Exploiting Partial Resistance to *Tomato yellow leaf curl virus* Derived from *Solanum pimpinellifolium* UPV16991

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ABSTRACT

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Tomato yellow leaf curl disease (TYLCD) causes great economic losses in tomato crops worldwide. Despite efforts undertaken by different research groups, there are no immune commercial plant materials available. Recently, our group reported partial resistance to TYLCD in line L102, derived from *Solanum pimpinellifolium* UPV16991. Resistance in this line is monogenic, with partial recessiveness and incomplete penetrance. Even though the resistance gene in L102 is not dominant, we also proved that levels of resistance were high in hybrids with different tomato lines. The objective of this work was to evaluate the level of resistance in plants which combined UPV16991-derived resistance and the *Ty-1* gene, both in heterozygosis. Most of the hybrids between *S. pimpinellifolium*- and *S. chilense*-derived resistant lines exhibited milder symptoms than heterozygotes for either *S. pimpinellifolium*- or *S. chilense*-derived resistance. In some of the hybrids, viral accumulation was also lower than in respective heterozygotes. Our results support the utility of resistance derived from UPV16991 combined with the *Ty-1* gene in increasing levels of resistance to TYLCD in tomato hybrids. This is the most practical approach to exploiting resistance derived from UPV16991, because it allows the development of hybrids without the need of fixing the resistance gene in both parents.

Tomato yellow leaf curl disease (TYLCD) produces great economic losses in tomato (*Solanum lycopersicum* L., syn. *Lycopersicon esculentum* Mill.) crops in many temperate areas (13,17). TYLCD is caused by a complex of viruses belonging to the genus *Begomovirus*, family *Geminiviridae* (3). Among viral species causing TYLCD, *Tomato yellow leaf curl virus* (TYLCV) has been detected infecting tomato worldwide.

Variable levels of resistance to TYLCD have been reported in several wild tomato relatives, such as *S. peruvianum* L. (syn. *L. peruvianum* (L.) Mill.), *S. chilense* (Dunal) Reiche (syn. *L. chilense* Dunal), *S. habrochaites* S. Knapp & D.M. Spooner (syn. *L. hirsutum* Humb. & Bonpl.), and *S. pimpinellifolium* L. (syn. *L. pimpinellifolium* (L.) Mill.) (8). Some of these resistance sources have been used to develop commercial cultivars, mainly several accessions of *S. chilense* and *S. peruvianum* (8). *S. habrochaites*-derived resistance has also been exploited, mostly in Asia (10) and Guatemala (11). In any case, no immune commercial materials have been made available to date; therefore, it is of

interest to make use of all existing resistance sources that show potential.

S. pimpinellifolium accessions have not been an important source in breeding programs for resistance to TYLCD (9). Recently, our research group derived an F₆ line from *S. pimpinellifolium* UPV16991 and characterized resistance in this line (16). It was determined that resistance is monogenic with partial recessiveness and incomplete penetrance. Considering these results, we proposed the use of this resistance in homozygosis for the development of vigorous hybrids or in combination with resistance genes from other sources. If successful, the second approach would be of greater interest, given that it would allow the development of hybrids, thereby avoiding the need to fix the resistance gene in both parents.

Several attempts to increase resistance to TYLCD have been made based on the accumulation of resistance genes from different sources. Kasrawi and Mansour (7) developed crosses from lines with resistance derived from *S. pimpinellifolium*, *S. peruvianum*, and *S. habrochaites*. Their results revealed that the level of resistance in progenies of interspecific crosses exceeded that of both parents. It has also been reported that the levels of resistance, not only to TYLCV but also to other begomoviruses infecting tomato in Guatemala, can be increased by combining *S. peruvianum*-derived resistance with resistance derived from *S. pimpinellifolium* and *S. habrochaites* (11). Similar results were

obtained in a study which included crosses between advanced breeding lines with variable levels of resistance derived from *S. peruvianum*, *S. pimpinellifolium*, and *S. chilense* (23). In all cases, F₁ combinations which involved highly resistant parents derived from different wild species showed higher resistance than that of any of the parents. Furthermore, even descendants of crosses involving one highly resistant line and another one with intermediate levels of resistance turned out to be highly resistant. The best results were obtained by combining *S. chilense*- and *S. pimpinellifolium*-derived resistance.

