature, 45 to 55%/85 to 100% relative humidity (day/night), 120 μmol s^{-1} m^{-2} or irradiance from Sylvania Grolux fluorescent tubes and a 14:10 (light/dark) photoperiod.

Eight large cages (115 × 70 × 60 cm) covered with antithrips mesh were used to rear *F. occidentalis* infectious populations. More than 500 thrips are estimated to be in each transmission cage. Ten susceptible plants infected with T isolate served as a inoculum source in each cage. The plants to be tested and the controls were placed in trays (34.5 × 23 × 6.5 cm) containing a sterilized mixture of peat and pearl (50:50, 5.5 cm deep). Six plants trays were placed in the cages to test for 2 months: Then the tested plants were cut and placed on the following tests plants in order to allow the larvae to pass from one group of plants to another. The sets of trials were carried out consecutively. In this way, the thrips were able to reinoculate the isolate from one trial to the next.

In all trials, the orientation and location of the trays, both intra and inter the cages, were periodically changed to ensure a homogeneous plant exposure to thrips.

Experimental design and evaluation criteria

In mechanical inoculation, 3 experiments were made: Inheritance of UPV 32 resistance, the relationship between UPV 32 and RDD and the relationship between UPV 32 and UPV 1. A split-plot like design was used. The inoculation chamber was divided into 3 blocks and each one was subdivided into 2 main plots, one for the HA isolate and the other for the T isolate. Each main plot was subdivided in 20 elemental plots. Five test plants and 1 susceptible control were set in each elemental plot. Ten white controls plants were included in each main plot. Plant distribution was made following 2 criteria. First, every genotype had a number of plants proportional to its sample size in each elemental plot. Second, developmental stage of plants was homogeneous in each main plot. When inoculating each elemental plot, the susceptible control was always the last inoculated plant. White controls were inoculated with inoculation buffer.

In thrips transmission, the plants were tested in 3 consecutive series, one for each experiment. A split-plot like design was also used. Each elemental plot consisted of an inoculation tray on which 10 plants were set, 8 of the genotypes tested and 2 controls. They were disposed in 2 rows of 5 plants. Controls were placed on a diagnonal, one position before the end of each row. The position of the tested plants in each tray was allotted randomly.

Table 1. Number of infected and non-infected plants in inoculation tests

Generations	Mechanical inoculation				Transmission by *Frankliniella occidentalis*	
	T-941117 isolate		HA-931100 isolate		T-941117 isolate	
	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected
Inheritance of UPV 32 resistance						
P1 (NE-1)	24	0	28	0	24	0
P2 (UPV 32)	0	28	1	29	6	17
F1 (NE-1 × UPV32)	0	25	2	28	8	16
F2 (NE-1 × UPV32)	16	66	29	75	39	43
BC1 (F1 × NE-1)	27	25	35	26	28	14
BC2 (F1 × UPV32)	0	50	3	48	10	32
RUTGERS (Control)	102	0	107	0	80	0
Relationship between UPV 32 and RDD resistance sources						
33 (UPV 32)	0	28	1	29	6	16
30 (NE-1 × UPV 32)	0	25	3	27	8	14
22 (RDD)	0	28	0	30	2	22
20 (NE-1 × RDD)	0	26	0	26	5	18
32 (UPV 32 × RDD)	0	27	0	25	5	19
[NE-1 × (UPV 32 × RDD)]	20	66	27	72	35	48
RUTGERS (Control)	40	0	40	0	34	0
Relationship between UPV 32 and UPV 1 resistance sources						
33 (UPV 32)	0	28	1	28	7	17
30 (NE-1 × UPV 32)	0	28	4	26	9	13
11 (UPV 1)	0	28	0	26	1	18
10 (NE-1 × UPV 1)	0	24	0	30	3	19
31 (UPV 32 × UPV 1)	3	22	5	24	6	15
[NE-1 × (UPV 32 × UPV 1)]	27	75	38	68	45	46
RUTGERS (Control)	40	0	40	0	34	0

