Sources of Resistance to *Pepino mosaic virus* (PepMV) in Tomato

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Additional index words. potexvirus, *Solanum lycopersicum*, virus resistance

Abstract. The disease incited by *Pepino mosaic virus* (PepMV) is currently a serious problem for tomato (*Solanum lycopersicum* L.) crops in several European countries. A collection of accessions from different *Solanum* species was screened to find sources of resistance to PepMV. All plants of *S. lycopersicum*, *S. lycopersicum* var. *cerasiforme*, *S. pennellii* Correll, *S. cheesmaniae* (L. Riley) Fosberg, *S. habrochaites* S. Knapp & D.M. Spooner, *S. neorickii* D.M. Spooner, G.J. Anderson & R.K. Jansen, *S. pimpinellifolium* L., *S. basendopogon* Bitter, *S. canense* Rydb., *S. caripense* Humb. & Bonpl. ex Dunal, and *S. muricatum* Aiton accessions showed a 100% systemic infection rate, high viral accumulation, and apparent symptoms. In some accessions of the species *S. chilense* (Dunal) Reiche and *S. peruvianum* L., a variable percentage of plants without systemic infection was observed. Although all plants of ECU-335 accession of *S. ochranthum* Dunal showed systemic infection by PepMV, the symptoms were mild and the levels of viral accumulation were low. PepMV was not detected in plants of AN-CA-214 accession of *S. pseudocapsicum* L. No symptoms were observed either on inoculated leaves or on growing leaves. The use of the latter two species is limited considering that they cannot be sexually crossed with cultivated tomato. As a result, *S. chilense* and *S. peruvianum* are the most promising species in the search for sources of resistance to PepMV.

In 2004, more than 4 million ha of tomato (*Solanum lycopersicum*) were cultivated worldwide, producing over 116 million tonnes (FAO, 2005). This crop is the second horticultural species in terms of cultivated surface area and production volume after potato. In the last decades, the viral diseases have become the main limiting factor in tomato crops along the Spanish Mediterranean coast and in many other areas in the world. In this respect, *Tomato spotted wilt virus* (TSWV) and *Tomato yellow leaf curl virus* (TYLCV) have been responsible for serious economic losses around the world (Goldbach and Peters, 1994; Picó et al., 1996; Roselló et al., 1996).

Recently, other viral diseases have appeared such as those incited by *Parietaria mottle virus* (PMoV), which is currently causing serious damage in Greece (Lisa et al., 1998), France (Marchoux et al., 1999), Italy (Roggero et al., 2000), and Spain (Aramburu, 2001); *Tomato infectious chlorosis virus* (TICV), identified in the United States (Duffus et al., 1996), Italy (Vaira et al., 2002), and Spain (Font et al., 2003); and *Tomato chlorosis virus* (ToCV), identified in Portugal (Louro et al., 2000), Spain (Navas-Castillo et al., 2000), and Greece (Dovas et al., 2002). Nevertheless, the viral disease that has caused the greatest impact on tomato crops is the one incited by *Pepino mosaic virus* (PepMV). This virus was first described in *Solanum muricatum* plants in Peru in 1974

(Jones et al., 1980). However, its presence was not reported again until 1999 when it was found in tomato crops in Holland. A few months later, its presence was reported in England and Germany in 100 ha infected (EPPO, 2000). In 2000, it was reported for the first time in the French region of Brittany and the Spanish provinces of Murcia, Almería, and the Canary Islands (Soler et al., 2000). It was later detected in Italy (Roggero et al., 2001), Canada, and the United States (French et al., 2001), Poland (Pospieszny and Borodynko, 2002), China (Zhang et al., 2003), and Hungary (Forray et al., 2004).

Currently, in Spain, the disease incited by PepMV affects plantations in the provinces of Alicante, Murcia, Almería, Granada, and the Canary Islands and represents a serious problem for tomato crops along the Spanish Mediterranean coast. The highest incidence of the disease appears in Murcia with a 75% to 90% rate of infected greenhouses. In many cases, losses reach 20% to 40% of production (Soler et al., 2000). In addition, many countries are reticent to buy Spanish tomatoes because PepMV has been occasionally detected in Spanish imported fruits (Zitikaite et al., 2004).