Consequently, even though the line derived from UPV16991 shows only partial resistance, it would be of interest to combine it with genes from other sources that confer higher levels of resistance. Among the reported TYLCV resistance sources, *S. chilense* LA1969 shows the highest levels (2,18,24). The resistance mechanism has been characterized in plant materials derived from LA1969 (12). Resistance in this accession is controlled by one major incompletely dominant gene, *Ty-1*, with at least two modifier genes (25). The *Ty-1* gene has been mapped on chromosome 6 (25). A cleaved amplified polymorphic sequence (CAPS) marker tightly linked to this gene has also been reported (15), which is essential in breeding programs aimed at the introgression of multiple resistance genes in the same plant material.

We have developed different crosses involving lines with *S. pimpinellifolium* UPV16991- and *S. chilense* LA1969-derived resistance. The objective of the work reported here was to evaluate the level of resistance in these plant materials, which included hybrids combining resistance from both sources.

MATERIALS AND METHODS

Plant material. The resistant plant materials used were seven lines with resistance derived from either *S. chilense* LA1969 or *S. pimpinellifolium* UPV16991 (Table 1). The two *S. chilense*-derived resistant lines were the BC₇S₁ generations from an initial NE-1 × LA3473 cross; NE-1 is a susceptible breeding line developed at the Institute for the Conservation and Improvement of Agrodiversity (COMAV, Spain) and LA3473 is homozygous for the *Ty-1* allele, and was kindly provided by the Tomato Genetics Resource Center (TGRC, United States). Of the BC₇S₁ plants, those

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that were homozygous for *Ty-1* were selected. Partial resistance to *Tomato yellow leaf curl Sardinia virus* (TYLCSV) was first described by our group in *S. pimpinellifolium* UPV16991 (19). This accession was collected in Piura (Peru) in an expedition of the COMAV. A breeding program for resistance to TYLCSV and TYLCV was then initiated. The five *S. pimpinellifolium*-derived resistant lines had been developed previously from an initial cross between the susceptible *S. lycopersicum* NE-1 and *S. pimpinellifolium* UPV16991. The F₁ generation was selfed, and descendants were evaluated for resistance to TYLCV and TYLCSV. The breeding program was continued with three plants (L102, L54, and L48) selected from the F₂

generation. After five to six selfing generations, several advanced lines were obtained from each of these plants. Five of these lines were used in this assay. The susceptible parents used were the old Fortuna C variety (hereafter FC) and three susceptible breeding lines developed at the COMAV: NE-1, P-73, and V-5-7. The crosses developed include *Ty-1* lines × susceptible, UPV16991 lines × susceptible (or reciprocal), *Ty-1* lines × UPV16991 lines, and UPV16991 lines × UPV16991 lines. The crosses are listed in Table 1. Eight plants of each of these crosses were included in the assay, as well as eight plants of each of the resistant lines and the susceptible control.

Inoculation techniques. Whitefly-mediated inoculation was used. The Spanish TYLCV isolate TYLCV-Mld[ES:72:97] (accession no. AF071228) was maintained in FC tomato plants by whitefly transmission. Infected plants and viruliferous whiteflies remained in muslin-covered cages inside a growth chamber. The conditions were: 25°C temperature, 60 to 65% and 95 to 99% relative humidity (day and night, respectively), 34 $\mu\text{Em}^{-2} \text{ s}^{-1}$ irradiance, and a 16- and 8-h (light and darkness, respectively) photoperiod. Whiteflies were biotype Q (supplied by F. Beitia, Instituto Valenciano de Investigaciones Agrarias, IVIA, Valencia, Spain). Plants to be inoculated were grown in pots in an insect-proof greenhouse with controlled light and temperature. Eight plants per genotype were caged with at least 20 whiteflies per plant at the three-true-leaf stage for 7 days. Plants were shaken daily to ensure that the distribution of the whiteflies was uniform. After the inoculation period, plants were sprayed with the insecticide imidacloprid and transferred to a new insect-proof cage for 2 days to avoid the spreading of whiteflies. Subsequently, plants were sprayed again with the insecticide, then transplanted to bigger pots and transferred to an insect-proof greenhouse, where they remained until the end of the assay. Plants were grown in 12-liter pots with coconut fibre and fertirrigated with the usual doses and regularity for tomato in the cultivation area (14).

