A SEARCH FOR RESISTANCE TO *CUCUMBER MOSAIC VIRUS* IN THE GENUS *LYCOPERSICON*

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SUMMARY

A search for resistance to *Cucumber mosaic virus* (CMV) within the genus *Lycopersicon* was made. Resistance to CMV isolates from eastern Spain were found in accessions of the species *L. hirsutum* (2 accessions), *L. chmielewskii* (1 accession), *L. pimpinellifolium* (1 accession) and *L. esculentum* (3 accessions). When these accessions were assayed against Fny-CMV, a strain more severe than the Spanish field isolates tested before, only *L. chmielewskii* CHM-47 was resistant; *L. hirsutum* HIR-25 showed mild symptoms and some plants were not infected. All the other accessions were susceptible, indicating that their resistance was strain-specific. Tolerance to the systemic necrosis induced by Ix-satRNA was not found in nine assayed accessions of *L. hirsutum*, *L. peruvianum*, *L. pimpinellifolium* and *L. esculentum.* In resistant plants of *L. chmielewskii* CHM -47 and *L. hirsutum* HIR-25 no accumulation of either Fny-CMV or LS-CMV was detected by ELISA in inoculated or upper leaves. Nevertheless, when scions of these plants were graft-inoculated with Fny-CMV they became infected. This indicates that the resistance factors in these accessions do not interfere with replication or cell-to-cell movement but, possibly with earlier stages of virus infection.

Key words: CMV, tomato, *Lycopersicon*, resistance.

INTRODUCTION

Cucumber mosaic virus (CMV) is one of the economically most important viruses worldwide in field-grown temperate vegetables (Tomlison, 1987). Spain is no exception: CMV is present in all horticultural areas (Fraile *et al*., 1997; Luis-Arteaga *et al*., 1998) and important losses have been reported in the main vegetable crops (Jordá *et al*., 1992; Alonso-Prados *et al*., 1997; Avilla *et al*., 1997). Because of the very wide host range of CMV, both in weeds and in crops, and its efficient non-persistent transmission by many species of aphids (Palukaitis *et al*., 1992), control based on the reduction of inoculum sources and inoculum spread is largely ineffective. Strategies based on genetic resistance have been hampered by the lack of resistance sources, and commercial varieties resistant to CMV exist only for cucumber (Havey, 1997). In Spain, CMV has been particularly severe in open-field tomato crops. During the last decade, severe epidemics of CMV, often supporting necrogenic satellite RNAs (satRNAs), have resulted in dramatic losses and in the disappearance of the crop in some of the more affected areas in eastern Spain (Jordá *et al*., 1992). This prompted us to start a search for resistance to CMV that could be used in breeding programs for tomato (*Lycopersicon esculentum* Mill).

Resistance to CMV has been reported in different species of the genus *Lycopersicon*, including *L. peruvianum* Mill (Kuriyama *et al*., 1971; Laterrot, 1980; Ciccarese *et al*., 1987; Stamova *et al*., 1990; Sotirova *et al*., 1992), *L. hirsutum* Humb and Bonpl (Gebré-Selasié *et al*., 1990; Laterrot, 1990), *L. pimpinellifolium* Mill (Nitzay, 1975; Ciccarese *et al*., 1987; Stoimenova *et al*, 1992), *L. chilense* Dun (Stamova *et al*., 1990; Stoimenova and Sotirova, 1991) and in varieties of *L. esculentum* (Yasui and Yamakawa 1978; Weber *et al.,* 1989; Stoimenova *et al*., 1992). Tolerance to CMV has been reported in *Solanum lycopersicoides* Dun (Phills *et al*., 1977), which can interbreed with some species of *Lycopersicon* (Taylor, 1986). Most of the reported resistance or tolerance was effective against some field strains of CMV, but not against others (Jacquemond and Laterrot, 1981; Laterrot, 1982; Ciccarese *et al*., 1987; Stamova *et al*., 1990), or was quantitative (Laterrot, 1986; Weber *et al*., 1989). Thus, although some introgression programs have been started (Laterrot, 1980, 1990), no genetic lines of tomato with effective resistance to CMV are available.