TSWV infection was monitored by systemic symptoms and confirmed by serological DAS-ELISA tests (Clark and Adams, 1977). Samples for DAS-ELISA test were periodically collected from plants showing systemic symptoms. Samples from symptomless plants were collected at the end of the experiment in order to verify that they were not infected. In mechanical inoculation, samples were collected from the apical non-mechanically inoculated region of the plant. In thrips transmission, the samples were collected from diverse parts of the plants were no feeding lesions were evident. In order to avoid as much as possible errors caused by escapes in mechanical inoculation, only the elemental plots in which the controls became infected were taken into account. In thrips transmission, the effectiveness of infection was complete.

Table 2. Monogenic model dependent on partial resistance and incomplete dominance

Generations		Model
P2 (UPV 32)	S[a]	p_{33}[c]
	R[b]	$1-p_{33}$
F1 (NE-1 × UPV32)	S	p_{30}[d]
	R	$1-p_{30}$
F2 (NE-1 × UPV 32)	S	$0.25 + 0.25p_{33} + 0.5p_{30}$
		R $0.75-(0.25p_{33} + 0.5p_{30})$
BC1 (F1 × NE-1)	S	$0.5 + 0.5p_{30}$
		R $0.5-0.5p_{30}$
BC2 (F1 × UPV 32)	S	$0.5p_{33} + 0.5p_{30}$
	R	$1-(0.5p_{33} + 0.5p_{30})$

[a] Susceptible.
[b] Resistant.
[c] Probability of infection of an homozygote resistant.
[d] Probability of infection of an heterozygote.

Table 3. Estimates of the proportion of infected plants in homozygotes resistant (p_{33}) and heterozygotes (p_{30}) in the inheritance of UPV 32 resistance experiment

	Mechanical inoculation HA-931100 isolate		Transmission by thrips T-941117 isolate	
	P_{33}	P_{30}	P_{33}	P_{30}
Estimate	0.033	0.075	0.232	0.316
Standard error	0.007	0.009	0.018	0.015
Confidence intervals (95%)	0.015–0.050	0.055–0.096	0.188–0.276	0.280–0.352
R-squared (adjusted for d.f.)	93.059		99.032	
$\chi^2_{Weighted}$	1.003		0.949	
P	0.800		0.813	

Table 4. General model of the inheritance of monogenic resistance of two sources. Partial resistance and incomplete dominance are considered

Generations		Model
33 (UPV 32)	S[a]	p_{33}[c]
	R[b]	$1-p_{33}$
30 (NE-1 × UPV 32)	S	p_{30}[d]
	R	$1-p_{30}$
22 (RDD)	S	p_{22}[e]
	R	$1-p_{22}$
20 (NE-1 × RDD)	S	p_{20}[f]
	R	$1-p_{20}$
32 (UPV 32 × RDD)	S	p_{32}[g]
	R	$1-p_{32}$
[NE-1 × (UPV 32 × RDD)]	S	$0.5r_1$[h] $+ 0.5(1-r_1)p_{30} + 0.5(1-r_1)p_{20} + 0.5r_1p_{32}$
	R	$0.5(1-r_1)(1-p_{30}) + 0.5(1-r_1)(1-p_{20}) + 0.5r_1(1-p_{32})$

[a] Susceptible.
[b] Resistant.
[c] Probability of infection of an homozygote for the resistance gene of UPV 32.
[d] Probability of infection of an heterozygote for the resistance gene of UPV 32.
[e] Probability of infection of an homozygote for the *Sw-5* resistance gene.
[f] Probability of infection of an heterozygote for the *Sw-5* resistance gene.
[g] Probability of infection of a double heterozygote for the resistance genes of UPV 32 and RDD (*Sw-5*).
[h] r_1 = Recombination fraction.