Experimental design. Plants for inoculation were divided into eight trays. One plant of each of the genotypes assayed was included in each of the trays. Plants were distributed randomly in each tray. After inoculation, plants were transplanted to bigger pots in a greenhouse to the end of the assay. The greenhouse was divided into two plots. Plants of each genotype were grouped in blocks of two plants and distributed randomly in the different plots. Conditions in the greenhouse were 25 and 18°C temperature (day and night, respectively), 80% relative humidity, and 16- and 8-h (light and darkness, respectively) photoperiod.

Disease assessment. Symptoms and viral accumulation were assessed at 15, 25,

35, 45, and 60 days post-inoculation (dpi). Symptoms were evaluated following the scale developed by Friedmann et al. (4), where 0 = no visible symptoms, inoculated plants show same growth and development as uninoculated plants; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling, and cupping, with some reduction in size, yet plants continue to develop; and 4 = very severe plant stunting and yellowing, and pronounced cupping and curling; plants cease to grow). Intermediate scores (0.5, 1.5, 2.5, and 3.5) were also used to obtain accurate evaluations. Viral accumulation was assessed by molecular hybridization. DNA was extracted following the procedure described by Crespi et al. (1) with some modifications. Approximately 150 mg of tissue from the upper canopy of the plant were taken at each date. The frozen tissue was crushed in 500 μl of extraction buffer (100 mM Tris-HCl [pH 8], 50 mM EDTA, 500 mM NaCl, 10 mM 2- β -mercaptoethanol, and 1% sodium dodecyl sulfate) and incubated at 65°C for 5 min. After addition of 150 μl of 5 M potassium acetate, samples were incubated on ice for 10 min. Later samples were centrifuged at 16,110 $\times g$ and the supernatant was recovered. Precipitation was carried out with isopropanol by centrifugation at 16,110 $\times g$ for 5 min, and DNA then was resuspended in 77 μl of distilled water. Each sample (1 μl) and a 10-fold dilution of the sample were denatured with 30 mM NaOH and 1 mM EDTA for 30 min and then blotted onto positively charged nylon membranes for hybridization. DNA was fixed by UV crosslinking. The probe used (kindly supplied by E. R. Bejarano, Universidad de Málaga, Spain) contained the intergenic region of the TYLCV-Mld[ES:72:97] isolate and was digoxigenin labeled by polymerase chain reaction. Hybridization was carried out following "The DIG System User's Guide for Filter Hybridization" (Roche Molecular Biochemicals). Membranes were prehybridized in standard hybridization buffer plus 50% deionized formamide for at least 1 h. Subsequent hybridization was done at 42°C overnight in fresh prehybridization solution containing denatured probe at 20 ng/ml. Washing steps and incubation with antibody were developed according to manufacturer's instructions. Detection was carried out with CSPD and direct exposure to a CCD camera for approximately 30 min (Intelligent Dark Box-II; Fujifilm, Tokyo).

Viral single-stranded DNA was quantified by comparison with a standard curve of TYLCV dotted on the same membrane (ranging from 25 ng to 1 μg).