In this paper we report work aimed at identifying sources of resistance to CMV that would be effective against the CMV isolates present in the main areas of tomato production in Spain. Spain is the second pro-

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ducer of tomato in Europe (FAO, 1996), and the main production areas are on the Mediterranean coast, Andalucía and the Canary Islands, where CMV epidemics are often dramatic (Jordá *et al*., 1992). We also report on the characterization of the resistance found in the potentially more interesting sources.

MATERIALS AND METHODS

Plant material. Data on the accessions of *Lycopersicon* spp. used in this work are listed in Table 1. Thirty-one accessions of eight species were chosen according to the following criteria: (I) to have represented all the species for which resistance to CMV had been reported. More accessions were included for those species in which resistance had been reported by several authors (*L. peruvianum* and *L. hirsutum*) than for those in which resistance had been reported only once (*L. pimpinellifolium* and *L. esculentum*); (II) to include at least one accession of some species in which resistance to CMV has not been reported, in order to have a broader representation of the different sections of the genus (*L. pennellii* D'Arcy*, L. chilense, L. cheesmanii* Riley and *L. chmielewskii* Rick, Kes, Fob and Holle). An accession of *S. lycopersicoides*, described as tolerant, and able to cross with some species of *Lycopersicon* (Phills *et al*., 1977; Taylor, 1986*)* was also included.

CMV isolates. Four isolates belonging to subgroup I, collected in eastern Spain in 1989-1991 (isolates L18, A21, 441 and C13, Aranda *et al*., 1993), plus strains Fny and LS, from New York, USA, belonging respectively to subgroup I and to subgroup II (Owen and Palukaitis, 1988) were multiplied in tobacco cv. 'Xanthinc', or tomato cv. 'Rutgers', and purified as described by Lot *et al*. (1972). The necrogenic satRNA Ix-satRNA (Moriones *et al*., 1991) was multiplied using Fny-CMV as helper.

Inoculation and screening procedures. Plants were mechanically inoculated when the first two true leaves were fully expanded (21-30 days after sowing according to the accession). Either sap of systemically infected leaves ground in 0.03 M sodium phosphate buffer pH 7.0, 0.2% diethylpyrocarbonate, or purified virus RNA (10 ml per plant of a 100 mg suspension in 0.1 Na_2HPO_4) were used as inocula. For graft inoculations, wedge or fluke grafts were made in the crown of CMV-infected, 35-40 day-old tomato plants. Axillary shoots 4 cm long of the accession to be tested were used as scions. Plants were maintained in a greenhouse (20-25°C, 16 h photoperiod) for mechanical inoculations, or in a growth chamber (23°C, with a relative humidity of 80%, 12 h photoperiod) for graft inoculations.

The presence of CMV in the inoculated plants was assessed by visual inspection of symptoms, and by DAS-ELISA (Clark and Adams, 1977) with commercial polyclonal antibodies to CMV (SANOFI Phytodiagnostic) as recommended by the manufacturer. In some experiments, the presence of CMV RNA and of CMV-satRNA in the inoculated plants was analyzed by dot blot hybridization using ^{32}P-labelled cRNA probes complementary to nucleotides 1389-1840 of Fny-CMV RNA3 (coat protein ORF, Owen *et al*., 1990) or to the whole B2-satRNA (Bernal and García-Arenal, 1994). The procedure described by Palukaitis and Zaitlin (1985) was followed.

Statistical analysis. The data analyzed in this work are number of infected plants over total inoculated ones. These data follow a binomial distribution, and non-parametric statistical tests were used for their analyses. The results for different accessions, replications, treatments or assessments were compared by contingency tests, based on the chi-square test for the distributions of resistant/susceptible plants for different events (Milton and Ysokos, 1994). Analyses were done using the STATGRAPHICS 4.2 software from Statistical Graphics Corporation.

