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Identification of genetic sources with attenuated *Tomato chlorosis virus*-induced symptoms in *Solanum* (section *Lycopersicon*) germplasm

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Abstract The whitefly-transmitted *Tomato chlorosis virus* (ToCV) (genus *Crinivirus*) is associated with yield and quality losses in field and greenhouse-grown tomatoes (*Solanum lycopersicum*) in South America. Therefore, the search for sources of ToCV resistance/tolerance is a major breeding priority for this region. A germplasm of 33 *Solanum* (*Lycopersicon*) accessions (comprising cultivated and wild species) was evaluated for ToCV reaction in multi-year assays conducted under natural and experimental whitefly vector exposure in Uruguay and Brazil. Reaction to ToCV was assessed employing a symptom severity scale and systemic virus infection was evaluated via RT-PCR and/or molecular hybridization assays. A subgroup of accessions was also evaluated for whitefly reaction in two free-choice bioassays carried out in Uruguay (with *Trialeurodes vaporariorum*) and Brazil (with *Bemisia tabaci* Middle-East-Asia-Minor1—MEAM1 = biotype B). The most stable sources of ToCV tolerance were identified in *Solanum habrochaites* PI 127827 (mild symptoms and low viral titers) and *S. lycopersicum* 'LT05' (mild symptoms but with high viral titers). These two accessions were efficiently colonized by both whitefly species, thus excluding the potential involvement of vector-resistance mechanisms. Other promising breeding sources were *Solanum peruvianum* (sensu lato) 'CGO 6711' (mild symptoms and low virus titers), *Solanum chilense* LA1967 (mild symptoms, but with high levels of *B. tabaci* MEAM1 oviposition) and *Solanum pennellii* LA0716 (intermediate symptoms and low level of *B. tabaci* MEAM1 oviposition). Additional studies are necessary to elucidate the genetic basis of the tolerance/resistance identified in this set of *Solanum* (*Lycopersicon*) accessions.

Keywords Virus resistance · Whitefly resistance · Tolerance · Virus screening

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Introduction

Whitefly (Hemiptera: Aleyrodidae)-transmitted viruses of the genus *Crinivirus* (family *Closteroviridae*) belong to the alphavirus-like supergroup of single-stranded, positive-sense RNA plant viruses with large (15.3–17.6 kb), complex, and segmented genomes (Mongkolsiriwattana et al. 2016). Two crinivirus species have been reported infecting tomato (*Solanum lycopersicum* L.) across the world: *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV) (Hanssen et al. 2010; Navas-Castillo et al. 2014). Among them, ToCV is considered an emerging tomato virus with global impact associated with significant yield and quality losses in all continents (Navas-Castillo et al. 2011, 2014). The host range of tomato-infecting criniviruses includes a wide array of domesticated and weed species, which may function as natural reservoirs of these viruses and their vectors (Wintermantel and Wisler 2006; Tzanetakis et al. 2013; Orfanidou et al. 2016). In South America, ToCV has been reported thus far as the only crinivirus infecting tomatoes (Barbosa et al. 2011; Arruabarrena et al. 2014; 2015) as well as other Solanaceae species (Fonseca et al. 2016; Boiteux et al. 2018) and associated weeds (Fonseca et al. 2013; Arruabarrena et al. 2015; Boiteux et al. 2016).

ToCV is transmitted in a semi-persistent, non-circulative manner by at least five whitefly species: *Trialeurodes abutilonea*, *T. vaporariorum*, *Bemisia tabaci* New World (*B. tabaci* NW species, formerly biotype A), *B. tabaci* Middle-East-Asia-Minor 1 (*B. tabaci* MEAM1 species, formerly biotype B), and *B. tabaci* Mediterranean (*B. tabaci* MED species, formerly biotype Q) (Wintermantel and Wisler 2006; Navas-Castillo et al. 2014). Some of these vectors can acquire the criniviruses very rapidly, and they can transmit them very efficiently (Wintermantel and Wisler 2006; Shi et al. 2018).

Tomato plants infected with ToCV develop characteristic yellowing symptoms similar to those induced by mineral (especially magnesium) deficiency. In early infection stages, the symptoms are more evident in the basal and middle leaves, while the apical region appears normal (Wisler et al. 1998). ToCV-induced symptoms in tomatoes often include interveinal foliar yellowing, leaf brittleness, reduced plant vigor, earlier senescence, and delay in fruit ripening (Tzanetakis et al. 2013). The viral latent period may vary from 3 to 4 weeks depending on the environmental conditions. Once the disease progresses, purple and necrotic areas may appear in leaves associated with the interveinal discoloration, contributing to the premature leaf senescence (Tzanetakis et al. 2013). Crinivirus infection remains confined to the phloem tissues, and most of the symptoms are the result of phloem plugging by large virus-associated inclusion bodies, which interfere with the normal nutrient transport in infected plants (Wisler & Duffus 2001). The decrease in the photosynthetic activity is the major cause of yield and quality losses in infected solanaceous hosts (Navas-Castillo et al. 2000; Fortes et al. 2012). Another peculiar feature of ToCV is its ability to interact with other viral tomato pathogens, altering the symptom expression or even leading to complete breaking-down of natural resistance factors of their host plants (García-Cano et al. 2006; Wintermantel et al. 2008).

In tomato, two major mechanisms have been identified to prevent or reduce infection by whitefly-transmitted viruses: (1) resistance/tolerance factors against viral pathogens after their invasion of the host cells and (2) resistance/tolerance factors interfering with virus-transmission efficiency by their vectors (Rodríguez-López et al. 2012; Rakha et al. 2017). Regarding resistance to the insect vector, it was demonstrated that the accumulation of defense compounds in glandular leaf trichomes interferes with the whitefly ability to visit, feed and colonize tomato accessions with these structures, which could reduce primary and secondary spread of whitefly-transmitted viruses (Mutschler and Wintermantel 2006; Rodríguez-López et al. 2011, 2012). The indirect effect of these insect-defense compounds on criniviruses incidence was observed in open field trials carried out in the Southern coast of California. A significant delay in TICV infection was observed in tomato accessions less preferred by *T. vaporariorum*, including *S. pennellii* LA0716 (an acylsugar-producing accession with type IV glandular trichomes) and *S. habrochaites* (with type VI trichomes and accumulation of methylketones and sesquiterpenes) (Mutschler and Wintermantel 2006). The *S. pimpinellifolium* accession 'TO-937' (which has type IV glandular trichomes and acylsugar accumulation) was found to be tolerant to mites (Fernández-Muñoz et al. 2003), *B. tabaci* MED (Rodríguez-López et al. 2011), and *B. tabaci* MEAM1 (Silva et al. 2014). However, the broad-spectrum

tolerance to pests of *S. pimpinellifolium* 'TO-937' was unable to avoid ToCV transmission and infection according to distinct field observations in Uruguay, Brazil, and Spain (García-Cano et al. 2010) as indicated by the high susceptibility levels of this accession.