Extracted plant DNA was quantified by fluorimetry (Hoefer DyNA Quant 200 fluorimeter, according to the manufacturer's instructions) in order to relate virus

Table 1. Plant materials analyzed, including the crosses developed

Susceptible <i>Solanum lycopersicum</i> cultivar Fortuna C (FC)
<i>Ty-1</i> lines (<i>S. chilense</i> -derived) ^a
Ch-1
Ch-2
UPV16991 lines (<i>S. pimpinellifolium</i> -derived) ^b
L48
L54-1
L54-2
L102-1
L102-3
<i>Ty-1</i> line × UPV16991 line crosses
Ch-1 × L102-1
Ch-1 × L102-2
Ch-1 × L102-3
Ch-1 × L54-1
Ch-1 × L54-2
Ch-2 × L102-2
Ch-2 × L54-1
Ch-2 × L54-2
Ch-2 × L102-3
UPV16991 line × UPV16991 line crosses
L48 × L54-1
L102-1 × L48
L102-1 × L54-1
Susceptible × <i>Ty-1</i> line crosses
FC × Ch-1
FC × Ch-2
UPV16991 line × susceptible (or reciprocal) crosses
L102-1 × NE-1 ^c
L102-1 × V-5-7 ^c
L102-1 × P-73 ^c
L102-1 × FC
L102-3 × NE-1
V-5-7 × L102-3
P-73 × L102-3
FC × L102-3
L54-1 × NE-1
L54-1 × V-5-7
P-73 × L54-1
FC × L54-1
L54-2 × NE-1
L54-2 × V-5-7
L54-2 × P-73
FC × L54-2

^a Lines homozygous for the *Ty-1* gene, selected among plants of the BC₃S₁ generation from the cross NE-1 × LA3473.

^b F₅ to F₆ generations from the initial NE-1 × *S. pimpinellifolium* UPV16991 cross.

^c Susceptible breeding lines developed at the Institute for the Conservation and Improvement of Agrobiodiversity (COMAV).

concentration to plant DNA present in each sample.

RESULTS

Parental lines. Plants of the susceptible control showed the first symptoms at 15 dpi. At 60 dpi, 100% of the plants exhibited severe symptoms (Fig. 1). For lines with *S. chilense*-derived resistance, only 40% of the plants showed some symptoms at some sampling dates. These symptoms were never scored over 1, and in almost all cases the plants recovered completely by 60 dpi (Fig. 1A). Variable responses were observed for *S. pimpinellifolium*-derived resistant lines (Fig. 1B). Generally, plants descendant from the L102 and L48-1 lines either remained asymptomatic or had slight symptoms. Only plants of one of the lines descendant from L102 displayed symptoms scored over 1.5 at 35 dpi, but these recovered by the end of the assay. One plant from a line descendant from L48

had symptoms scored higher than 2 from 35 dpi until the end of the assay. L54-derived lines behaved differently. Plants descendant from L54-2 remained symptomless. However, plants derived from L54-1 had symptom severity scores greater than 2 at 45 dpi. Symptoms in lines descendant from L54-1 were more severe later, although they were systematically lower than in the susceptible control FC and in no case did plants cease to grow.

Viral DNA was detected in all plants assayed (Fig. 1). Viral accumulation followed a similar pattern in most of the resistant genotypes studied, with maximum accumulation occurring at 25 dpi. In the susceptible FC control, the maximum viral accumulation was detected at 35 dpi.

Both *S. chilense*-derived lines accumulated similar, low amounts of virus. Viral accumulation in lines derived from *S. pimpinellifolium* was variable depending on the genotype. Lines descendant from

L102 and L48 showed similar levels of viral accumulation, higher than those found in *S. chilense*-derived lines. Differences were found in lines descendant from L54. L54-2 registered the lowest viral accumulation among these lines, not only those derived from *S. pimpinellifolium* but also those derived from *S. chilense*. In addition, viral accumulation was delayed in L54-2, because the virus was first detected at 35 dpi. However, L54-1, which showed the most severe symptoms, accumulated a significantly higher amount of virus at 25 dpi although, from 35 dpi, viral accumulation was similar to that detected in the resistant lines.