RESULTS

Screening for resistance to Spanish isolates of CMV in the genus *Lycopersicon*. The thirty two accessions of *Lycopersicon* and *S. lycopersicoides* listed in Table 1 were tested for resistance to CMV by mechanical inoculation with a mixture of leaf sap from tobacco plants infected with CMV isolates 441, L18, A21 and C3. The number of plants tested varied for the different accessions according to their expected genetic variability (Rick, 1982; Miller and Tanskley, 1990). In the design of the experiment more plants per treatment were included for accessions of out breeding self-incompatible species (*L. chilense, L. hirsutum* and *L. peruvianum)*, than for accessions of the out breeding self-compatible ones (*L. pennellii* and *L. pimpinellifolium),* or of the inbreeding species (*L. cheesmanii* and *L. esculentum).* In some cases, particularly in accessions of *L. hirsutum*, poor germination resulted in lower actual plant numbers. Plants of tomato cv. 'Rutgers' were included as positive, susceptible, controls.

Table 1. Details of accessions of *Lycopersicon* and *Solanum* used in this work.

Species	Subspecies	Code	Accession	Origin[a]
L. chilense		CHI-45	LA 1971	Tacna, Peru **TGRC**
L. peruvianum	*humifusum*	PE-06	UPV 851	**UPV**
L. peruvianum		PE-07	LA 2172	Cajamarca, Peru. **TGRC**
L. peruvianum		PE-08	CMVsel INRA	**INRA**
L. peruvianum		PE-09	PI 126486	**USDA**
L. peruvianum		PE-10	PI 127831	Acabandia, Peru. **USDA**
L. peruvianum	*glandulosum*	PE-11	PI 126448	Rio Canda, Peru. **USDA**
L. peruvianum		PE-64	PE-23	Peru. **Nuez and Cuartero, 1984**
L. hirsutum	*typicum*	HIR-24	PE-39	Peru. **Nuez and Cuartero, 1984**
L. hirsutum	*typicum*	HIR-25	PE-43	Peru. **Nuez and Cuartero, 1984**
L. hirsutum	*typicum*	HIR-27	LA-1777	Ancash, Peru. **TGRC**
L. hirsutum	*typicum*	HIR-28	PI 126449	Road to Yeso, Peru. **USDA**
L. hirsutum	*glabratum*	HIR-29	PI 134627	**USDA**
L. hirsutum	*glabratum*	HIR-30	PI 247087	Loja. Ecuador **USDA**
L. hirsutum	*glabratum*	HIR-31	LA 2124	Chinchuipe, Ecuador. **TGRC**
L. hirsutum	*glabratum*	HIR-32	GI 1560	**ITV Wageningen**
L. hirsutum	*glabratum*	HIR-33	GI 1290	**ITV Wageningen**
L. pennellii		PEN-50	LA 716	Arequipa, Peru. **TGRC**
L. pennellii		PEN-51	PE-47	Peru. *Nuez y Cuartero* , **1984**
L. chmielewskii		CHM-47	LA 2678	Cusco, Peru. **TGRC**
L. cheesmanii	*minor*	CHE-46	LA 1401	Isabela, Galapagos. **TGRC**
L. esculentum	*cerasiforme*	CER-57	WVA 100	**INRA**
L. esculentum	*cerasiforme*	PYR-58	UPV 776	**UPV**
L. esculentum	*cerasiforme*	PYR-66	Mex-118	Mexico. **Nuez and Cuartero, 1984**
L. esculentum	*cerasiforme*	CER-68	Reg 1085	Peru. **Univ. La Molina. Perú**
L. pimpinellifolium		PIM-54	PE-8	Peru. **Nuez y Cuartero, 1984**
L. pimpinellifolium		PIM-55	LA 1607	Lima, Peru. **TGRC**
L. pimpinellifolium		PIM-56	PE-9	Peru. **Nuez and Cuartero, 1984**
L. pimpinellifolium		PIM-62	CIAPAN-10	**CIAPAN**
L. pimpinellifolium		PIM-63	CIAPAN-11	**CIAPAN**
L. pimpinellifolium		PIM-69	PE-2	Peru. **Nuez y Cuartero, 1984**
S. lycopersicoides		LYC-49	UPV 919	**UPV**

[a] Shown in bold are the institutions providing the accession (or the reference).
UPV Universidad Politécnica de Valencia, Spain.
INRA Ministère de l'Agriculture "Station d'Amélioration des Plantes Maraîchères". Avignon, France.
TGRC Tomato Genetics Resource Center. Department of Vegetable Crops, University of California Davis, California, USA.
CIAPAN Centro de Investigaciones Agrícolas del Pacífico Norte. Culiacan, Mexico.
USDA United States Department of Agriculture, USA.
ITV Institute for Horticultural Plant Breeding. Wageningen, The Netherlands.