Data analysis

Yates' correction for continuity has been used, in χ^2 test (Little and Hills, 1978). Parameters of the model have been estimated by using least square regression method, taking as weights the inverse of the variances (joint scaling test from Cavalli described in Mather and Jinks, 1971). Each generation variance has been obtained from the sample size and average, due to its binomial distribution. The joint fit of all generations in an experiment to the proposed model is evaluated by using χ^2 weighted statistic obtained by the expression (Mather and Jinks, 1971): χ^2 weighted = Σ [(Observed relative frequency - Expected relative frequency)^2 × Weight].

Results

All susceptible controls became infected, in mechanical inoculation and by thrips. In mechanical inoculation, the first symptoms appeared 12 or 13 days after the inoculation with HA isolate, and after 15 or 16 days with T isolate. In thrips transmission, the first

Table 5. Estimates of the proportions of infected plants in homozygotes (p_{33}) and heterozygotes (p_{30}) for UPV 32 resistance gene, homozygotes (p_{22}) and heterozygotes (p_{20}) for *Sw-5* gene and the double heterozygote (p_{32}) for both genes. Independent segregation is assumed

	Mechanical inoculation HA-931100 isolates		Transmission by thrips T-941117 isolate				
	P33	P30	P33	P30	P22	P20	P32
Estimate	0.033	0.099	0.272	0.348	0.083	0.206	0.198
Standard error	0.0008	0.0012	0.013	0.013	0.008	0.011	0.011
Confidence intervals (95%)	0.031–0.035	0.095–0.102	0.240–0.305	0.316–0.380	0.064–0.102	0.178–0.234	0.171–0.225
R-squared (adjusted for d.f.)	99.93				99.49		
$\chi^2_{Weighted}$	0.004				0.293		
P	0.945				0.588		

symptoms appeared at 18–20 days; after 33–35 days all susceptible plants had become completely infected.

HA isolate proved to be more aggressive than T isolate in mechanical inoculation. In general, lower infection levels were observed in mechanically inoculated plants than in those tested by *F. occidentalis* transmission. In thrips transmission, the resistances tested were partially overcome, since infected plants always appeared in every generation (Table 1).

Inheritance of UPV 32 resistance

Mechanical inoculation. All plants of the susceptible parent (NE-1) became infected (Table 1). With T isolate, no plant of the resistant parent plant (UPV 32) showed any symptoms, thus indicating complete resistance. F1 infected plants were not found, indicating dominance of resistance. The observed infected : non-infected ratio in segregant generations F2, BC1 and BC2 suggests a monogenic dominant control (χ^2 = 1.04, P = 0.31 for F2; χ^2 = 0.02, P = 0.89 for BC1).

With HA isolate, there is partial overcoming of resistance and incomplete dominance. A percentage of 3.3 of UPV 32 and 7.6 of F1 (NE-1 × UPV 32) were infected. In order to establish the genetic model of the resistance, it is necessary to modify the previously mentioned model (T isolate) by introducing these effects. The parameters p_{33} and p_{30} quantify the proportion of plants infected in homozygotes and heterozygotes respectively for the resistance gene. The resistance levels are quantified by the p_{33} parameter. The result $p_{33} < p_{30}$ indicates partial dominance. The new genetic model can be seen in Table 2, and the estimates by weighted least square regression in Table 3. The fit to the model is highly significant (R^2 adj. = 93.05%, $\chi^2_{weighted}$ = 1.003, P = 0.80).

Inoculation by thrips. Partial overcoming of resistance and incomplete dominance is also observed, but in this case, all generations showed higher infection levels than in HA mechanical inoculation (Table 1). The fit to the partial resistance and dominance model is highly satisfactory (Table 3).