Typical leaf symptoms associated with PepMV infection are yellow mosaics, puffiness, and leaf filiformity. Dark green grooves appear on stems and sepals. Infected plants show a severe reduction of fruit set. Fruits usually show a mosaic of different shades of red only on ripe fruits, which is incited by an irregular distribution of lycopene on the surface and reduces their commercial value (Soler et al., 2000). The appearance of these symptoms depends on environmental factors, mainly temperature.

Symptom severity is reduced when temperature rises above 25 °C and sometimes disappears altogether. In this case, plants may show spotted ripe fruits without symptoms on the leaves or may have systemic infection with high levels of viral accumulation without showing symptoms.

During the 1998–1999 season in Murcia, a sudden wilting of tomato plants was reported and in many cases caused the irreversible collapse of the plants. Studies carried out on collapsed and noncollapsed plants reported a clear association between PepMV and tomato collapse (Soler-Aleixandre et al., 2005b). This syndrome could constitute another symptom associated with infection by this potexvirus. This has contributed to the fact that PepMV is now considered to be one of the most serious limiting factors in the profitability of tomato crops in Spain. In Holland and the United Kingdom, the appearance of severe mosaic and filiformity symptoms, associated with PepMV, also cause serious losses (Tomassoli, 2001). Fruit spotting causes the most damage in tomato crops in Italy (Roggero et al., 2001).

PepMV can be mechanically transmitted very efficiently. In the field, the disease spreads fast, even more than *Potato virus X* (PVX), and can be transmitted more efficiently than *Tomato mosaic virus* (ToMV) (Wright and Mumford, 1999). It is transmitted through the touching of plants with contaminated hands, during the handling of infested tools, and even by clothes brushing against plants. Although it has been detected in tomato seeds (Salomone and Roggero, 2002), there is no evidence of transmission to descendants. No specific transmission vectors have been reported. The transmission role by contact of pollinating insects such as *Bombus terrestris* and *B. canariensis*, used in greenhouse crops, has been reported (Lacasa et al., 2003). Because of the lack of severe symptoms in infected plants, growers are reluctant to eliminate the infected ones and, therefore, the disease spreads quickly through greenhouses (Soler et al., 2000).

The highly efficient mechanical transmission of this disease has made the adoption of cultural practices unsuccessful in preventing its spread. The development of varieties that incorporate resistance to the virus would allow effective control of the disease. In this study, results of a screening for resistance to PepMV in a collection of accessions from different *Solanum* species are presented.

Material and Methods

Plant material. A total of 229 accessions from 15 different species of the *Solanum* genus were assayed for resistance to PepMV. The screening included accessions of *Solanum basendopogon* (one accession), *S. canense* (one), *S. caripense* (5), *S. cheesmaniae* (2), *S. chilense* (13), *S. habrochaites* (42), *S. lycopersicum* (54), *S. lycopersicum* var. *cerasiforme* (3), *S. muricatum* (9), *S. neorickii* (5), *S. ochranthum* (one), *S. pennellii* (9), *S. peruvianum* (45), *S. pimpinellifolium* (38),

Received for publication 14 June. 2006. Accepted for publication 28 July. 2006.

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Table 1. Response of *Solanum basendopogon*, *S. canense*, *S. caripense*, *S. cheesmaniae*, *S. chilense*, *S. habrochaites*, *S. lycopersicum*, *S. lycopersicum* var. *cerasiforme*, *S. muricatum*, *S. neorickii*, *S. ochranthum*, *S. pennellii*, *S. peruvianum*, *S. pimpinellifolium*, and *S. pseudocapsicum* accessions to mechanical inoculation with the LE-2002 PepMV isolate.