Twenty-five days after inoculation (dpi) the presence of CMV in the inoculated plants was screened by ELISA, as most plants in most accessions did not show clear symptoms. The results are shown in Table 2. Significant differences in the percentage of infected plants, as compared with the susceptible control (80% infection) were found for 11 accessions belonging to the species *L. chilense, L. hirsutum L. chmielewskii, L. esculentum* and *L. pimpinellifolium.* To check that these differences were not due to inoculation failures, all ELISA-negative and symptomless plants were re-inoculated ten days after the initial screening. A new set of plants of tomato cv. 'Rutgers' was re-inoculated. The results of the second inoculation were again screened by ELISA, and are shown in Table 2. The percentage infection found for the different accessions in both inoculations did not differ significantly in a contingency analysis ($P > 0.93$), when the species of *L. hirsutum* and *L. peruvianum* were excluded from the analysis (data not shown). In these two species a high percentage of ELISA positive plants were eliminated after the first inoculation. Thus, the different percentage of infection found reflects different susceptibilities to CMV among the accessions. Accessions CHM-47 of *L. chmielewskii,* CER-57, CER-68 and PYR-66 of *L. esculentum* var. *cerasiforme*, HIR-25 and HIR-27, of *L. hirsutum* and PIM-55, PIM-63 and PIM-69 of *L. pimpinellifolium*, differed from the control at a significance level of at least 0.05 in both inoculations, and were considered resistant.

Screening for resistance to Fny-CMV in the genus *Lycopersicon.* Infection by the field isolates of CMV in the above experiment did not result in clear symptoms in many of the assayed accessions, and the symptoms shown by the susceptible control, tomato cv. 'Rutgers', were not severe (not shown). Thus, a second screening was done using a severe strain of CMV, Fny. For this, a smaller number of accessions was considered, including those with the better levels of resistance in the experiment above. Some non-resistant accessions of different species were also included in order to characterize CMV symptoms in a broader range of *Lycopersicon* species. Also, inoculations with the necrogenic Ix-satRNA supported by Fny-CMV were made in a few accessions from species in which resistance had been reported: *L. esculentum* (3 accessions), *L. hirsutum* (3), *L. peruvianum* (2) and *L. pimpinellifolium* (1). Since in these two experiments we were interested in comparing the symptoms shown by the different accessions, purified virus and satellite RNAs were used as inocula for maximum homogeneity among treatments. The presence of CMV and/or satRNA was analyzed by dot-blot hybridization 20 dpi.

The results are shown in Table 3. Infection by Fny-CMV resulted in stunting, mosaic and extreme reduction of the leaf lamina ('shoe-string') in most accessions. Assessment of infection by visual inspection of symptoms and by dot-blot gave consistent results ($P = 0.88$ in a contingency test). Thus, no accession was found showing tolerance to Fny-CMV. However, some accessions of *L. hirsutum* (HIR-25, HIR-27, HIR-30 and HIR-31) developed milder symptoms. Only accession CHM-47, of *L. chmielewskii*, showed resistance as compared with the susceptible control. Among the rest, accession HIR-25 showed a lower percentage of infection, but the difference from the susceptible control was not significant. When Fny-CMV was co-inoculated with Ix-satRNA, all assayed accessions behaved like the susceptible control. For all accessions, all plants that were infected by Fny-CMV were also infected by Ix-satRNA and showed systemic lethal necrosis (Table 3). Thus, no accession was found susceptible to CMV and resistant or tolerant to CMV-satRNA.