In relation to resistance and/or tolerance to criniviruses, some promising genetic sources were found in *Solanum* (section *Lycopersicon*) germplasm. Stable levels of resistance to ToCV were identified in the lines '802-11-1' [derived from selfing the line 'IAC-CN-RT', obtained from an interspecific cross of *S. lycopersicum* × *S. peruvianum* (lato sensu) LA444-1] and the line '821-13-1', which was derived from *S. chmielewskii* LA1028 after two generations of selfing (García-Cano et al. 2010). Even though some plants displayed systemic infection, '802-11-1' and '821-13-1' exhibited significant resistance to ToCV accumulation and attenuated ToCV-induced symptoms (i.e. virus-infected plants displaying only mild symptoms). In addition, a subgroup of *S. chilense*, *S. lycopersicum*, and *S. corneliomulleri* accessions exhibited good levels of resistance under field conditions. However, the resistant reaction of these accessions was not stable in controlled inoculation assays under greenhouse conditions (García-Cano et al. 2010). More recently, Mansilla-Córdova et al. (2018) identified superior levels of ToCV resistance in *S. habrochaites* (PI 127826 and PI 134417), *S. peruvianum* (LA444-1 and LA0371) and several *S. lycopersicum* × *S. peruvianum* hybrids (e.g. 'IAC-68F-22-2-24-1', 'IAC-CN-RT', 'IAC-14-2-49', and 'IAC-14-2-85') in free-choice assay using viruliferous *B. tabaci* MEAM1. When these accessions were inoculated at non-choice vector conditions, only 'IAC-CN-RT' was confirmed as being resistant to ToCV.

Even after these pioneering breeding efforts so far there are no commercial tomato varieties with either resistance or tolerance to criniviruses. The present work is an attempt to combine the information generated by a diverse set of greenhouse assays as well as some field observations in distinct tomato crop seasons as well as in two distinct geographic regions of South America (Uruguay and Central Brazil). We characterize a wide range of differential reactions of a *Solanum* (*Lycopersicon*) germplasm collection (comprising cultivated and wild species) to ToCV and against two of its vectors (*T. vaporariorum* and *B. tabaci* MEAM1) in multi-year trials conducted under natural and experimental whitefly vector exposure.

Materials and methods

Solanum (section *Lycopersicon*) germplasm

A germplasm collection of 33 *Solanum* (*Lycopersicon*) accessions from diverse origins (Table 1) was used in a set of five independent bioassays. This germplasm comprised 16 commercial tomato hybrids from distinct seed companies, two open pollinated (OP) cultivars, six breeding lines, and ten wild accessions belonging to the species S. *pimpinellifolium* (2 accessions), *S. chilense* (1 accession), *S. peruvianum* (1 accession), *S. pennellii* (1 accession), and *S. habrochaites* (4 accessions). This germplasm was evaluated in three independent bioassays for ToCV reaction (collectively named as ToCV trials) and in two independent free-choice bioassays for whitefly reaction (which were collectively named as WF trials).

Evaluation criteria employed to characterize the reaction of *Solanum* (*Lycopersicon*) germplasm to ToCV

The accessions of *S. pimpinellifolium* 'TO-937' (García-Cano et al. 2010) and the *S. lycopersicum* 'Santa Clara *Sw*-5/*Sw*-5' were employed as susceptible controls in all bioassays for reaction to ToCV. Three criteria employed to evaluate the reaction to ToCV are described below:

(1) *Assessment of ToCV-induced symptom severity*—this evaluation was carried out employing a visual scale for assessment of virus-induced symptom severity (developed in the present work; Online Resource 1) where: 0 = no visible symptoms, 1 = interveinal chlorosis restricted to basal leaves, 2 = interveinal chlorosis in basal and middle leaves, presence of some necrotic areas, 3 = intense interveinal chlorosis and overall yellowing, with presence of necrotic areas. (2) *Evaluation of systemic ToCV infection via reverse transcription polymerase chain reaction (RT-PCR)*—Total plant RNA was extracted from foliar tissue with TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. Total RNA was diluted in 50 µL of

Table 1 List of *Solanum* (section *Lycopersicon*) accessions evaluated for *Tomato chlorosis virus* (ToCV) reaction in three different bioassays (ToCV trails) in Uruguay and Central Brazil

Solanum (*Lycopersicon*) accession	Origin	ToCV trials
S. chilense LA1967	Tomato genetics resource center	3[a]
S. habrochaites PI 126445	USDA-GRIN	1, 3
S. habrochaites PI 126925	USDA-GRIN	1
S. habrochaites PI 127827	USDA-GRIN	1, 2, 3
S. habrochaites PI 134417	USDA-GRIN	3
S. lycopersicum 'BRS Nagai'	F_1 hybrid (Embrapa–Agrocinco)	1
S. lycopersicum 'BRS Portinari'	F_1 hybrid (Embrapa–Agrocinco)	1
S. lycopersicum 'Cetia'	F_1 hybrid (Clause)	1
S. lycopersicum 'Santa Clara' (*sw*-5/*sw*-5)	OP commercial cultivar	3
S. lycopersicum 'Compack'	F_1 hybrid (De Ruiter)	1
S. lycopersicum 'Dominique'	F_1 hybrid (Hazera)	1
S. lycopersicum 'Elpida'	F_1 hybrid (Enza Zaden)	1, 2
S. lycopersicum 'Gostomiel'	F_1 hybrid (Syngenta)	3
S. lycopersicum 'Ivanhoé'	F_1 hybrid (Rijk Zwaan)	1
S. lycopersicum 'Kumato'	F_1 hybrid (Syngenta)	3
S. lycopersicum 'Santa Clara (*Sw*-5/*Sw*-5)'	Tospovirus R inbred line	1, 2, 3
S. lycopersicum 'LT05'	Inbred line (INIA—Uruguay)	1, 2, 3
S. lycopersicum 'LT17'	Inbred line (INIA—Uruguay)	1, 2, 3
S. lycopersicum 'Matrero'	F_1 hybrid (Seminis)	1, 2
S. lycopersicum 'Montenegro'	F_1 hybrid (Rijk Zwaan)	1
S. lycopersicum 'Nemo Netta'	F_1 hybrid (Nirit)	1
S. lycopersicum 'Paronset'	F_1 hybrid (Syngenta)	1
S. lycopersicum 'Swanson'	F_1 hybrid (De Ruiter)	1, 2
S. lycopersicum 'Torry'	F_1 hybrid (Syngenta)	1, 2
S. lycopersicum 'TX 468 RG'	Begomovirus R inbred line	3
S. lycopersicum 'Tyerno'	F_1 hybrid (Syngenta)	1
S. lycopersicum 'Viradoro'	OP Cultivar (Embrapa—Brazil)	3
S. lycopersicum × *S. pimpinellifolium* 'BC3F3'	Inbred line (CSIC—Spain)	3
S. lycopersicum × *S. pimpinellifolium* 'BC5F5'	Inbred line (CSIC—Spain)	3
S. pennellii LA0716	Tomato Genetics Resource Center	3
S. peruvianum (sensu lato) 'CGO 6711'	International Germplasm	1
S. pimpinellifolium PI 126931	USDA-GRIN	1
S. pimpinellifolium 'TO-937'	CSIC—Spain	1, 2, 3