Ty-1 line × susceptible crosses. Descendants from both crosses involving resistant *S. chilense*-derived lines with FC responded in a similar way. Approximately 30% of the plants remained symptomless throughout the entire assay. Some of the other plants started showing symptoms at

Fig. 1. Average symptom score (left) and viral accumulation (right) in the resistant lines derived from **A**, *Solanum chilense* and **B**, *S. pimpinellifolium* and the susceptible control (FC), after whitefly-mediated inoculation with *Tomato yellow leaf curl virus*. Symptom score was evaluated using a scale from 0 (symptomless) to 4 (severe symptoms). Viral accumulation is expressed in nanograms of viral DNA per micrograms of total DNA extracted. Error bars represent standard error.

15 dpi (Fig. 2). At 35 dpi, the most severe symptoms were observed, ranging from 1 to 3 on the scale used. From that date on, plants recovered and symptoms decreased. At the end of the assay, 70 and 60% of the plants from crosses with Ch-1 and Ch-2, respectively, showed no symptoms, and only 10% of plants showed symptoms scored over 2. For both genotypes and for all sampling dates, the average symptom score was considerably lower than the score for the susceptible parent and the midparent value, and higher than the score for the resistant parent.

The timing of viral accumulation in heterozygous plants involving *S. chilense*-derived lines followed the aforementioned prevalent tendency (Fig. 2). Viral accumulation in the FC × Ch-1 and FC × Ch-2 crosses was similar to the accumulation found in the resistant parents Ch-1 and Ch-2, and lower than the midparent value.

UPV16991 line × susceptible crosses. Responses in crosses between susceptible genotypes and lines with *S. pimpinellifolium*-derived resistance depended on both the susceptible and resistant parents, as well as the interaction between them. The highest symptoms were observed at 60 dpi. The percentage of symptomatic plants at 60 dpi varied depending on the hybrid considered, ranging from 40 to 100% (*data not shown*). In all hybrids, some of the plants showed severe symptoms, with percentages that were also variable. The only tendency to be highlighted was the fact that, in crosses with FC, the percentage of plants displaying severe symptoms was always higher with respect to mild symptoms (<2). The average symptom score in the hybrids with L102-1 and L102-3 was always similar or higher than in the resistant parent (Fig. 3). In the hybrids with L54-2, symptoms were higher than in the resistant parent. Otherwise, symptoms

were similar or lower in the hybrids than the midparent value. The midparent value was calculated based on symptoms in FC, which was the susceptible control included in the assay. Our experience in previous work (*unpublished data*) showed that symptom development in NE-1, V-5-7, and P-73 is comparable with the symptoms in FC. Unexpectedly, the best response, compared with the response in the parent line, was obtained in three of the hybrids involving the parent line which showed the lowest levels of resistance (L54-1).

Viral accumulation in crosses involving L102-1 was similar to or higher than viral accumulation in the resistant parent (Fig. 3). Hybrids with L102-3 accumulated amounts of virus similar to the resistant parent. In a way similar to symptom expression, viral accumulation in hybrids with L54-1 was significantly lower than that found in the *S. pimpinellifolium*-derived parent. However, in crosses with L54-2, viral accumulation was higher than accumulation in the resistant parent.

UPV16991 line × UPV16991 line. At 60 dpi, the average symptom score was similar in all the crosses involving two lines with resistance derived from *S. pimpinellifolium* (Fig. 4) but the response was different in the three hybrids. Seven of the eight plants analyzed from the L48-1 × L54-1 cross showed symptoms, but they showed mild symptoms. However, only five and four of the plants from L102-1 × L48-1 and L102-1 × L54-1 crosses, respectively, showed more severe symptoms (*data not shown*). For all the hybrids, there was a delay in time of symptom development with respect to both parents; the first symptoms in plants of the three hybrids appeared at 35 dpi (*data not shown*). In both crosses with L54-1, the hybrids showed a response similar to that of the other, more resistant parent. Plants from

the hybrid L102-1 × L48-1 showed a response similar to that of both parents.

