Sources of Resistance to Pepino mosaic virus (PepMV) in Tomato
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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. pennelli Correll, S. cheesemaniae (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. basendapotog Bitter, S. canense Rydb., S. caripense Humb. & Bonpl. & 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 most important horticultural species in terms of cultivated surface and production value 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, Almeria, 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 (Farray et al., 2004).

Currently, in Spain, the disease incited by PepMV affects plantations in the provinces of Alicante, Murcia, Almeria, 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 reluctant to buy Spanish tomatoes because PepMV has been occasionally detected in Spanish imported fruits (Zitikaitė et al., 2004).

Typical leaf symptoms associated with PepMV infection are yellow mosaics, puffiness, and leaf filimorphism. Dark green spots with brighter 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 withering 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 filimorphism 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 basendapotog (one accession), S. canense (one), S. caripense (5), S. cheesemaniae (2), S. chilense (12), S. habrochaites (42), S. lycopersicum (54), S. lycopersicum var. cerasiforme (3), S. muricatum (9), S. neorickii (5), S. ochranthum (one), S. pennelli (9), S. peruvianum (45), S. pimpinellifolium (38),
Table 1. Response of *Solanum basendopogon*, *S. canense*, *S. caripense*, *S. cheesmaniae*, *S. chilense*, *S. habrochaites*, *S. lycopersicum*, *S. lycopersicum* var. *ceresiforme*, *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 | Mean Maximum Absorbance | Total Percent of Plants With Systemic Infection |
|-----------|-----------------------------|-------------------------|-----------------------------------------------|
| **S. basendopogon** |                             |                         |                                               |
| BIRM/S-1975 | 3.00                       | 3.02                    | 1.01                                          | 100.0 |
| **S. canense** |                             |                         |                                               |
| BIRM/S-1815 | 3.17                       | 3.06                    | 1.02                                          | 100.0 |
| **S. caripense** |                             |                         |                                               |
| PI-243342  | 3.33                       | 2.45                    | 0.82                                          | 100.0 |
| 261        | 2.00                       | 2.49                    | 0.83                                          | 100.0 |
| ECU-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 |
| **S. cheesmaniae** |                             |                         |                                               |
| GLP-36     | 3.8                        | 2.96                    | 0.99                                          | 100.0 |
| GLP-3      | 2.4                        | 2.99                    | 1.00                                          | 100.0 |
| **S. chilense** |                             |                         |                                               |
| 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 |
| **S. habrochaites** |                             |                         |                                               |
| ECU-923    | 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** |                             |                         |                                               |
| LA-2774    | 2.6                        | 2.71                    | 0.91                                          | 92.3  |
| **S. lycopersicum** |                             |                         |                                               |
| GLP-36     | 3.6                        | 2.75                    | 0.92                                          | 78.6  |
| 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                       | 0.92                    | 0.27                                          | 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                    | 0.39                                          | 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    | 1.0                        | 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 |
| **S. lycopersicum** |                             |                         |                                               |
| UPV-32     | 3.5                        | 1.48                    | 0.49                                          | 100.0 |

continued
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 | Mean Maximum Absorbance | Total Percent of Plants With Systemic Infection |
|-------------------------|----------------------------|-------------------------|-----------------------------------------------|
| 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 |
| S. lycopersicum var. cerasiforme |            |                         |                                               |       |
| 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                        | 3.0                   | 1.00                                          | 100.0 |
| S. muricatum var. cerasiforme |                     |                         |                                               |       |
| ECU-46                  | 1.79                       | 2.33                    | 0.78                                          | 100.0 |
| Viri                    | 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 × 6–21              | 2.75                       | 3.03                    | 1.01                                          | 100.0 |
| S. neorickii            |                            |                         |                                               |       |
| 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 |
| S. ochranthum var. cerasiforme | 0.6                  | 0.14                    | 0.04                                          | 100.0 |
| S. pennellii            |                            |                         |                                               |       |
| 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 |
| S. peruvianum           |                            |                         |                                               |       |
| 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-973                 | 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 |

Note: The numbers in Table 1 represent the mean maximum symptom index, mean maximum absorbance, and total percent of plants with systemic infection for each accession. 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. The rest of accessions showed moderate or severe symptoms.
Only in some S. chilense and S. peruvianum accessions were detected plants without systemic infection. 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 accessions 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 Leivelula 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

Table 1. (continued) Response of Solanum brevipes, S. canense, S. caripense, S. cheesmanii, S. chilense, S. habrochaites, S. lycopersicum, S. lycopersicum var. cerasiforme, S. muricatum, S. neoricii, S. ochranthum, S. pennelli, S. peruvianum, S. pimpinellifolium, and S. pseudocapsicum accessions to mechanical inoculation with the LE-2002 PepMV isolate.

| Accession     | Mean Maximum Symptom Index | Mean Maximum Absorbance | Mean Absorbance Index | Total Percent of Plants With Systemic Infection |
|---------------|----------------------------|-------------------------|-----------------------|------------------------------------------------|
| AN-CA-214     | 0.0                       | -                       | -                     | 0.0                                             |

*Solanum pimpinellifolium*

| Accession | Mean Maximum Symptom Index | Mean Maximum Absorbance | Mean Absorbance Index | Total Percent of Plants With Systemic Infection |
|-----------|---------------------------|-------------------------|-----------------------|------------------------------------------------|
| 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                    | 0.39                  | 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                    | 0.58                  | 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                    | 0.86                  | 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-600   | 2.8                       | 3.01                    | 1.00                  | 100.0                                          |

*Mean 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).

*Ratio of mean maximum DAS-ELISA absorbance of the plants with systemic infection for each accession.

*Mean value of the maximum DAS-ELISA absorbance of each accession to mean maximum absorbance of Fortuna-C.

*Percentage of plants that showed systemic infection at least once during the duration of the assay.

Of 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.
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 were obtained in some parts of the plant at low concentrations.

The accession AN-CA-214 of *S. pseudo-

Capsicum* 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-Alexandre et al., 2009b). 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. pseudcapsicum* 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 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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