Accession	Mean Maximum Symptom Index ^z	Mean Maximum Absorbance ^y	Absorbance Index ^x	Total Percent of Plants With Systemic Infection ^w
<i>S. basendopogon</i>				
BIRM/S-1975	3.00	3.02	1.01	100.0
<i>S. canense</i>				
BIRM/S-1815	3.17	3.06	1.02	100.0
<i>S. caripense</i>				
PI-243342	3.33	2.45	0.82	100.0
261	2.00	2.49	0.83	100.0
EC-36	2.67	2.62	0.88	100.0
BIRM/S-1034	2.67	2.75	0.92	100.0
E-7	3.42	3.06	1.02	100.0
<i>S. cheesmaniae</i>				
GLP-36	3.8	2.96	0.99	100.0
GLP-3	2.4	2.99	1.00	100.0
<i>S. chilense</i>				
PER-551	1.0	0.79	0.26	100.0
LA-470	1.1	1.03	0.30	30.0
ECU-527	0.88	0.97	0.33	80.0
PER-522	0.9	1.08	0.36	100.0
PER-526	0.75	1.15	0.39	100.0
PER-542	1.0	1.18	0.39	100.0
LA-1932	1.77	1.79	0.60	100.0
LA-1963	2.3	2.26	0.76	84.6
LA-1971	3.2	2.61	0.87	88.2
LA-1968	3.5	2.35	0.89	83.3
LA-2762	2.6	2.71	0.91	92.3
LA-2774	2.2	2.73	0.91	100.0
LA-458	3.6	2.75	0.92	78.6
<i>S. habrochaites</i>				
ECU-436	1.6	3.00	1.00	100.0
ECU-910	2.77	3.02	1.01	100.0
ECU-922	2.0	0.44	0.15	100.0
ECU-926	1.93	0.56	0.19	100.0
ECU-968	1.2	0.60	0.20	100.0
ECU-911	2.27	0.59	0.20	100.0
ECU-921	2.20	0.61	0.21	100.0
ECU-908	2.33	0.65	0.22	100.0
ECU-764	0.78	0.68	0.23	100.0
ECU-800	1.25	0.78	0.26	100.0
ECU-753	1.35	0.77	0.26	100.0
ECU-869	1.23	0.89	0.30	100.0
ECU-847	0.9	0.93	0.31	100.0
ECU-872	2.13	1.01	0.33	100.0
ECU-851	1.57	1.01	0.34	100.0
ECU-870	1.40	1.11	0.37	100.0
ECU-924	1.47	1.09	0.37	100.0
ECU-888	1.80	1.10	0.37	100.0
ECU-862	2.27	1.13	0.38	100.0
ECU-854	2.13	1.31	0.44	100.0
ECU-758	2.27	1.33	0.45	100.0
ECU-849	0.5	1.38	0.46	100.0
ECU-846	1	1.40	0.47	100.0
ECU-865	1.00	1.42	0.48	100.0
ECU-856	1.17	1.52	0.51	100.0
ECU-950	2.5	1.56	0.52	100.0
ECU-760	1.57	1.63	0.55	100.0
ECU-852	1.4	1.78	0.60	100.0
ECU-850	2.27	1.83	0.61	100.0
ECU-859	1.40	1.81	0.61	100.0
ECU-776	2.7	1.89	0.63	100.0
ECU-868	1.87	1.87	0.63	100.0
ECU-863	1.50	2.08	0.70	100.0
ECU-855	1.47	2.14	0.72	100.0
ECU-860	1.73	2.16	0.72	100.0
ECU-871	1.97	2.48	0.81	100.0
ECU-864	1.73	2.45	0.82	100.0
ECU-909	1.80	2.50	0.84	100.0
ECU-816	2.8	2.68	0.90	100.0
ECU-923	2.20	2.75	0.92	100.0
ECU-524	2.8	2.81	0.94	100.0
ECU-434	2.8	2.85	0.95	100.0
<i>S. lycopersicum</i> ^v				
UPV-32	3.5	1.48	0.49	100.0

continued

and *S. pseudocapsicum* (one). Also, lines NE-1 and Fortuna-C of *S. lycopersicum* were included in all assays as susceptible controls. Seeds were treated at a temperature of 80 °C for 24 h to eliminate any possible contamination by ToMV. Afterward, they were soaked in 5% sodium hypochlorite for 10 min to facilitate germination. The number of plants tested per accession varied between 10 and 20 by the lack of uniformity in the germination of the seeds. Once germinated, seedling were transplanted to 8 × 6 × 6 cm pots and grown at 25 °C/18 °C (day/night) with a 14-h day length and relative humidity of 65%/95% to 100% (day/night). Plants were subjected to irradiance of 65 to 85 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ obtained through the use of GroLux tubes (Sylvania, Danvers, Mass.). Two plants per accession were kept as controls: one as an uninoculated control and another as a water-inoculated control. These controls were kept separately to avoid infection with PepMV.