Relationship between the UPV 32 and RDD resistance sources

Mechanical inoculation. In inoculation with T isolate, infected plants did not appear in UPV 32 or in F1 (NE-1 × UPV 32). This confirms the total resistance and complete dominance of UPV 32 gene in these conditions. The same behaviour was observed in the line and carrying *Sw-5* gene: RDD, NE-1 × RDD and UPV 32 × RDD. In NE-1 × (UPV 32 × RDD) cross 23.25% of the plants became infected. Since UPV 32 and RDD show monogenic dominant control of resistance, this result is in accordance with an independent segregation of both genes (χ^2 = 0.062, P = 0.803). We propose to name the UPV 32 resistance gene Sw-6.

In HA mechanical inoculation, *Sw-5* gene behaved in a similar way as in T inoculation, showing total resistance and complete dominance. However, partial overcoming of both resistance and dominance of *Sw-6* gene was detected. A total of 3.33% and 10% of infected plants appeared in UPV 32 and NE-1 × UPV 32 respectively. The NE-1 × (UPV 32 × RDD) cross, with 27.27% of infection, shows that *Sw-5* and *Sw-6* genes segregate independently. The partial resistance and incomplete dominance model previously

Table 6. Estimates of the proportions of infected plants in homozygotes (p_{33}) and heterozygotes (p_{30}) for the *Sw-6* gene, homozygotes (p_{11}) and heterozygotes (p_{10}) for the UPV 1 resistance gene and the double heterozygote (p_{31}) for both genes. Independent segregation is assumed

	Mechanical inoculation				Transmission by thrips				
	T-941117 isolate	HA-931100 isolate			T-941117 isolate				
	P_{31}	P_{33}	P_{30}	P_{31}	P_{33}	P_{30}	P_{11}	P_{10}	P_{31}
Estimate	0.112	0.034	0.144	0.186	0.291	0.432	0.052	0.147	0.306
Standard error	0.011	0.009	0.016	0.018	0.019	0.020	0.010	0.014	0.019
Confidence intervals (95%)	0.075–0.149	0.010–0.058	0.102–0.186	0.140–0.233	0.245–0.337	0.384–0.479	0.027–0.078	0.112–0.182	0.260–0.351
R-squared (adjusted for d.f.)	96.94		96.90				99.19		
$\chi^2_{Weighted}$	0.215		0.756				0.616		
P	0.642		0.384				0.432		

described can be used. Under mechanical inoculation, no plants infected in homozygotes (p_{22}) and heterozygotes (p_{20}) for *Sw-5* gene were found for either isolate. The proportion of infected plants in the double heterozygote for the *Sw-6* and *Sw-5* genes is quantified by p_{32} parameter. The possible linkage between those gene is represented by r_1, the recombination fraction. The generalized model is shown in Table 4. The recombination fraction estimate and its standard deviation is $r_1 = 0.494 \pm 0.039$, thus, not differing significantly from 0.5. The fit to model, assuming independent segregation, is extremely high (Table 5).

Inoculation by thrips. Sw-6 gene showed the same response pattern as in HA mechanical inoculation trial. Notwithstanding, in thrips transmission there is a lower resistance than that experienced with mechanical inoculation. In contrast to HA mechanical inoculation, the *Sw-5* gene showed partial resistance and dominance. However, *Sw-5* resistance was higher than that of *Sw-6* (Table 1). The double heterozygote for both genes (UPV 32 × RDD) showed a similar level of infection to the heterozygote for *Sw-5* gene (NE-1 × RDD). The percentage of infection (41.46%) of the NE-1 × (UPV 32 × RDD) cross can be satisfactorily explained by the previously described model, assuming independent segregation (Table 5).