[a]Numbers correspond to the experiments in which each accession was evaluated: 1 = fall 2013 trial in Uruguay, 2 = fall 2014 trial in Uruguay, 3 = fall 2015 trial in Brazil

DEPC-treated water. Virus detection by RT-PCR was carried out essentially as described by Dovas et al. (2002). One step RT-PCR was performed using the HS-11/HS-12 primer pair, which amplifies a fragment of ≈ 587 bp corresponding to the heat shock protein (HSP-70) gene homolog. This genomic region is highly conserved across *Crinivirus* species. RT-PCR reaction was performed using the following conditions: a first step at 42 °C for 60 min, second step at 50 °C for 2 min, third step at 94 °C for 5 min, 35 cycles subdivided in step #1: 30 s at 95 °C, step #2: 30 s at 43 °C, and step #3: 15 s at 72 °C, followed by a

final extension step (72 °C for 2 min). Afterwards, ToCV infection was confirmed by assaying the RT-PCR products as templates in multiplex nested-PCR with the TIC-3/TIC-4 and ToC-5/ToC-6 primer pairs (Dovas et al. 2002). These assays allowed the determination of TICV-specific (≈ 263 bp) and ToCV-specific (≈ 463 bp) amplicons. Nested RT-PCR assays were performed using the following conditions: a first denaturizing step at 95 °C for 1 min, 40 cycles subdivided in 20 s at 95 °C, 15 s at 60 °C, and 10 s at 72 °C, followed by one final extension step (72 °C for 2 min). The final RT-PCR products were separated by 1.5% agarose gel electrophoresis in 0.1X TBE buffer and stained with ethidium bromide to determine presence/absence of the target DNA. Direct Sanger sequencing was carried out (at the Genomic Analysis Lab, CNPH, Brasília–DF, Brazil) with a subset of gel-purified PCR amplicons (PureLink PCR micro kit®; Invitrogen, Carlsbad, CA, USA) in order to confirm the identity of the putative ToCV-specific bands. (3) *Analysis of systemic ToCV infection via dot-blot and tissue-print hybridization*—Molecular hybridization assays with a ToCV RNA-specific probe were performed using the methodology developed by García-Cano et al. (2006). In the first assay for assessment of ToCV reaction (ToCV trial #1), dot-blot hybridization was implemented with a standardized drop of total RNA from each plant placed on a positively charged nylon membrane (Roche Diagnostics GmbH, Mannheim, Germany). Total RNA of the plant was extracted from leaves from the middle section of the plants using the TRIzol® reagent (Invitrogen, Carlsbad, CA, USA). For standardization, RNA of each sample was diluted or concentrated so that each drop contained 1 μg of total RNA in a volume of 1.5 μL. For the ToCV trial #2, tissue-print hybridization was implemented using fresh cross sections of petioles obtained from basal leaves of each plant, which were directly squash-blotted on the membrane. For the ToCV trial #3, tissue-print hybridization was also implemented from cross-sections of leaf petioles using basal and middle leaves of each evaluated plant (two leaves per plant), which were also directly squash-blotted on the membrane. The membranes with RNA drop or tissue prints were oven-fixed at 80 °C for 2 h and then hybridized with a ToCV RNA specific probe containing a fragment of the ToCV coat protein gene. The probe was synthesized from total RNA using M380/M381 ToCV-specific primers (Fortes et al. 2012), which amplify a fragment of 436 bp. The fragment was cloned in pGEM®-T Easy vector (Promega Corporation, Madison, WI, USA). To generate the antisense RNA probe labeled with dioxigenin-11-UTP, the linearized plasmid (containing the 436 bp amplicon) was subjected to *in vitro* transcription with T7 RNA polymerase. Chemiluminescent detection was done with CDP-Star Detection Reagent (Roche Diagnostics GmbH, Mannheim, Germany). The probe synthesis, hybridization and chemiluminescent detection were performed using the DIG-labeling and detection kit (Roche Diagnostics GmbH, Mannheim, Germany) and following the protocols supplied by the manufacturer. Development of the chemiluminescent reaction and analysis of blots were performed by a ChemiDoc XRS+ System (Bio-Rad; Hercules, CA, USA).

Screening *Solanum* (*Lycopersicon*) germplasm for reaction to ToCV in Uruguay (ToCV trial #1)

This bioassay was conducted with 23 *Solanum* accessions (Table 1) during the fall season of 2013 under conditions of natural ToCV infection in a plastic greenhouse in Salto (Uruguay). Healthy seedlings were grown in an insect-proof glasshouse and 30 days after sowing five plants of each accession (with four fully expanded true leaves) were transplanted direct to the soil under the plastic house. No insecticide applications were employed during the entire assay in order to promote an abundant presence of ToCV-carrying *T. vaporariorum* adults. Previous experience indicates that under these experimental conditions high levels of ToCV infection are observed, following the virus spread from either infected susceptible commercial tomato crops or weed hosts in the surrounding areas (Arruabarrena et al. 2014, 2015). Disease severity was rated in all individual plants at 100 days after planting (DAP) using our previously described visual symptom scale. At this evaluation time, five plants of the most susceptible and five plants of the most resistant accessions were analyzed for systemic ToCV infection by RT-PCR. Also, five plants of a selected group of accessions were analyzed by dot-blot hybridization.

Evaluation of a selected subgroup of *Solanum* (*Lycopersicon*) accessions for reaction to ToCV in Uruguay (ToCV trial #2)

This experiment was carried out in Salto (Uruguay) during the fall season of 2014 under similar conditions as described in the ToCV trial #1. A selected group of nine *Solanum* (*Lycopersicon*) accessions that displayed contrasting reactions to the virus in the ToCV trial #1 (Table 1) was re-evaluated to confirm the germplasm responses previously observed and to study the rate of symptom development. Healthy seedlings were grown in an insect proof glasshouse and 30 days after sowing ten plants of each accession (at the four-leaf growth stage) were transplanted to 5 L-pots (filled with sterile substrate) and kept under a plastic house. Three plots (three to four plants) per genotype were employed in a complete randomized design. Disease severity was rated on individual plants using our visual symptom scale, and average plot ratings were calculated for each evaluation date (50, 70, and 90 DAP). The presence as well as the crinivirus identity was confirmed by RT-PCR. The systemic presence of ToCV in the plants was evaluated by tissue-print hybridization in duplicates along with the first and the last visual evaluation of the symptoms (i.e. 50 and 90 DAP).