Virus isolate and plant inoculation. PepMV isolate LE-2002 used in this study was obtained from tomato-infected plants collected in the region of Murcia (East of Spain). LE-2002 induces severe mosaics and leaf distortions and the full-length sequence of the genomic RNA was determined (López et al., 2005). The nucleotide sequence share identities higher than 99% with the strain reported in Europe (European tomato strain), which is the prevalent genotype in Spain (López et al., 2005; Pagan et al., 2006). To obtain the inoculum, leaves of tomato plants infected with LE-2002 isolate were crushed with a mortar and pestle until a uniform paste was obtained. The paste was mixed with sterile distilled water at a ratio of 1:5 (w/v) and filtered through a muslin cloth. Plants were mechanically inoculated at the fourth true leaf stage. The previously prepared inoculum was delivered to the four leaves using a cotton swab. The inoculation was repeated 7 d later, in new developed leaves, to guarantee infection and to prevent escapes.

Symptoms evaluation and plant analysis. Throughout the inoculation assays, the symptoms shown by each of the inoculated plants were recorded and compared with the non-inoculated controls. A global index was used for the degree of symptoms observed (mosaic, filimorphism, and leaf curling): 0, absence of symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms; 4, very severe symptoms or dead plant.

Apical leaves, developed after the second inoculation, were taken at 30 and 60 d after inoculation (DAI). Leaves extracts of each plant were tested by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977) using specific antiserum against PepMV (DSMZ GmbH, Braunschweig, Germany). Samples were mashed in extraction buffer at a dilution of 1:20 (w/v) and 0.1 mL of the extract obtained was analyzed. The absorbance of the serologic reaction was measured using a Model 550 Microplate Reader (Bio Rad, Hercules, Calif.). Samples were considered to

Table 1. (continued) Response of *Solanum basendopogon*, *S. canense*, *S. caripense*, *S. cheesmaniae*, *S. chilense*, *S. habrochaites*, *S. lycopersicum*, *S. lycopersicum* var. *cerasiforme*, *S. muricatum*, *S. neorickii*, *S. ochrantum*, *S. pennellii*, *S. peruvianum*, *S. pimpinellifolium*, and *S. pseudocapsicum* accessions to mechanical inoculation with the LE-2002 PepMV isolate.

Accession	Mean Maximum Symptom Index ^z	Mean Maximum Absorbance ^y	Absorbance Index ^x	Total Percent of Plants With Systemic Infection ^w
CM-L-56	2.5	1.97	0.66	100.0
CB-1	2.1	2.54	0.85	100.0
CA-1	3.1	2.62	0.88	100.0
CL-1	2.6	2.70	0.9	100.0
NE-1 (susceptible control)	2.5	2.71	0.91	100.0
CS-1	2.7	2.74	0.92	100.0
CC-1	2.4	2.85	0.95	100.0
CO-1	3.2	2.91	0.97	100.0
ECU-930	3.07	2.97	1.00	100.0
Fortuna-C (susceptible control)	3.25	2.99	1.00	100.0
V-L-168	3.7	3.01	1.01	100.0
<i>S. lycopersicum</i>				
var. <i>cerasiforme</i>				
AN-L-133	2.9	2.90	0.97	100.0
GLP-22	3.7	3.00	1.00	100.0
GLP-26	3.4	3.00	1.00	100.0
<i>S. muricatum</i>				
EC-46	1.79	2.33	0.78	100.0
Virú	2.63	2.91	0.97	100.0
M-1	1.29	2.91	0.97	100.0
152-A x 6-7	0.75	2.98	0.99	100.0
Lima	0.83	3.00	1.00	100.0
EC-12	2.71	3.00	1.00	100.0
11-48	1.96	3.00	1.00	100.0
37-A	2.33	3.00	1.00	100.0
7-2 x 6-21	2.75	3.03	1.01	100.0
<i>S. neorickii</i>				
PE-51	2.7	2.55	0.85	100.0
PE-50	2.7	2.76	0.92	100.0
PE-52	4	2.89	0.97	100.0
ECU-446	3.8	2.90	0.97	100.0
ECU-448	4	2.92	0.98	100.0
<i>S. ochrantum</i>				
ECU-335	0.6	0.14	0.04	100.0
<i>S. pennellii</i>				
ECU-831	2.18	1.30	0.43	100.0
ECU-840	2.35	1.35	0.45	100.0
ECU-832	3.47	2.55	0.85	100.0
ECU-836	3.53	2.55	0.85	100.0
ECU-837	3.63	2.74	0.92	100.0
LA-716	2.4	2.76	0.92	100.0
ECU-835	3.50	2.81	0.94	100.0
PE-45	3.6	2.91	0.97	100.0
PE-47	3.5	3.02	1.01	100.0
<i>S. peruvianum</i>				
ECU-947	0.5	0.53	0.18	100.0
CIAPAN-16	0.5	0.70	0.23	77.8
PI-212407	3.0	0.74	0.38	90.0
ECU-778	1.25	0.80	0.41	100.0
PI-126441	2.37	0.90	0.46	100.0
PI-251311	3.5	0.94	0.48	95.0
ECU-946	2.7	0.97	0.32	100.0
LA-1537	2.05	1.09	0.55	100.0
ECU-953	2.97	1.11	0.37	100.0
ECU-944	2.73	1.13	0.38	100.0
ECU-969	3.5	1.22	0.62	100.0
ECU-771	2.67	1.73	0.58	100.0
ECU-765	2.97	1.78	0.59	100.0
ECU-964	2.60	1.91	0.64	100.0
CIAPAN-15	0.7	1.92	0.64	83.3
ECU-804	1.97	1.99	0.67	100.0
ECU-766	2.5	2.21	0.74	100.0
ECU-798	2.63	2.23	0.75	100.0
ECU-775	2.6	2.28	0.76	100.0
ECU-777	2.5	2.32	0.78	100.0
ECU-779	2.77	2.33	0.78	100.0
ECU-783	2.63	2.36	0.79	100.0
ECU-788	2.83	2.51	0.84	100.0
ECU-784	2.53	2.52	0.84	100.0
ECU-807	2.47	2.56	0.86	100.0
ECU-952	2.97	2.60	0.87	100.0
ECU-813	3.23	2.61	0.87	100.0