Analysis of CMV infection in accessions HIR-25 and CHM-47. The reaction of *L. chmielewskii* CHM-47 and *L. hirsutum* HIR-25 to CMV infection was further analyzed. Plants of these accessions that were resistant to inoculation by Fny-CMV were selfed. The infection of the resulting progeny by the severe Subgroup I strain Fny and by a mild strain in Subgroup II (LS-CMV) was analyzed. Plants at the five leaf stage were inoculated on the fourth leaf with purified viral RNA. The presence of CMV was analyzed by ELISA 6 dpi in the inoculated leaf, 13 dpi in the eighth leaf and 25 dpi in the eleventh leaf. The results are shown in Table 4. All assayed plants of tomato cv. 'Rutgers' were infected by both Fny and by LS isolates. No plant of *L. hirsutum* HIR-25 or of *L. chmielewskii* CHM-47 was infected by LS-CMV, and only one plant of *L. hirsutum* HIR-25 was infected by Fny-CMV. In this plant, Fny-CMV infection was already detected in the inoculated leaf at 6 dpi.

Resistant plants in the above experiment were grown on, and from them scions were obtained that were grafted onto Fny-CMV-infected 35-40 day-old 'Rutgers' tomato plants. Of ten attempted grafts, only two with *L. hirsutum* HIR-25 and four with *L. chmielewskii* CHM-47 were successful. The presence of CMV was analyzed by ELISA in the youngest expanded leaf of the scions 20 and 40 days after grafting (dpg).

The results, shown in Table 5, indicate that plants resistant to mechanical inoculation with Fny were susceptible to graft inoculation. Although the ELISA analysis was not quantitative, absorbance data suggested that *L. hirsutum* plants supported higher levels of Fny than *L. chmielewskii*. Symptoms in the scion were also milder for *L. chmielewskii* than for *L. hirsutum* (Table 5).

Table 2. Susceptibility of thirty two accessions of the genera *Lycopersicon* and *Solanum* to a mixture of Spanish isolates of CMV.

Species	Accession	N° [a]	Inoculation [b]	Reinoculation [b]	Difference [c]
L. chilense	CHI-45	119	28**	73	> 44.83**
L. peruvianum	PE-06	85	85	25**	< 60.00**
L. peruvianum	PE-07	75	58	0**	< 57.15**
L. peruvianum	PE-08	107	78	20**	< 57.29**
L. peruvianum	PE-09	115	75	40	< 35.00*
L. peruvianum	PE-10	106	75	30*	< 45.00*
L. peruvianum	PE-11	145	79	60	< 18.79
L. peruvianum	PE-64	145	40	37	> 3.34
L. hirsutum	HIR-24	26	55	50	< 5.00
L. hirsutum	HIR-25	42	35*	10**	< 25.00
L. hirsutum	HIR-27	54	37*	10**	< 26.37
L. hirsutum	HIR-28	58	84	30*	< 53.34*
L. hirsutum	HIR-29	50	60	20**	< 40.00
L. hirsutum	HIR-30	57	77	28*	< 49.65*
L. hirsutum	HIR-31	61	67	73	> 6.06
L. hirsutum	HIR-32	105	67	45	< 22.22
L. hirsutum	HIR-33	105	67	34	< 33.33
L. pennellii	PEN-50	89	50	40	< 10.00
L. pennellii	PEN-51	84	50	55	> 5.00
L. chmielewskii	CHM-47	96	23**	20**	< 2.73
L. cheesmanii	CHE-46	46	50	40	< 10.00
L. esculentum	CER-57	50	30*	20**	> 10.00
L. esculentum	PYR-58	50	90	50	< 40.00*
L. esculentum	PYR-66	50	10**	10**	> 0.00
L. esculentum	CER-68	43	13**	23*	> 9.73
L. pimpinellifolium	PIM-54	50	80	40	< 40.00**
L. pimpinellifolium	PIM-55	48	10**	30*	> 20.00
L. pimpinellifolium	PIM-56	50	10**	40	> 30.00
L. pimpinellifolium	PIM-62	49	40	30*	< 10.00
L. pimpinellifolium	PIM-63	49	30*	30*	0.00
L. pimpinellifolium	PIM-69	49	20**	10**	< 10.00
S. lycopersicoides	LYC-49	24	58	100	> 42.85*
L. esculentum	Rutgers		80	80	0.00

[a] Number of inoculated plants.
[b] Data are shown as percentage of plants positive for CMV in ELISA. Significance of the differences from the susceptible control in contingency tests is indicated.
[c] Differences between percentage of negative plants in ELISA after inoculation and reinoculation.
* $P \leq 0.05$, ** $P \leq 0.01$.