Response of the hybrids with respect to viral accumulation was comparable with that described for symptom development (Fig. 4).

Ty-1 line × UPV16991 line crosses. The average symptom score for all hybrids between *S. chilense*-derived and *S. pimpinellifolium*-derived resistant lines was under 1.5 for all sampling dates (Fig. 5). The first symptoms appeared at 25 or 35 dpi. At the end of the assay, the average was under 1 in all cases. Individual scores were also no higher than 1.5 for six of the hybrids. The exceptions were hybrids involving L54-1 and hybrid Ch-2 × L102-3, in which between 10 and 20% of the plants, respectively, were scored 2 or 2.5 at some date. The average symptom score in hybrids was lower than the score for plants heterozygous for the resistance derived from *S. pimpinellifolium*. It also was lower than the symptom score in *S. pimpinellifolium* homozygotes, with the only exception being crosses with L54-2, which did not exceed the resistance found in the *S. pimpinellifolium*-derived parent. In plants of the hybrids *S. chilense* × *S. pimpinellifolium*, there was a delay in time of symptom development compared with response in Ch-1 × FC and Ch-2 × FC crosses. In crosses Ch-1 × L102-1, Ch-1 × L102-2, Ch-1 × L102-3, and Ch-2 × L102-2, symptom score in the hybrids was significantly lower than in those heterozygous for *Ty-1* until 60 dpi. At 60 dpi, only the symptom score in Ch-2 × L102-2 was significantly lower than in heterozygotes for *Ty-1*. Plants of the Ch-2 × L102-2 hybrid, which remained symptomless throughout the assay, were even better than both lines homozygous for *Ty-1*.

Viral accumulation peaked for most of the crosses at 25 dpi (Fig. 5). Levels of

Fig. 2. Average symptom score (left) and viral accumulation (right) in *Ty-1* line × susceptible crosses after whitefly-mediated inoculation with *Tomato yellow leaf curl virus*. *Ty-1* lines. Symptom score was evaluated using a scale from 0 (symptomless) to 4 (severe symptoms). Viral accumulation is expressed in nanograms of viral DNA per micrograms of total DNA extracted. Error bars represent standard error.

virus accumulated in both crosses with L54-1 were high, similar to the amounts detected in L54-1. Viral accumulation also was high in crosses between Ch-1 and L102-2 and L102-3. Accumulation in the rest of the hybrids was significantly lower. In most of the hybrids, viral accumulation was similar to or lower than viral accumulation in heterozygote plants for *S.*

pimpinellifolium-derived resistance and similar to viral accumulation in heterozygous for *S. chilense*-derived resistance.

DISCUSSION

Despite the efforts undertaken by different research groups since TYLCD was first reported, no immune commercial materials are available to date. Therefore, it is of

interest to make use of all promising sources of resistance. Moreover, strategies such as combining resistance genes from different sources already have been used to achieve higher levels of resistance and make it more durable. Our work shows that *S. pimpinellifolium* UPV16991-derived resistance may be useful when combined with the *Ty-1* gene.

Fig. 3. Average symptom score at 60 days post inoculation (upper) and maximum viral accumulation (lower) in UPV16991 line x susceptible crosses after whitefly-mediated inoculation with *Tomato yellow leaf curl virus*. UPV16991 lines, the susceptible control, and the midparent (MP) value of each cross (for symptom score, and based on symptom development in the susceptible control [FC]) are included to facilitate comparisons. Symptom score was evaluated using a scale from 0 (symptomless) to 4 (severe symptoms). Viral accumulation is expressed in nanograms of viral DNA per micrograms of total DNA extracted. Error bars represent standard error.