continued

be infected (DAS-ELISA-positive) when absorbance was greater than three times the average absorbance of the samples from healthy control plants (≈ 0.06 absorbance units). Samples were considered to be non-infected (DAS-ELISA-negative) when the absorbance value was below this threshold. The absorbance values of the immunoenzymatic reaction were used as an indirect estimate of the viral accumulation (Ding et al., 1995, Lankes, 2003). To be able to compare the viral accumulation levels between accessions, an absorbance index (I) was calculated as the ratio of maximum mean absorbance of an accession to maximum mean absorbance of the susceptible Fortuna-C control.

Plants without systemic infection at 60 DAI (DAS-ELISA-negative in apical uninoculated leaves) were minutely analyzed at 75 DAI to confirm the absence of PepMV. For this purpose, samples of the stem were taken every 10 cm starting at the stem base and were analyzed along with samples from apical leaves and roots. Plants tested DAS-ELISA-negative in each of the samples were also analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) to control again for the absence of PepMV (López et al., 2005).

Results

Screening assay. Yellow mosaic was observed at 7 DAI in the inoculated leaves of the controls NE-1 and Fortuna-C. Leaf mosaic did not appear on the uninoculated leaves, but at 30 to 45 DAI, development of filimorphism, or curling of the leaflets, was observed (data not shown). Throughout the assay, the symptoms became more acute, particularly the filimorphism, and all susceptible control plants became systemically infected in all assays (Table 1).

All plants from the accessions of *Solanum basendopogon*, *S. canense*, *S. caripense*, *S. cheesmaniae*, *S. lycopersicum*, *S. lycopersicum* var. *cerasiforme*, *S. muricatum*, *S. neorickii*, and *S. pennellii* showed systemic infection (Table 1). The absorbance was very high in most of the accessions and the symptoms consisted of moderate to very severe mosaics and filimorphism in leaves. In some of the accessions, the incidence of the disease was so strong that up to 33.3% of the plants died. Only one (1.85%) *S. lycopersicum* accession (UPV-32) and two (22.2%) *S. pennellii* accessions (ECU-831 and ECU-840) showed an absorbance index lower than 0.5.

All plants from the accessions of *S. habrochaites* and *S. pimpinellifolium* also showed systemic infection (Table 1). Nevertheless, in these species, variable degrees of viral accumulation were observed. A total of 22 (52%) *S. habrochaites* accessions and 12 (31%) *S. pimpinellifolium* accessions gave absorbance values lower than 50% ($I < 0.5$) of the corresponding susceptible Fortuna-C control. This reduction in the absorbance did not mean that there was an absence of symptoms. Only some *S. habrochaites* accessions (ECU-764, ECU-847, and ECU-849) showed mild symptoms. The rest of accessions showed moderate or severe symptoms.