Evaluation of a selected subgroup of *Solanum* (*Lycopersicon*) accessions for reaction to ToCV in Brazil (ToCV trial #3)

This experiment was conducted in Brasilia–DF (Central Brazil) during the fall season of 2015 under greenhouse in a region where ToCV occurrence is endemic (Macedo et al. 2014). A selected group of six *Solanum* (*Lycopersicon*) accessions that displayed contrasting reactions to ToCV in the previous assays and ten novel accessions of breeding interest were evaluated (Table 1). Healthy seedlings were grown in an insect proof glasshouse and 20 days after sowing (i.e. at stage of two fully expanded true leaves) were placed inside a greenhouse containing eggplant and tomato plants with severe ToCV symptoms (Fonseca et al. 2016) and harboring a high population of viruliferous *B. tabaci* MEAM1 adults, which is the only whitefly vector species present thus far in Central Brazil (Blawid et al. 2015). Ten days later, seedlings were transplanted in 5 L-pots with a mixture of soil and commercial substrate on the definitive greenhouse. No insecticide applications were employed inside the greenhouse, aiming to promote higher levels of natural infection and spread of ToCV by allowing abundant presence of *B. tabaci* MEAM1 adults. Three plots per accession (six plants each) were organized in a completely randomized design. Disease severity was rated on individual plants using our visual symptom scale, and an average plot ratings were calculated at 70 DAP. The systemic presence of the virus was evaluated in two central plants of each plot (six plants per accession) along with the last evaluation of symptoms (done at 70 DAP) by tissue-print hybridization. In addition, Sanger sequencing was carried out (at the Genomic Analysis Lab, CNPH, Brasília–DF, Brazil) with a subset of gel-purified PCR amplicons (PureLink PCR micro kit®; Invitrogen, Carlsbad, CA, USA) in order to confirm the identity of the putative ToCV-specific bands.

Evaluation of a selected subgroup of *Solanum* (*Lycopersicon*) accessions to whitefly species infestation (WF trails)

A subgroup of accessions was evaluated in two independent bioassays for reaction to distinct whitefly species. Two experiments were carried out: (1) *Evaluation of Solanum (Lycopersicon) accessions to T. vaporariorum in Uruguay (WF trial #1)*—The same subgroup of accessions evaluated in the ToCV trial #2 were also evaluated for *T. vaporariorum* oviposition in a free-choice bioassay as a way to estimate potential differences of insect preference among accessions. Evaluation was performed under natural infestation by counting of whitefly eggs at 70 DAP over the same plants of ToCV trial #2. The two central plants of each plot (in the first block) and one plant of each plot (in the second and third blocks) were evaluated providing a total of four plants (replicates) per accession. From each plant, the first fully developed leaf counted from the apex was taken and ten disks of 0.8 cm in diameter were removed from the foliar tissue. The counting of eggs was performed on the abaxial surface of each disc using a stereo-microscope. Values of the same plant were averaged and expressed as the number of eggs per cm^2 of leaf. (2) *Evaluation of Solanum (Lycopersicon) accessions to B. tabaci MEAM 1 in Brazil (WF trial #2)*—The same subgroup of accessions evaluated in ToCV trial #3 was also evaluated for *B. tabaci*

MEAM 1 oviposition in a free-choice bioassay. Tomato seedlings were transplanted to pots filled with 5 L of sterile substrate. Thirty-days after sowing, ten plots per treatment (one plant each) were arranged in a completely randomized design within a greenhouse. Heavily *B. tabaci* MEAM 1-infested cucumber (*Cucumis sativus* L.) plants with adults and fourth instar nymphs were placed into the greenhouse for 24 h. After that, cucumber plants were removed from the substrate and left on the benches for 3 days in order to promote aviruliferous whitefly movement towards tomato plants under evaluation. Twenty-one days after exposure to the insects, eggs on the leaves were counted in the laboratory with the help of a stereomicroscope. The counting was performed in the abaxial surface of leaf disks (0.8 cm in diameter), which were excised from three different leaflets per plant (= six disks per plant). Countings of the same plant were averaged and expressed as the number of eggs per cm^2 of leaf tissue.

Statistical analyses

For the assessment of the plant reaction to ToCV, the ordinal symptom severity values (0–3) were expressed by the average of the mean values of each replicate. In the ToCV trial #2 and ToCV trial #3, the nonparametric Kruskal-Wallis analysis of variance was implemented, followed by non-parametric multiple comparisons of ranks used for testing significant difference among treatments (Zar 1984). In the ToCV trial #2, disease progress curves were constructed based on symptom ratings, and the area under the symptom progress curve (AUSPC) was determined using the following formula: $\text{AUSPC} = \Sigma\ [(Si + S(i+1))(T(i+1) - vTi)]/2$ with Si = mean symptom score values at date i, and Ti = time (in days) at date i. AUSPC was used as the single variable for disease progression description. For WF preference trials, due to lack of independence of the treatments in vector free-choice tests, the nine tomato accessions evaluated for *T. vaporariorum* oviposition in Uruguay and the 16 accessions evaluated for *B. tabaci* MEAM1 oviposition in Brazil were ranked (within each replication) from the least preferred to the most preferred accession (Menezes et al. 2005). The summation of the ranks obtained for each accession in relation to the potential maximal ranking (either nine or 16) was calculated. The Friedman's test for block design was performed to determine whether there were differences among tomato accessions based upon the rank summation (Conover 1998). Multiple comparisons based upon rank summation differences were conducted between pairs of tomato accessions, using the sequential Holm's adjustment for significance level (Holm 1979).