Relationship between the UPV 32 and UPV 1 resistance sources (R31)

In general, the results for each one of the 3 combinations of isolate and inoculation methods follow a very similar pattern to that observed in the relationship between the UPV 1 and UPV 32 resistance trials. *Sw-6* gene shows total resistance and complete dominance in T mechanical inoculation, and partial resistance and incomplete dominance in HA mechanical inoculation and T inoculation by thrips. UPV 1 resistance gene, as in *Sw-5* gene, shows total resistance and complete dominance in mechanical inoculation (T and HA), and partial resistance and incomplete dominance in thrips inoculation. However, in this test there is a unexpectedly high level of infection for the UPV 32 × UPV 1 cross, which was not observed in UPV 32 × RDD cross (Table 1). The results were fitted by using the formerly described general model (Table 4). The p_{22}, p_{20}, p_{32} and r_1 parameters have been substituted for p_{11}, p_{10}, p_{31} and r_2, respectively. The results fit the model well, assuming independent segregation (Table

Table 7. Summary of results and conclusions

Experiment	Mechanical inoculation				Transmission by thrips		Genetic control
	T-941117 isolate		HA-931100 isolate		T-941117 isolate	conclusions	
Inheritance of UPV 32 resistance	$\mathbf{p}_{33}$[b] = 0 $\mathbf{p}_{30}$ = 0	$p_{33} = p_{30}$	$\mathbf{p}_{33}$ = 0.033 $\mathbf{p}_{30}$ = 0.075	$p_{33} < p_{30}$	$\mathbf{p}_{33}$ = 0.232 $\mathbf{p}_{30}$ = 0.316	$p_{33} < p_{30}$	*Sw-6*
Relationship between UPV 32 and RDD resistance sources	$\mathbf{p}_{33}$ = 0 $\mathbf{p}_{30}$ = 0 $\mathbf{p}_{22}$ = 0 $\mathbf{p}_{20}$ = 0 $\mathbf{p}_{32}$ = 0 $\mathbf{r}_1$ = 0.5	$p_{33} = p_{30}$ $p_{22} = p_{20}$ $p_{32} = p_{20}$	$\mathbf{p}_{33}$ = 0.033 $\mathbf{p}_{30}$ = 0.099 $\mathbf{p}_{22}$ = 0 $\mathbf{p}_{20}$ = 0 $\mathbf{p}_{32}$ = 0 $\mathbf{r}_1 = 0.494 \pm 0.039$	$p_{33} < p_{30}$ $p_{22} = p_{20}$ $p_{32} = p_{20}$	$\mathbf{p}_{33}$ = 0.272 $\mathbf{p}_{30}$ = 0.348 $\mathbf{p}_{22}$ = 0.083 $\mathbf{p}_{20}$ = 0.206 $\mathbf{p}_{32}$ = 0.198 $\mathbf{r}_1$ = 0.5	$p_{33} < p_{30}$ $p_{22} < p_{20}$ $p_{32} = p_{20}$	*Sw6* segregates independently of *Sw-5*
Relationship between UPV 32 and UPV 1 resistance sources	$\mathbf{p}_{33}$ = 0 $\mathbf{p}_{30}$ = 0 $\mathbf{p}_{11}$ = 0 $\mathbf{p}_{10}$ = 0 $\mathbf{p}_{31}$ = 0.112[g] $\mathbf{r}_2$ = 0.5	$p_{33} = p_{30}$ $p_{11} = p_{10}$ $p_{31} > p_{10}$	$\mathbf{p}_{33}$ = 0.034 $\mathbf{p}_{30}$ = 0.144 $\mathbf{p}_{11}$ = 0 $\mathbf{p}_{10}$ = 0 $\mathbf{p}_{31}$ = 0.186[g] $\mathbf{r}_2$ = 0.5	$p_{33} < p_{30}$ $p_{11} = p_{10}$ $p_{31} > p_{10}$	$\mathbf{p}_{33}$ = 0.291 $\mathbf{p}_{30}$ = 0.432 $\mathbf{p}_{11}$ = 0.052 $\mathbf{p}_{10}$ = 0.147 $\mathbf{p}_{31}$ = 0.306[g] $\mathbf{r}_2$ = 0.5	$p_{33} < p_{30}$ $p_{11} < p_{10}$ $p_{31} > p_{10}$	*Sw-6*segregates independently of UPV 1 resistance gene
Resistance conclusions	TR[c] (*Sw-6 Sw-6*) TR (UPV1 gene UPV1 gene) TR (*Sw-5 Sw-5*)	CD[d] (*Sw-6*) CD (UPV1 gene) CD (*Sw-5*)	PR[e] *Sw-6 Sw-6*) PR (UPV1 gene UPV1 gene) PR (*Sw-5 Sw-5*)	ID[f] (*Sw-6*) CD (UPV1 gene) CD (*Sw-5*)	PR (*Sw-6 Sw-6*) TR (UPV1 gene UPV1 gene) TR (*Sw-5 Sw-5*)	ID (*Sw-6*) ID (UPV1 gene) ID *Sw-5*)	