Table 1. (continued) Response of *Solanum basendopogon*, *S. canense*, *S. caripense*, *S. cheesmaniae*, *S. chilense*, *S. habrochaites*, *S. lycopersicum*, *S. lycopersicum* var. *cerasiforme*, *S. muricatum*, *S. neorickii*, *S. ochranthum*, *S. pennellii*, *S. peruvianum*, *S. pimpinellifolium*, and *S. pseudocapsicum* accessions to mechanical inoculation with the LE-2002 PepMV isolate.

Accession	Mean Maximum Symptom Index ^z	Mean Maximum Absorbance ^y	Absorbance Index ^x	Total Percent of Plants With Systemic Infection ^w
ECU-802	2.97	2.62	0.88	100.0
ECU-801	3.03	2.63	0.88	100.0
PE-20	2.28	2.65	0.89	100.0
ECU-785	3.17	2.66	0.89	100.0
ECU-809	3.20	2.67	0.89	100.0
ECU-793	2.77	2.67	0.89	100.0
ECU-803	2.67	2.68	0.90	100.0
CIAPAN-17	1.4	2.69	0.9	100.0
ECU-791	2.77	2.71	0.91	100.0
PE-18	1.5	2.77	0.93	100.0
ECU-790	2.90	2.78	0.93	100.0
PI-126944	2.4	2.79	0.93	100.0
PE-19	2.53	2.84	0.95	100.0
PI-143679	1.0	2.87	0.96	100.0
LA-1708	2.7	2.88	0.96	100.0
ECU-811	3.53	2.88	0.96	100.0
PI-126935	1.4	2.93	0.98	100.0
ECU-821	3.63	2.97	0.99	100.0
<i>S. pimpinellifolium</i>				
LA-1587	3.0	0.35	0.12	100.0
ECU-879	2.17	0.54	0.18	100.0
ECU-951	3.57	0.57	0.19	100.0
ECU-877	2.40	0.65	0.22	100.0
ECU-878	2.60	0.76	0.25	100.0
ECU-853	1.92	0.76	0.25	100.0
ECU-893	3.5	0.94	0.31	100.0
ECU-873	1.9	0.97	0.32	100.0
ECU-701	1.57	1.08	1.09	100.0
ECU-966	2.47	1.08	0.36	100.0
ECU-874	2.83	1.12	0.38	100.0
ECU-875	2.47	1.18	0.39	100.0
ECU-749	2.97	1.18	0.40	100.0
ECU-742	3.07	1.54	0.52	100.0
ECU-745	2.83	1.56	0.52	100.0
ECU-693	1.53	1.62	0.54	100.0
ECU-727	3.13	1.69	0.57	100.0
ECU-711	3.37	1.69	0.57	100.0
ECU-739	2.57	1.76	0.59	100.0
ECU-748	3.17	2.18	0.73	100.0
ECU-738	2.70	2.28	0.76	100.0
ECU-867	3.2	2.29	0.77	100.0
ECU-744	2.60	2.32	0.78	100.0
ECU-750	2.77	2.48	0.83	100.0
ECU-707	3.30	2.52	0.84	100.0
ECU-712	3.23	2.57	0.86	100.0
ECU-713	3.63	2.60	0.87	100.0
ECU-692	3.17	2.64	0.88	100.0
ECU-710	3.27	2.68	0.90	100.0
ECU-736	3.07	2.70	0.90	100.0
ECU-709	2.87	2.74	0.92	100.0
ECU-702	3.27	2.80	0.94	100.0
ECU-604	2.4	2.93	0.98	100.0
ECU-603	3.2	2.95	0.99	100.0
ECU-579	3.6	2.97	0.99	100.0
ECU-927	3.23	2.97	1.00	100.0
ECU-928	3.10	2.99	1.00	100.0
ECU-660	2.8	3.01	1.00	100.0
<i>S. pseudocapsicum</i>				
AN-CA-214	0.0	-	-	0.0

^zMean value of the maximum symptom index of the plants of each accession (0, absence of symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms; 4, very severe symptoms or dead plant).