Table 3. Susceptibility of different accessions of the genus *Lycopersicon* to Fny-CMV and to Ix-satRNA.

Species	Accession	Infected plants					
		Fny -CMV			Fny -CMV + Ixsat RNA		
		Symptoms [a]		Hybridization [b]	Symptoms [a]		Hibridization [c]
L. chilense	CHI-45	st	100	100			
L. peruvianum	PE-07	st, sh, m	100	100	sn	100	100
L. peruvianum	PE-08	st, m	90	90	sn	100	100
L. peruvianum	PE-10	st, sh, m	70	80			
L. peruvianum	PE-64	st, sh, m	80	80			
L. hirsutum	HIR-25	*sh, cl*	77	77	sn	80	80
L. hirsutum	HIR-27	*st, mot*	90	90			
L. hirsutum	HIR-30	*st, m, sh*	88	77	sn	100	100
L. hirsutum	HIR-31	*sh, m,mot*	87	87	sn	100	100
L. hirsutum	HIR-32	st, sh, mot	90	90			
L. pennellii	PEN-51	st, cl	100	100			
L. chmielewskii	CHM-47	st, m	60 *	60 *			
L. cheesmanii	CHE-46	sh, cu	100	100			
L. esculentum	CER-57	st, m, fil	90	80			
L. esculentum	PYR-58	sh, m	90	90	sn	100	100
L. esculentum	PYR-66	m, sh	88	88	sn	93	93
L. esculentum	CER-68	st, m, sh	100	100	sn	100	100
L. pimpinellifolium	PIM-55	st, m,cu	100	100			
L. pimpinellifolium	PIM-56	st, m, cu	90	100	sn	100	100
L. pimpinellifolium	PIM-62	st, m,sh	100	100			
L. pimpinellifolium	PIM-69	st, mot, cu	90	90			
	Rutgers	st, m, sh	100	100	sn	100	100

[a] Data are percentage of plants showing symptoms. 8-10 plants per accession were inoculated with Fny-CMV and 10-15 plants with Fny-CMV+Ix sat RNA. sn: systemic necrosis; st: stunt; sh: shoestring; m: mosaic; mot: mottling; cl: chlorosis; cu: curl; fil: filimorphism; *mild symptoms.*

[b] Percentage of positive plants in dot blot hybridizations with an RNA probe complementary to CMV CP ORF.

[c] Percentage of positive plants in dot blot hybridizations with an RNA probe complementary to B2-satRNA. All the positive plants in this test were also positive for CMV CP.

In all cases significance of differences from the susceptible control in a contingency test are indicated.

* $P \leq 0.05$.

A second graft inoculation experiment was done, including grafts of 'Rutgers' tomato scions on infected tomato rootstocks. Results (Table 5) showed again that all the scions of *L. hirsutum* and *L. chmielewskii* were infected. Again, lower levels of antigen were detected in *L. chmielewskii* than in *L. hirsutum* scions, which gave absorbance values in ELISA similar to the scions of 'Rutgers' tomato.

DISCUSSION

Screening for resistance to a mixture of four field isolates of CMV from the area where tomato crops have been more severely affected by CMV in Spain, showed that nine accessions from four species of *Lycopersicon* had some degree of resistance. Two of these accessions (HIR-25 and HIR-27) belonged to *L. hirsutum*, a

Table 4. Accumulation of Fny-CMV and LS-CMV in different leaves of plants of tomato cv. 'Rutgers', *L. chmielewskii* CHM-47 and *L. hirsutum* HIR-25*.