[b] p_{ij} = Probability of infection of a plant of genotype ij. i = 0 (*Sw* +); 1 (UPV1 gene); 2 (*Sw-5*); 3 (*Sw-6*).
[c] = Total resistance.
[d] = Complete dominance.
[e] = Partial resistance.
[f] = Incomplete dominance.
[g] = Loss of resistance due to physiological effects.

6), in both mechanical inoculation (T and HA) and thrips transmission.

Discussion

We present a line of *L. esculentum* (UPV 32) with resistance introgressed from *L. peruvianum*. UPV 32 resistance is probably controlled by a single gene. This gene may show incomplete penetrance, giving rise to partial overcoming of resistance and gene dosage effect, which result in incomplete dominance. Both resistance and dominance are conditioned by thrips transmission, and the aggressiveness of the isolate (sensu Vanderplank, 1968) (Table 7). When inoculating the T isolate mechanically, UPV 32 gene shows total resistance. Nothwithstanding, this resistance diminishes in thrips transmission, leading to an increase of 23% to 28% infected plants. The difference in infection levels between the two inoculation methods was also observed previously (Díez et al., 1995; Roselló et al., 1997). One possible explanation for the lower level of infection in the mechanical inoculation could be the appearance of defective interfering (DI) RNA molecules (Inoue-Nagata et al., 1997) which would result in symptom attenuation. However, this is unlikely to have occurred considering that first generation inoculum, proceeding from thrips transmission, were used. In all probability, the greater efficiency of the vector transmission is due to the fact that the plants are submitted to a high number of infectious thrips that ensure a continuous and large scale inoculation. Moreover, the thrips can efficiently inoculate young, very susceptible, tissues. Nevertheless, the overcoming of the resistance in some UPV 32 plants in thrips transmission can not be explained by the appearance of virulence in the T isolate (sensu Vanderplank, 1968). The high number of thrips in the cages would have reinoculated the virulent variant of the isolate in plants with the UPV 32 resistance gene, and therefore all the plants would have become susceptible in a similar way to that observed in the TSWV resistance found in *Capsicum chinense* (Moury et al., 1997). Moreover, using the adopted methodology, in the case of virulence appearing in a trial, the resistance would have been completely overcome in subsequent trials. We have carried out 3 thrips transmission trials, both independently and consecutively, and in all 3 trials have observed similar levels of infection in the plants with the UPV 32 resistance gene. These results, together with those of a previous study (Roselló et al., 1997) in which the same methodology was adopted, and where partial resistance was also detected, confirm that no virulence appeared in the T isolate. Consequently, the partial loss of resistance is due to incomplete gene penetrance.

The results of the mechanical inoculation trials show the HA isolate to be more aggressive than T isolate, diminishing this gene resistance by 3%. Nevertheless, it is observed that although the UPV 32 resistance is partially conditioned by aggressiveness, it is not completely overcome as occurs with the *Sw-1*^{a}, *Sw-1*^{b}, *sw-2*, *sw-3* and *sw-4* genes (Finlay, 1953).

There is complete dominance when T isolate is mechanically inoculated. However, when this isolate is transmitted by thrips, the proportion of resistant gene heterozygote infected plants increases between 8% and 12% more than in the homozygotes. In HA mechanical inoculation, this effect on incomplete dominance ranges from 4% to 12%.