Results

Screening of *Solanum* (section *Lycopersicon*) germplasm for reaction to ToCV in Uruguay (ToCV trial #1)

Twenty-three *Solanum* (section *Lycopersicon*) accessions were initially evaluated for ToCV reaction under natural infection conditions in Salto, Uruguay (Table 2). The vector (*T. vaporariorum*) population was high and ToCV spread was uniform. This was somewhat expected for this region during the time of the year when the bioassay was carried out. Typical symptoms of interveinal chlorosis (initially restricted to basal leaves) were observed as soon as 45 DAP. In susceptible accessions, the symptoms progressed to chlorosis in the middle leaves and finally in apical leaves as well (Fig. 1). Necrotic areas were observed associated with the yellowing leaf sectors by the end of the experiment. The five plants of the susceptible controls (*S. lycopersicum* 'Santa Clara *Sw*-5/*Sw*-5' and *S. pimpinellifolium* 'TO-937') displayed typical disease symptoms with severity ranging from medium-high (1.8 ± 0.1) to high (2.6 ± 0.2), respectively (Table 2). Plants of the accessions with the lowest (= *S. habrochaites* PI 127827) and highest (= *S. lycopersicum* 'Matrero') levels of symptom severity displayed systemic virus infection as indicated by RT-PCR assays (Table 2, Fig. 2 a). Among the 17 *S. lycopersicum* accessions under evaluation, a broad range of symptom severity was observed, from mild (attenuated) symptoms restricted to the basal leaves (e.g. 'LT05', 'Swanson', 'BRS Nagai', and 'Elpida') to overall chlorosis and yellowing with necrotic areas (e.g. 'Tyerno', 'Matrero', 'Montenegro', and 'Cetia'). Among wild species accessions, *S. habrochaites* PI 127827, *S. habrochaites* PI 126445, and *S. peruvianum* 'CGO 6711' displayed mild chlorosis symptoms in some plants, while *S. pimpinellifolium* PI 126931, *S. habrochaites* PI 126925, and *S. pimpinellifolium* 'TO-

Table 2 Evaluation to *Tomato chlorosis virus* (ToCV) reaction of a collection of commercial hybrids, breeding lines, and wild *Solanum* (section *Lycopersicon*) germplasm under natural infection conditions in a greenhouse in Salto, Uruguay (*ToCV trial #1*). Fall, 2013

Solanum (*Lycopersicon*) accession	Symptom severity[a]
S. habrochaites PI 127827	0.1 ± 0.1
S. lycopersicum 'Swanson'	0.2 ± 0.1
S. habrochaites PI 126445	0.5 ± 0.0
S. lycopersicum 'BRS Nagai'	0.5 ± 0.0
S. lycopersicum 'LT05'	0.5 ± 0.0
S. lycopersicum 'Elpida'	0.6 ± 0.1
S. peruvianum 'CGO 6711'	0.6 ± 0.2
S. lycopersicum 'Paronset'	0.8 ± 0.1
S. lycopersicum 'Compack'	0.9 ± 0.3
S. lycopersicum 'Ivanhoé'	1.0 ± 0.0
S. lycopersicum 'Dominique'	1.2 ± 0.2
S. lycopersicum 'Nemo Netta'	1.4 ± 0.2
S. lycopersicum 'Torry'	1.6 ± 0.2
S. lycopersicum 'LT17'	1.7 ± 0.1
S. lycopersicum 'BRS Portinari'	1.7 ± 0.3
S. lycopersicum 'Santa Clara (*Sw*-5/ *Sw*-5)'	1.8 ± 0.1
S. pimpinellifolium PI 126931	2.0 ± 0.0
S. lycopersicum 'Cetia'	2.2 ± 0.2
S. habrochaites PI 126925	2.4 ± 0.1
S. lycopersicum 'Montenegro'	2.4 ± 0.2
S. pimpinellifolium 'TO-937'	2.6 ± 0.2
S. lycopersicum 'Matrero'	2.7 ± 0.1
S. lycopersicum 'Tyerno'	2.7 ± 0.1

[a]Evaluation carried out at 100 days after planting, where 0 = absence of ToCV-induced symptoms and 3 = severe ToCV-induced symptom expression. Results are mean ± standard error for five plants

937' displayed severe symptoms in all plants. The dot-blot hybridization with a ToCV RNA-specific probe is shown for three accessions classified as having high symptom severity and four accessions classified as mild symptom severity (Table 2, Fig. 2b). Strong hybridization signals were detected in *S. lycopersicum* plants in both high (e.g. 'Matrero' and 'Santa Clara *Sw*-5/*Sw*-5') and mild (e.g. 'LT05') symptom severity groups of accessions. In the wild species, some plants with low hybridization signals (Fig. 2b) were observed in high symptom severity accessions (e.g. *S. pimpinellifolium* 'TO-937') as well as in the mild symptom severity accessions (e.g. *S. habrochaites* PI 127827, *S. habrochaites* PI 126445, and *S. peruvianum* 'CGO 6711').

Evaluation of selected *Solanum* (*Lycopersicon*) germplasm for reaction to ToCV in Uruguay (ToCV trial #2)

Nine accessions evaluated in ToCV trial #1 were selected for a second assay in order to confirm the first observations and to improve the characterization of the potential resistance/tolerance mechanisms. In this assay, the natural population of the vector (*T. vaporariorum*) was present in moderate levels. Each evaluated plant was analyzed for presence of the virus by tissue-blot hybridization. All accessions displayed 100% of the plants with systemic ToCV infection, except for *S. habrochaites* PI 127827, *S. pimpinellifolium* 'TO-937', and *S. lycopersicum* 'Santa Clara *Sw*-5/*Sw*-5', which each had eight plants infected out of ten (Table 3, Online Resource 2). Assessments carried out at 90 DAP are summarized in Table 3. As for the ToCV trial #1, the susceptible controls (*S. pimpinellifolium* 'TO-937' and *S. lycopersicum* 'Santa Clara *Sw*-5/*Sw*-5') displayed high levels of symptom severity with values of 2.4 ± 0.1 and 2.1 ± 0.1, respectively. Taking this reference, it was possible to identify three accessions ('LT05', PI 127827 and 'Elpida') with significantly lower symptom severity level at the end of the evaluation period, and were classified as having high tolerance. 'Matrero' and