	Fny-CMV			LS-CMV		
leaf	Inoculated (4th)	8th	11th	Inoculated(4th)	8th	11th
dpi	6	13	25	6	13	25
Tomato Rutgers						
Mock	0.22	0.21	0.23	0.22	0.21	0.23
Plant 1	0.22	0.63 **	1.73 **	0.00	2.22 **	2.45 **
Plant 2	0.86 **	0.61 **	2.09 **	0.00	2.12 **	2.32 **
Plant 3	0.84 **	0.67 **	1.85 **	0.00	2.00 **	2.01 **
Plant 4	0.88 **	0.60 **	2.03 **	0.00	2.08 **	2.32 **
Plant 5	0.24	0.63 **	2.15 **	0.00	2.34 **	2.09 **
L. hirsutum HIR-25						
Mock	0.25	0.23	0.18	0.25	0.23	0.18
Plant 1	0.61 **	0.60 **	2.27 **	0.00	0.00	0.00
Plant 2	0.06	0.13	0.00	0.00	0.02	0.00
Plant 3	0.00	0.01	0.00	0.00	0.00	0.00
Plant 4	0.20	0.00	0.00	0.00	0.08	0.00
Plant 5	0.15	0.02	0.00	0.00	0.00	0.00
L. chmielewskii CHM-47						
Mock	0.24	0.14	0.21	0.24	0.14	0.21
Plant 1	0.00	0.00	0.00	0.00	0.00	0.00
Plant 2	0.00	0.00	0.00	0.00	0.00	0.00
Plant 3	0.00	0.00	0.00	0.10	0.00	0.00
Plant 4	0.00	0.00	0.00	0.00	0.03	0.00
Plant 5	0.10	0.00	0.00	0.00	0.00	0.00

* For mock-inoculated plants data are absorbance values at 405 nm. For inoculated plants data are absorbance values at 405 nm after substraction of the values for the corresponding mock-inoculated plants.

** Indicates absorbances at least twice as high as those of mock-inoculated plants.

species that has been reported as a promising source of resistance to CMV (Laterrot, 1990). Accessions HIR-25 and HIR-27 have not been reported previously as resistant to CMV. On the other hand, accession HIR-30 (PI 247087), reported to be a good source of resistance to CMV (Gebré-Selasié *et al.*, 1990; Laterrot, 1990), showed only a moderate level of resistance on re-inoculation of the non-infected plants. Resistance was also found in accessions of *L. pimpinellifolium* (PIM-69) and of *L. esculentum* var. *cerasiforme* (CER-57, PYR 66, CER-68). These two species may be particularly interesting for breeding, as they are closely related to the domestic tomato, and resistance would be easier to introgress in tomato lines. Resistance to CMV had previously been reported in other accessions of *L. pimpinellifolium* (Nitzay, 1975; Ciccarese *et al.*, 1987; Stoimenova *et al.*, 1992) and *L. esculentum* (Weber *et al.*, 1989; Stoimenova *et al.*, 1992). The high percentage of accessions in these two species that showed resistance to CMV is to be stressed.

An accession of *S. lycopersicoides* was described as tolerant to CMV (Phills *et al.*, 1977). From our screening we cannot conclude if this is also the case regarding the Spanish CMV isolates, since they induced very mild symptoms, or none, in all assayed accessions.

Table 5. Graft inoculation of plants of *L. chmieleewskii* CHM-47 and *L. hirsutum* HIR-25 with Fny-CMV.

Scion	20 dpg *	30 dpg *	40 dpg *	Symptoms 30 dpg
1st experiment				
L. hirsutum HIR-25				
Negative control	0.23		0.22	
Graft 1	1.64**		3.23 **	Severe mosaic
Graft 2	1.75 **		2.76 **	Severe mosaic
L. chmieleewskii CHM-47				
Negative control	0.17		0.15	
Graft 1	0.32 *		1.39 **	Mild mosaic
Graft 2	0.53 **		2.86 **	Mild mosaic
Graft 3	0.75 **		0.98 **	Mild mosaic
Graft 4	0.59 **		1.04 **	Mild mosaic
2nd experiment				
L. hirsutum HIR-25-2				
Negative control		0.14		
Graft 1		2.35**		Severe mosaic
Graft 2		1.77**		Severe mosaic
Graft 3		2.43**		Severe mosaic
Graft 4		1.56**		Severe mosaic
L. chmieleewskii CHM-47-2				
Negative control		0.12		
Graft 1		1.93**		Mild mosaic
Graft 2		2.02**		Mild mosaic
Graft 3		1.26**		Mild mosaic
Graft 4		0.95**		Mild mosaic
Graft 5		1.87**		Mild mosaic
Graft 6		0.73**		Mild mosaic
Rutgers				
Negative control		0.11		
Graft 1		2.36**		Severe mosaic
Graft 2		1.73**		Severe mosaic
Graft 3		2.56**		Severe mosaic

In all cases the rootstock was tomato cv. 'Rutgers'.