^yMean value of the maximum DAS-ELISA absorbance of the plants with systemic infection for each accession.

^xRatio of mean maximum DAS-ELISA absorbance of each accession to mean maximum absorbance of Fortuna-C.

^wPercentage of plants that showed systemic infection at least once during the duration of the assay.

^vOf the 54 accessions tested from *S. lycopersicum*, only 12 are included because the results of the remaining 42 accessions were consistent with those observed for the 12 reported accessions.

In bold font, accessions showing some DAS-ELISA-negative plant.

Only in some *S. chilense* and *S. peruvianum* accessions were detected plants without systemic infection (Table 1). Seven (53.8%) *S. chilense* accessions (ECU-527, LA-458, LA-470, LA-1963, LA-1968, LA-1971, and LA-2762) showed variable percentages of presumably resistant plants without systemic infection or symptoms. The same was true in four (8.9%) *S. peruvianum* accessions (CIAPAN-15, CIAPAN-16, PI-212407, and PI-251311). The only accession of *S. ochranthum* (ECU-335) showed systemic infection in all plants at 30 DAI, although the symptoms were mild (symptom index = 0.6). In addition, only 13.3% of the plants were systemically infected at 60 DAI and the levels of absorbance were low with mean absorbance values of 0.134 at 30 DAI and 0.030 at 60 DAI (data not shown). Finally, PepMV was not detected in any of the plants of accession AN-CA-214 of *S. pseudocapsicum* (Table 1). No symptoms at all were observed in the inoculated leaves or in the other leaves developed after inoculation.

Analysis of plants without systemic infection. Plants without systemic infection at 60 DAI were minutely analyzed at 75 DAI to confirm the absence of PepMV. The samples of stem, apical leaves and roots of all Fortuna-C plants showed infection by PepMV (Table 2). All *S. chilense* and *S. peruvianum* plants without systemic infection in the previous assay, except three plants of LA-470, showed infection by PepMV at least in one of the analyzed samples (apical leaves, stem, and root) at 75 DAI (Table 2). *S. chilense* and *S. peruvianum*-infected plants showed a basipetal distribution of the virus with the maximum level of viral accumulation in the basal part of the plant stems. None of the samples of *S. ochranthum* and *S. pseudocapsicum* tested were DAS-ELISA-positive (Table 2). DAS-ELISA-negative plants of accessions LA-470, ECU-335, and AN-CA-214 were RT-PCR analyzed to confirm absence of PepMV. The presence of the virus was detected in all plants of LA-470 and ECU-335 identified as DAS-ELISA-negative, but not in AN-CA-214 plants of *S. pseudocapsicum* (Fig. 1).

Discussion

The results obtained suggest that *S. chilense* is the most promising *Solanum* species in the search for sources of resistance to PepMV. This species has been also used for introgressing resistance to the fungus *Leveillula taurica* (Stamova and Yordanov, 1987) and to other viruses such as *Tomato yellow leaf curl virus* (TYLCV) (Zamir et al., 1994), *Cucumber mosaic virus* (CMV) (Stamova and Chetelat, 2000), and *Tomato spotted wilt virus* (TSWV) (Canady et al., 2001) in the cultivated tomato. Otherwise, some accessions of *S. habrochaites*, *S. peruvianum*, and *S. pimpinellifolium* showed significant reduction in absorbance after being inoculated with PepMV as described previously (Soler-Aleixandre et al., 2005a). It would be necessary to carry out

field assays to determine if this reduction is sufficient to attenuate the damage of spotted fruits and abortion of flowers incited by PepMV.

Higher levels of resistance were detected in accession ECU-335 of *S. ochranthum*. Although 100% of ECU-335 plants showed systemic infection by PepMV at first, the absorbance was low. This accumulation decreased gradually with time. The drastic disappearance of symptoms could be the result of this reduction of accumulation of the virus in the plant organs. In this sense, all inoculated plants became infected initially and afterward, all of them showed no infection in the apical leaves. The positive absorbance values detected in some plants at 30 DAI could be because the samples were taken from inoculated leaves, in which PepMV had been deposited during inoculation. This is highly improbable because 30 d had elapsed between the last inoculation and the first sample-taking. During this time, four new leaves developed, which were precisely the ones that were sampled. DAS-ELISA analysis indicates that virus had disappeared in the inoculated plants of ECU-335. RT-PCR analysis of these plants suggests that the virus is randomly distributed in some parts of the plant at low concentrations.