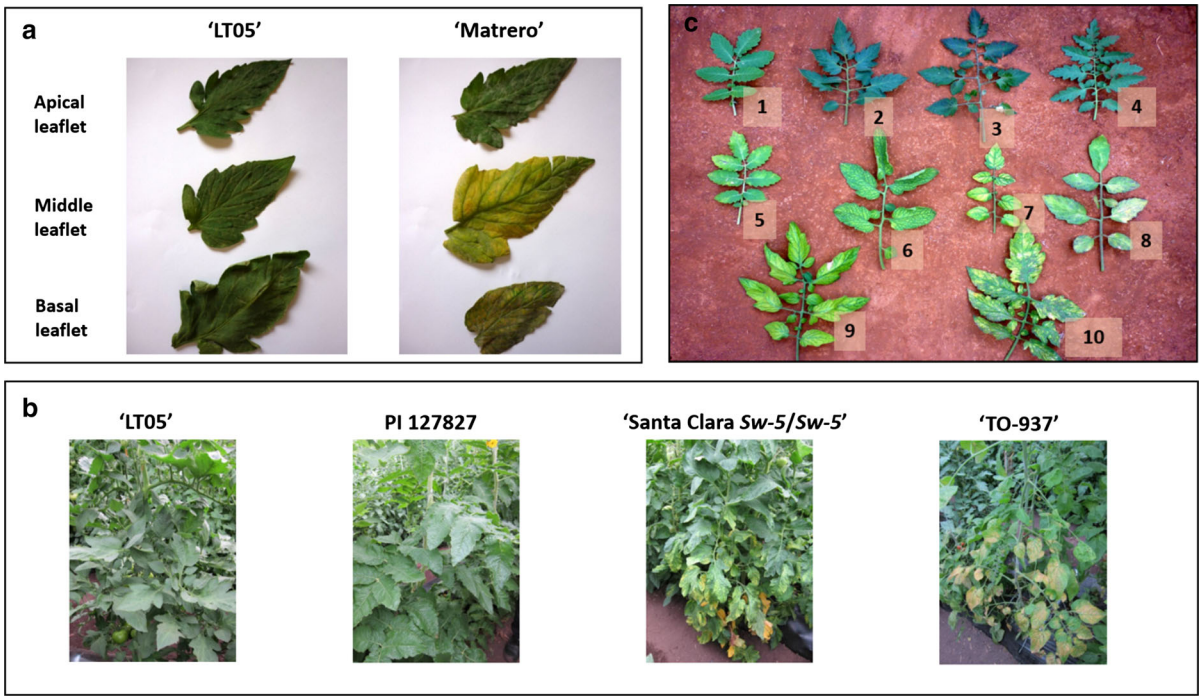

Fig. 1 **a** Trial #1: Carried out at 100 days after sowing, displaying differences of *Tomato chlorosis virus* (ToCV)-induced symptoms in the leaflets of the tolerant line *S. lycopersicum* 'LT05' (symptom severity value of 0.5 ± 0.0) and the susceptible F_1 hybrid 'Matrero' (symptom severity value of 2.7 ± 0.1). Position of the leaflet in the plant is indicated at the left of the figure. **b** Trial #2: Carried out at 70 days after sowing, displaying peculiar aspects of the ToCV-tolerant accessions *S. lycopersicum* 'LT05' and *S. habrochaites* PI 127827 in contrast with the susceptible standards (*S. pimpinellifolium* 'TO-937' and *S. lycopersicum* 'Santa Clara *Sw*-5/*Sw*-5'). **c** Trial #3: Carried out at 70 days after sowing, displaying differences in ToCV-induced symptoms in the middle leaves of distinct accessions: (1) *S. habrochaites* PI 127827; (2) *S. lycopersicum* 'LT05'; (3) *S. lycopersicum* 'Gostomiel'; (4) *S. chilense* LA1967; (5) *S. habrochaites* PI 134417; (6) *S. lycopersicum* 'Santa Clara *Sw*-5/*Sw*-5'; (7) *S. pimpinellifolium* 'TO-937'; (8) *S. lycopersicum* × *S. pimpinellifolium* 'BC5F5'; (9) *S. lycopersicum* 'LT17' and (10) *S. lycopersicum* 'Viradoro'

'LT17' did not differ from the sensitive controls. 'Swanson' and 'Torry' could not be differentiated from either the highly tolerant or the sensitive accessions, and these were classified as having intermediate levels of tolerance. The AUSPC values indicated that the highly tolerant accessions had a significantly slower symptom development rate (i.e. lower AUSPC values) relative to the susceptible controls (Table 3).

Evaluation of *Solanum* (*Lycopersicon*) germplasm for reaction to ToCV in Brazil (ToCV trial #3)

This bioassay was carried out in Brasília–DF (Brazil) under controlled inoculation conditions with high *B. tabaci* MEAM1 pressure (with an average of more than 30 whitefly adults per plant) at early (seedling) growth stage. This assay was composed of 11 accessions previously evaluated in trials in Uruguay plus a subgroup of five accessions of breeding interest that were not previously evaluated (Table 4). ToCV infection symptoms evolved rapidly and the final evaluation was carried out at 70 DAP. At that time, tissue-blot hybridization signals indicated that all six plants of each *Solanum* (*Lycopersicon*) accession under evaluation were positive for systemic presence of ToCV (Table 4, Online Resource 3). High levels of symptom severity were confirmed once again in *S. lycopersicum* breeding line 'Santa Clara *Sw*-5/*Sw*-5' and *S. pimpinellifolium* 'TO-937' (both used as susceptible controls). On the other hand, it was possible to confirm again the low disease severity observed in plants of the *S. lycopersicum* 'LT05' and *S. habrochaites* PI 127827. Plants of *S. pennellii* LA0716, *S. habrochaites* PI 126445, and *S. chilense* LA1967 displayed low symptom severity under these

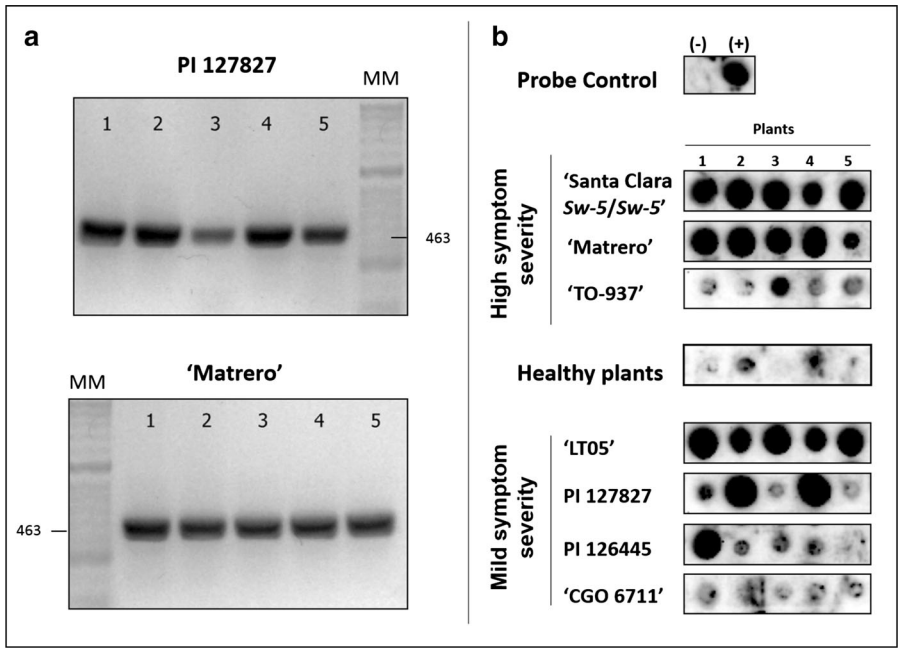

Fig. 2 **a** Evaluation of *Solanum* (section *Lycopersicon*) accessions for reaction to *Tomato chlorosis virus* (ToCV at 100 days after planting. All five plot plants of *S. habrochaites* PI 127827 (with mild symptom severity = tolerant) and *S. lycopersicum* 'Matrero' (high symptom severity = sensitive) were analyzed for ToCV systemic invasion by reverse transcription-polymerase chain reaction (RT-PCR). The expected (≈ 463-bp) ToCV-specific amplicons were observed in all plants. **b** Evaluation carried out at 100 days after planting. All five plants of each plot of the three accessions classified as sensitive and four accessions classified as tolerant were analyzed for presence of ToCV by a standardized dot-blot hybridization using a ToCV-derived probe. All drops contain 1.0 µg of total host plant RNA