* For negative controls (scions grafted on uninfected plants) data are absorbance values at 405 nm. For graft-inoculated plants data are absorbance values at 405 nm after substraction of the values of the corresponding controls.

** Indicates absorbances at least twice as high as those of controls.

It is interesting to note that no accession of *L. peruvianum,* a species often reported as a resistance source to CMV (Kuriyama *et al*., 1971; Jacquemond and Laterrot, 1981; Ciccarese *et al*., 1987; Stamova *et al*., 1990; Sotirova *et al*., 1992) was rated as resistant in our assay. Accessions that appeared to be so showed a high percentage of infection after re-inoculation of the non-infected plants. This was also the case for accession PE-08, that has been described as resistant to CMV (Laterrot, 1982) but susceptible to CMV+sat RNA (Jacquemond and Laterrot, 1981) and was used to introgress its resistance into commercial lines of tomato (Laterrot, 1982; 1986). The different behaviour of accessions of *L. peruvianum* upon inoculation and re-inoculation might be related to a tendency to escape infection, rather than to resistance.

Other discrepancies between our results and those earlier reported might be due to differential interactions of certain accessions of *Lycopersicon* with different CMV strains. Accession-isolate specific interactions have been reported for the pathosystem *Lycopersicon*-CMV (Ciccarese *et al.,* 1987; Stamova *et al*., 1990), and

are apparent when comparing the results of inoculation with the Spanish isolates of CMV and with Fny-CMV. Most of the accessions that were resistant to the Spanish CMV isolates proved to be susceptible to the more severe Fny. The only exception was accession CHM-47 of *L. chmielewskii,* a species for which resistance had not been described by other authors. CHM-47 showed a good degree of resistance to Spanish CMV field isolates and to Fny-CMV, all in Subgroup I of CMV strains, as well as to LS-CMV, in Subgroup II, suggesting that the resistance of this accession is quite general and thus could be of interest in breeding programs. This species is genetically distant from *L. esculentum* and more closely related to *L. peruvianum* and *L. hirsutum* (Miller and Tanskley, 1990), but at odds with these, it crosses easily with tomato and lacks many of the undesirable agronomic traits of *L. hirsutum* and *L. peruvianum* (Taylor, 1986).

In most of the assayed accessions, Fny-CMV induced stunting, mosaic and severe reduction of leaf lamina, as in tomato. No evidence for tolerance was found, although four of five assayed accessions of *L. hirsutum* consistently showed milder symptoms than accessions of other species. Tolerance to the systemic necrosis induced by Ix-satRNA was also absent in nine accessions belonging to four species, including *L. hirsutum* (3 accessions) and *L. peruvianum* (2 accessions). This is in contrast with results from White and Kaper (1987), who reported frequent tolerance to CMV-satRNA-induced systemic necrosis in these two species.

Because *L. chmielewskii* CHM-47 showed good resistance to all assayed CMV isolates, and *L. hirsutum* HIR-25 showed good resistance to the Spanish ones and mild symptoms after Fny-CMV infection, an attempt to characterize the nature of their resistance was made. The progeny derived by selfing plants of these accessions that were resistant to Fny-CMV, proved to be resistant to LS-CMV and to Fny-CMV for most of the plants. In the resistant plants, CMV antigen was not detected in the inoculated leaf 6 dpi, or in the upper leaves at 25 dpi, suggesting that resistance interferes with replication and/or cell-to-cell movement of the virus. Nevertheless, scions from these resistant plants became infected by Fny-CMV when grafted upon Fny-CMV-infected tomatoes. This shows that resistance to CMV in accessions HIR-25 and CHM-47 does not interfere with replication or movement of the virus, but rather appears to interfere with early events after mechanical inoculation in an, as yet, undetermined way.

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