The experiments on relationship between resistance sources show that UPV 32 gene segregates independently of the other resistant genes coming from *L. peruvianum* studied in this paper. The allelic relationship with other resistance genes, especially with the dominant alleles *Sw-1*^{a} and *Sw-1*^{b}, remains to be checked. However, due to the strong crossability barriers that exist between *L. peruvianum* on one hand, and *L. pimpinellifolium* and *L. esculentum* on the other (Rick, 1979), it is unlikely that a resistant gene transference took place between them. Furthermore, using our isolates we have carried out trials with the Pearl Harbour (*Sw-1*^{a}, *sw-4*) and Manzana (*Sw-1*^{b}, *sw-3*) cultivars and they resulted as being susceptible. We propose to give the name *Sw-6* to the UPV 32 resistance gene.

The resistance of RDD (*Sw-5*) and UPV 1 is not isolate-dependent. Although it is total when inoculating mechanically, in thrips transmission it is incomplete (5% to 8% of homozygotes are infected), as described in previous experiments (Roselló et al., 1997). It has also been observed that the infection level in heterozygotes for RDD (*Sw-5*) resistance is higher than the level reached by heterozygotes for UPV 1 resistance gene (Table 7). The joint data analysis of all thrips transmission trials give us (α = 5%) p_{20} = 0.177–0.257 and p_{10} = 0.102–0.170 as confidence intervals. Consequently, UPV 1 resistance gene confers significantly higher resistance level than RDD (*Sw-5*) in heterozygosis. This lower dependence of UPV 1 resistance on the gene dosage makes it very interesting for the development of commercial hybrids.

The high level of infection in UPV 32 × UPV 1 cross, which does not appear in UPV 32 × RDD cross, could be due to physiological reasons. During the seedling stage, before inoculation, the plants of this cross showed poorer growth development and less vigour than the other progenies. It is likely that the infection level rises under these physiological conditions. A similar effect has been observed in corn. In this case, plants experiencing poorer growing conditions suffered from more severe levels of infection caused by the Maize dwarf mosaic virus (MDMV) (Van Gessel and Coble, 1993). The explanation could be that the combination of genome fragments introgressed in UPV 32 and UPV 1 from *L. peruvianum* cause detrimental effects on the vigour of this hybrid.

The mechanism of TSWV resistance coming from *L. peruvianum* remains unknown. The behaviour after mechanical infection of the plants carrying the *Sw-5* gene described by Stevens et al. (1992) suggests that this gene acts with a hypersensitive response. However, on many occasions, the local lesions associated with this type of response are not evident. Furthermore, this mechanism is usually conditioned by temperature (Van Loon, 1975; Beier et al., 1979) and to the present date, there does not exist any evidence that the resistance conferred by the *Sw-5* locus is desestabilized by temperature. In our experiments, we have found that the resistance proceeding from *L. peruvianum* shows a dependence on the gene dosage in conditions of high inoculation pressure by thrips, confirming therefore the observations made on the *Sw-5* gene by Stevens et al. (1992). This dependence on the gene dosage is normally associated in the literature in two types of resistance mechanisms: single-cell infection response and virus multiplication restriction (Ponz and Bruening, 1986; Fraser, 1990). However, further studies are needed to understand clearly the mechanism of this resistance.

In summary, *L. peruvianum* showed a wide variability in TSWV resistance. The resistance genes characterized in this study show different levels of protection according to their original source. The resistance conferred by *Sw-5* locus is especially suitable for use in breeding programmes. Nevertheless, the partial resistance and dominance effects observed in thrips transmission make it advisable to search for new TSWV resistance sources. *L. peruvianum* has proved capable of providing sources that, either alone or joined, assure higher protection levels.

Finally, it is important to underline that the genetic models proposed in this work allow for the contrast of results from trials in which infected plants appear in resistant genotypes. Effects such as gene penetrance and gene dosage can be quantified by the parameters of the model. This would allow a wider study of the genetics of partial resistance.

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