conditions. Interestingly, plants of the *S. lycopersicum* cultivars 'Kumato' and 'Gostomiel' (carrying the "*green-flesh*" – *gf* mutation) displayed medium to low symptom severity. The remaining *S. lycopersicum* accessions evaluated in this assay were found to be susceptible to ToCV, including two *S. lycopersicum* × *S. pimpinellifolium* lines ('BC3F3' and 'BC5F5') derived from an introgression program aiming to incorporate the insect tolerance traits (high acylsugar and type IV glandular trichomes) from *S. pimpinellifolium* 'TO-937' into cultivated tomato cv. 'Moneymaker' (Rodriguez-López et al. 2011, Escobar-Bravo et al. 2016).

Evaluation of *Solanum* (*Lycopersicon*) germplasm to *T. vaporariorum* in Uruguay (WF trial #1)

The same subgroup of accessions tested in the ToCV trial #2 was additionally estimated for whitefly preference by evaluation of oviposition (Table 3). Accessions displayed varying levels of *T. vaporariorum* oviposition. However, the differences observed among these accessions were not significant. For the accessions under evaluation, there was no significant correlation between ToCV symptom severity and *T. vaporariorum* preference based upon oviposition.

Evaluation of *Solanum* (*Lycopersicon*) germplasm to *B. tabaci* MEAM 1 in Brazil (WF trial #2)

A subgroup of 16 tomato accessions with contrasting reactions to ToCV were also evaluated against *B. tabaci* MEAM 1 in a free-choice bioassay in Brazil (Table 4). The *S. pennellii* accession LA0716 displayed the highest level of resistance to *B. tabaci* MEAM1 based on oviposition ($6.0 \times 10^{-3} \pm 3.0 \times 10^{-3}$ eggs/cm^2), but this value was not statistically different from oviposition rates of *S. pimpinellifolium* 'TO-937' and *S. habrochaites* PI 134417 with 5.62 ± 2.29 and 4.24 ± 1.93 eggs/cm^2, respectively. However, it was not possible to differentiate these latter two accessions from a final subgroup of materials that were characterized by presenting the highest levels of oviposition (high susceptibility). 'TO937'

Table 3 Evaluation of reaction to *Tomato chlorosis virus* (ToCV) and whitefly (*T. vaporariorum*) oviposition in nine selected *Solanum* (section *Lycopersicon*) accessions under greenhouse conditions and natural infection. Salto, Uruguay. (*ToCV trial #2*). Fall, 2014

Solanum (*Lycopersicon*) accession	No. of infected plants/ Total no. of evaluated plants[w]	AUSPC[x]	Symptom severity[y]	Eggs (cm^2)[z]
S. lycopersicum 'LT05'	10/10	3.3 ± 1.7^{a}	0.1 ± 0.1^{a}	1.25 ± 0.24
S. habrochaites PI 127827	8/10	5.0 ± 5.0^{a}	0.2 ± 0.2^{a}	0.75 ± 0.31
S. lycopersicum 'Elpida'	10/10	10.8 ± 2.5^{ab}	0.3 ± 0.1^{ab}	1.30 ± 0.10
S. lycopersicum 'Swanson'	10/10	14.6 ± 3.4^{ab}	0.4 ± 0.1^{abc}	1.00 ± 0.22
S. lycopersicum 'Torry'	10/10	27.5 ± 8.0^{abc}	0.8 ± 0.3^{abcd}	1.10 ± 0.58
S. lycopersicum 'LT17'	10/10	49.6 ± 5.4^{bc}	1.7 ± 0.2^{bcd}	0.55 ± 0.38
S. lycopersicum 'Matrero'	10/10	50.0 ± 9.8^{bc}	1.8 ± 0.4^{bcd}	1.73 ± 0.37
S. lycopersicum 'Santa Clara (*Sw*-5/ *Sw*-5)'	8/10	65.4 ± 5.6^{c}	2.1 ± 0.1^{cd}	0.90 ± 0.47
S. pimpinellifolium 'TO-937'	8/10	72.1 ± 4.1^{c}	2.4 ± 0.1^{d}	0.70 ± 0.17

[w]Evaluation carried out at 50 and 90 days after planting. Plants were analyzed for presence of ToCV by tissue-print hybridization using a ToCV-derived probe

[x]Area under severity progress curve (AUSPC). Values are displayed as mean ± standard error of three replicates. Treatments within the column followed by different letter are significantly different ($p < 0.05$) by non-parametric multiple comparisons of ranks after Kruskal–Wallis test. (H = 22.73; p = 0,0037)

[y]Evaluation carried out at 90 days after planting, where 0 = no conspicuous ToCV-induced symptoms; 3 = severe ToCV-induced symptoms. Values are mean ± standard error of three replicates. Treatments within the column followed by different letter are significantly different ($p < 0.05$) by non-parametric multiple comparisons of ranks after Kruskal–Wallis test. (H = 21.94; p = 0.0046)

[z]Whitefly (*T. vaporariorum*) eggs per cm^2 of leaflet. Values are mean ± standard error of four plants (replicates). No statistical differences between ranks sums of accessions were observed for the Friedman's test (Fr = 10.271; p = 0.246520)

was only significantly better than the accession PI 126445, whereas *S. habrochaites* PI 134417 displayed lower ovipositon levels than five accessions (Table 4).

Discussion

To date, there is no commercial tomato cultivar reported with adequate levels of resistance to ToCV. This may be a result of a reduced priority in breeding for resistance against this virus when compared with the solid efforts conducted for begomoviruses (genus *Begomovirus*, family *Geminiviridae*) resistance due to their prevalence in all major tomato-producing regions around the world (Giordano et al., 2005; Pereira-Carvalho et al. 2015). In addition, for most tomato farmers, ToCV infection is often misdiagnosed as being induced by nutritional deficiency with no conspicuous effects on fruit yield and quality. More recently, after the massive deployment of begomovirus resistant hybrids (Boiteux et al. 2007), the losses induced by ToCV infection are becoming more evident. In this new scenario, growers and the tomato agribusiness sector are demanding genetic solutions to this problem. However, tomato breeding programs are facing some difficulties since the currently available sources of resistance (García-Cano et al. 2010; Mansilla-Córdova et al. 2018) are not easily incorporated into elite commercial material.