The accession AN-CA-214 of *S. pseudocapsicum* showed total resistance to mechanical inoculation with PepMV. No symptoms associated with PepMV infection were observed. Likewise, none of the inoculated leaves showed signs of developing local lesions that could suggest a hypersensitive response. This allows the ruling out of this mechanism as being responsible for resistance. RT-PCR results confirm that plants of this accession remain virus-free.

The stem analysis of *S. chilense* and *S. peruvianum* plants, selected as resistant at 60 DAI, clearly demonstrates the existence of an asymmetric distribution of the virus in the stem. This type of distribution had already been detected in tomato plants infected by PepMV in the region of Murcia (Soler-Aleixandre et al., 2005b). The lowest absorbance values were obtained in the youngest parts of the plants. In some plants, the presence of the virus in the apical leaves was not detected. This distribution could explain that some plants of *S. chilense* and *S. peruvianum* were DAS-ELISA-negative in apical leaves at 60 DAI and DAS-ELISA-positive in the stem at 75 DAI. These results mean that in future searches for sources of resistance, the absence of the virus in plants classified as resistant must be checked.

Having materials with a high level of resistance to PepMV at one's disposal is a great achievement. Nevertheless, *S. pseudocapsicum* cannot be crossed with cultivated tomato plants. In this case, somatic hybridization or other techniques could allow the transfer of the identified resistance to commercial tomato varieties. The identification of reduced viral accumulation in some plants of the accession LA-470 of *S. chilense* could

Table 2. Serologic analysis by DAS-ELISA of plants classified as resistant in the screening assay.

Accession	No. of Selected/Infected Plants ^z	Mean Absorbance				
		A ^y	S30 ^x	S20 ^w	S10 ^v	R ^u
<i>S. chilense</i>						
ECU-527	4/4	0,21	0,51	0,85	1,45	1,50
LA-458	2/2	0,45	1,60	2,31	2,88	2,91
LA-470	7/4	0,01	0,02	0,50	0,08	0,11
LA-1963	2/2	0,65	1,14	1,74	1,80	2,31
LA-1968	3/3	0,91	1,37	2,01	2,21	2,38
LA-1971	2/2	0,23	1,35	1,84	2,45	2,18
LA-2762	1/1	0,36	1,46	2,11	2,75	2,62
<i>S. peruvianum</i>						
CIAPAN-15	3/3	0,42	0,98	1,11	1,54	1,84
CIAPAN-16	4/4	0,12	0,78	0,95	0,98	1,45
PI-212407	3/3	0,30	0,12	0,85	1,31	1,70
PI-251311	2/2	0,11	0,24	0,27	0,78	1,11
<i>S. ochranthum</i>						
ECU-335	13/0	0,01	0,00	0,01	0,01	0,01
<i>S. pseudocapsicum</i>						
AN-CA-214	15/0	0,01	0,01	0,02	0,01	0,01
<i>S. lycopersicum</i>						
Fortuna-C (susceptible control)	12/12	1,68	2,17	2,84	3,06	2,92

^zNumber of plants selected as resistant in screening assay/number of plants infected in at least one of the analyzed zones.

^yApical leaf sample.

^xStem sample 30 cm from stem base.

^wStem sample 20 cm from stem base.

^vStem sample 10 cm from stem base.

^uRoot sample.

In bold font, accessions showing some DAS-ELISA-negative plant.

Fig. 1. Reverse transcriptase-polymerase chain reaction detection of PepMV in plants classified as resistant from accessions LA-470 (*S. chilense*), ECU-335 (*S. ochranthum*), and AN-CA-214 (*S. pseudocapsicum*) and Fortuna-C (*S. lycopersicum*) as susceptible control. The templates used were apical leaves (lanes 1, 4, 7, and 10), stem (lanes 2, 5, 8, and 11) and stem base (lanes 3, 6, 9, and 12). C⁺ and C⁻: positive and negative NE-1 controls from infected and noninoculated plants, respectively. M, molecular size marker.

allow the development of varieties resistant to PepMV because hybrids with tomato can be obtained. However, search for new sources of resistance in this species must be thoroughly advanced, because this could be useful for the short term in the development of varieties of tomato resistant to PepMV.

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