S. chilense has been reported as the best source of resistance to TYLCD when compared with resistance found in other wild tomato relatives (20,24). Accordingly, accession LA1969 was reported as highly resistant because plants remained symptomless and viral DNA was rarely detected (24). The *Ty-1* gene is the major gene controlling resistance in this accession (25). This gene, when studied in a tomato background, showed incomplete dominance, and even homozygous plants developed mild disease symptoms (6,25). In other works, plants homozygous for *Ty-1* have been described as asymptomatic (5,12) and not as carriers of viral DNA (12) when inoculated with different viral species causing TYLCD. In our work, 40% of the plants in both lines tested (Ch-1 and Ch-2) showed slight symptoms at some sampling date though, at 60 dpi, plants recovered and were symptomless. Discrepancies found in different assays may be due to differences in either inoculation or detection procedures, or in the background in which the resistance gene is expressed. In any case, in our work, resistance found in lines homozygous for *Ty-1* was higher than that found in most of the *S. pimpinellifolium*-derived lines, with respect to symptom development and levels of viral accumulation. Only L54-2 displayed similar symptoms and accumulated lower amounts of viral DNA than did Ch-1 and Ch-2.

The response in lines with *S. pimpinellifolium*-derived resistance to TYLCV was variable. Lines descendant from L102 and L48 had similar symptom development and viral accumulation. Most of the plants had slight symptoms and viral accumulation was approximately half that detected in the susceptible control. These results also were consistent with the monogenic

control with partial recessiveness and incomplete penetrance previously proposed for resistance in line L102 (16). Progeny from L54 behaved differently. L54-1 had low levels of resistance under the inoculum pressure of this assay. On the other hand, L54-2 had resistance similar to the *S. chilense*-derived lines. It must be highlighted that all L54-2 plants were resistant; therefore, the gene or genes controlling resistance in this line may have complete penetrance, unlike what was stated previously regarding the resistance gene in L102 (16). This is a hypothesis that should be tested by analyzing more plants of this line. These results suggest that the gene or genes controlling resistance in L54-2 could differ from the reported gene in L102. Kasrawi and Mansour (7) also found variability when comparing several lines derived from *S. pimpinellifolium* LA121, in which the average symptom score ranged from 0.25 to 2.07 on a scale from 0 (symptomless) to 4 (severe symptoms).

The *Ty-1* gene was first described as incompletely dominant because resistance in heterozygotes was slightly lower than in homozygotes (25). Again, there were differences among studies, because some authors described *Ty-1* as dominant (5). In addition, resistance in some commercial hybrids is based on plants heterozygous for *Ty-1*, and most of these hybrids show high resistance to TYLCD (*personal communication* from breeders). In our experiments, heterozygotes for *Ty-1* had more severe symptoms than homozygotes, supporting the incomplete dominance of the *Ty-1* gene. There was an important effect of the genetic background in heterozygous plants for *S. pimpinellifolium*-derived resistance. Values for symptom score and viral accumulation were, with exceptions, between

those of the *S. pimpinellifolium* and *S. lycopersicum* parents; however, there was variability depending on both the susceptible and the resistant parent. There was also an important effect caused by the interaction between both parents, given that the highest levels of resistance were not obtained for hybrids involving the most resistant parent. Kasrawi and Mansour (7) found similar results when evaluating hybrids between lines with resistance derived from different *S. pimpinellifolium* accessions and the susceptible cv. Special Back. The response in the hybrids did not always correlate with the response in the resistant parent. In our work, the most unexpected result was the response in hybrids involving L54-1, the least resistant line derived from *S. pimpinellifolium*. Three of these hybrids showed high levels of resistance, despite the fact that L54-1 showed only low levels of resistance. One possible explanation is that resistance is better expressed in the hybrids due to genetic background effects.

The most relevant results of this work involve the response in the hybrids between *S. pimpinellifolium*- and *S. chilense*-derived resistant lines. As expected, these hybrids exhibited higher levels of resistance than heterozygotes for *S. pimpinellifolium*-derived resistance, given that they displayed significantly lower symptoms and accumulated either similar or lower viral DNA amounts. The only exceptions were hybrids with L54-1, in which viral accumulation was higher than in heterozygotes for *S. pimpinellifolium*-derived resistance. When compared with heterozygotes for *Ty-1*, the hybrids showed a delay in time of symptom development. In addition, the symptom score in the hybrids was generally lower than in heterozygotes for

Fig. 4. Average symptom score at 60 days post inoculation (left) and maximum viral accumulation (right) in UPV16991 line × UPV16991 line crosses, after whitefly-mediated inoculation with *Tomato yellow leaf curl virus*. UPV16991 lines and the midparent (MP) value of each cross are included to facilitate comparisons. Symptom score was evaluated using a scale from 0 (symptomless) to 4 (severe symptoms). Viral accumulation is expressed in nanograms of viral DNA per micrograms of total DNA extracted. Error bars represent standard error.

Ty-1, with some exceptions at 60 dpi. It is also remarkable that symptoms were mild in all of the hybrid plants. These results contrast with results in heterozygotes for both *S. pimpinellifolium*- and *S. chilense*-derived resistance, in which, at 60 dpi or beforehand, some of the plants showed severe symptoms (≥ 2). Response in the hybrids was better than in *S. pimpinellifolium*-derived homozygotes. Moreover, all

plants of the hybrid Ch-2 $\times$ L102-2 remained symptomless and accumulated lower amounts of viral DNA, thus exceeding even the resistance level in lines homozygous for the *Ty-1* gene. However, this hybrid was the exception, given that, generally, lines homozygous for the *Ty-1* gene showed higher levels of resistance than the hybrids combining *S. pimpinellifolium*- and *S. chilense*-derived resistance. The

possibility of achieving high levels of resistance by combining *S. chilense*- and *S. pimpinellifolium*-derived resistance has already been reported by Vidavsky et al. (23). Similarly to our results, they found that, even if the *S. pimpinellifolium* parent had intermediate levels of resistance, when crossed with a highly resistant line derived from *S. chilense*, highly resistant descendants resulted.

Fig. 5. Average symptom score (left) and viral accumulation (right) in **A**, Ch-1 $\times$ UPV16991 line crosses and **B**, Ch-2 $\times$ UPV16991 line crosses after whitefly-mediated inoculation with *Tomato yellow leaf curl virus*. Symptom score was evaluated using a scale from 0 (symptomless) to 4 (severe symptoms). Viral accumulation is expressed in nanograms of viral DNA per micrograms of total DNA extracted. Error bars represent standard error.

Therefore, resistance derived from *S. pimpinellifolium* UPV16991 is useful when combined with the *Ty-1* gene in the development of tomato hybrids. This is the most practical approach to exploiting the resistance derived from UPV16991. Currently, the tomato market is mainly based on hybrid cultivars (22). Furthermore, preferred resistance genes are those which do not need to be fixed in both parents (21). Therefore, the use of resistance genes in homozygosis is not frequent in current breeding programs. Combining the *Ty-1* gene and resistance derived from *S. pimpinellifolium* UPV16991 could allow the achievement of higher levels of resistance than the levels obtained with *Ty-1* gene in heterozygosis. This would be the most useful approach to exploit resistance found in this source.

The *S. pimpinellifolium*-derived lines available are consecutive selfings from the initial *S. lycopersicum* NE-1 $\times$ *S. pimpinellifolium* UPV16991 cross and they still have too much *S. pimpinellifolium* background. As a previous step to exploit resistance derived from this source, future work will include the introgression of the resistance gene into *S. lycopersicum* backgrounds. For this purpose, it would be advisable to identify a molecular marker linked to the resistance gene derived from UPV16991 in order to accelerate the breeding program. Once advanced lines which incorporate the UPV16991-derived resistance in *S. lycopersicum* background are achieved, crosses of these lines with homozygous lines for *Ty-1* should be developed in order to select those with higher levels of resistance. Finally, it would be of interest to further characterize the high resistance found in L54-2, in order to confirm whether it differs from the resistance previously reported in L102.

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