The present work summarizes our breeding efforts to identify and characterize potential novel sources of resistance/tolerance to ToCV, involving an extensive set of bioassays (under both natural and controlled viruliferous and aviruliferous whitefly exposure) with a diverse *Solanum* (*Lycopersicon*) germplasm collection. These assays were carried out in vegetable-producing areas in South America where severe ToCV infections are reported in tomatoes as well as in several other cultivated and weed Solanaceae species (Arruabarrena et al. 2014, 2015; Macedo et al. 2014; Fonseca et al. 2016; Boiteux et al. 2018).

Table 4 Evaluation of reaction to *Tomato chlorosis virus* (ToCV) and of whitefly (*Bemisia tabaci* MEAM 1) oviposition in 16 *Solanum* (section *Lycopersicon*) accessions. Assays carried out under greenhouse conditions in Brasilia–DF, Brazil (*ToCV trial #3*). Fall, 2015

Solanum (*Lycopersicon*) accession	No. of infected plants/No. of total analyzed plants[x]	Symptom severity[y]	Eggs (cm^2)[z]
S. pennellii LA0716	6/6	0.2 ± 0.1^{a}	0.006 ± 0.003^{d}
S. habrochaites PI 126445	6/6	0.3 ± 0.2^{ab}	25.85 ± 8.90^{a}
S. chilense LA1967	6/6	0.3 ± 0.1^{ab}	21.83 ± 5.78^{ab}
S. lycopersicum 'LT05'	6/6	0.5 ± 0.1^{ab}	10.86 ± 1.34^{abc}
S. habrochaites PI 127827	6/6	0.6 ± 0.3^{abc}	8.38 ± 2.00^{abc}
S. lycopersicum 'Gostomiel'	6/6	0.9 ± 0.1^{abcd}	15.36 ± 3.86^{abc}
S. lycopersicum 'Kumato'	6/6	1.2 ± 0.1^{abcde}	9.91 ± 2.79^{abc}
S. habrochaites PI 134417	6/6	1.4 ± 0.1^{abcde}	4.24 ± 1.93^{cd}
S. lycopersicum × *S. pimpinellifolium* 'BC3F3'	6/6	2.0 ± 0.2^{bcde}	8.41 ± 3.06^{abc}
S. lycopersicum 'TX 468 RG'	6/6	2.1 ± 0.2^{cde}	23.28 ± 6.99^{ab}
S. pimpinellifolium 'TO-937'	6/6	2.3 ± 0.1^{cde}	5.62 ± 2.29^{bcd}
S. lycopersicum 'LT17'	6/6	2.4 ± 0.2^{de}	6.81 ± 2.34^{abc}
S. lycopersicum × *S. pimpinellifolium* 'BC5F5'	6/6	2.6 ± 0.1^{e}	7.75 ± 1.47^{abc}
S. lycopersicum 'Viradoro'	6/6	$2.6 \pm 0.1e$	24.10 ± 6.60^{ab}
S. lycopersicum 'Santa Clara (*Sw*-5/*Sw*-5)'	6/6	2.6 ± 0.0^{e}	10.12 ± 1.38^{abc}
S. lycopersicum 'Santa Clara (*sw*-5/*sw*-5)'	6/6	2.7 ± 0.1^{e}	17.04 ± 3.42^{ab}

[x]Evaluation carried out at 70 days after planting. The two central plants from each plot (six plants) were analyzed for presence of ToCV by tissue-print hybridization using a ToCV-derived probe

[y]Evaluation carried out at 70 days after planting, where 0 = no conspicuous ToCV-induced symptoms; 3 = severe ToCV-induced symptoms. Values are displayed as mean ± standard error of three replicates. Treatments within the column followed by different letter are significantly different ($p < 0.05$) by non-parametric multiple comparisons of ranks after Kruskal–Wallis test (H = 40.86; $p = 0.0003$)

[z]Whitefly (*Bemisia tabaci* MEAM 1) eggs per cm^2 of the sampled leaflet. Values are displayed as mean ± standard error for ten plants (replicates). Different letters within the column indicate significant difference between the rank sum of each treatment (Friedman multiple pair-wise test, followed by sequential Holm adjustment: $p < 0.05$). (Fr = 63.560; $p < 0.0001$)

The use of terms related to the response of plants to a virus infection was taken from Cooper and Jones (1983). In this way, when we describe the behavior of virus in the plant (e.g. viral titer) we will refer to resistance/susceptibility, whereas when we describe the response of the plant to the disease (e.g. symptom severity) we will refer to tolerance/sensitivity. Consistent infections were observed in the sensitive controls (which were included in all independent trials), confirming the high ToCV pressure in across all assays. Most of the cultivated tomato (*S. lycopersicum*) accessions were systemically infected by ToCV and displayed high to medium symptom severity levels (= sensitivity). However, it was possible to identify the *S. lycopersicum* inbred line 'LT05' (high tolerance in ToCV trial #1, #2 and #3) and in a second step the commercial F_1 hybrids 'Elpida' (high tolerance in ToCV trial #1 and #2) and 'Swanson' (high tolerance in ToCV trial #1 and intermediate in #2). All *S. lycopersicum* were preferred by *T. vaporariorum* based upon oviposition values, indicating that tolerance to ToCV found in some accessions is likely not to be related to vector resistance mechanisms. For these tolerant accessions, it was possible to observe, in the conditions described for ToCV trial #2, a lower rate of symptom progression when compared with more sensitive accessions.

Previous studies have identified a subgroup of *S. lycopersicum* accessions with high levels of field tolerance to ToCV-induced symptoms expression in Spain (García-Cano et al. 2010). However, this tolerance was not expressed under high vector

pressure conditions. Based upon our experiments, we cannot exclude the possibility that the lower severity of symptoms observed in 'Elpida' and 'Swanson' would be stable under either high disease pressure conditions or under longer crop production cycles. However, the *S. lycopersicum* breeding line 'LT05' confirmed the low severity of ToCV-induced symptoms across all bioassays corresponding to three different environmental conditions. In this case, all evaluated plants displayed strong hybridization signals in the dot-blot assays, indicating systemic infection of ToCV and, therefore, we discarded the potential involvement of resistance mechanisms related to limitation of virus infection (i.e. multiplication and transport within the plant). In this way, employing the concept defined by Cooper and Jones (1983), we identify a stable source of tolerance to ToCV-induced symptom development in the inbred line 'LT05'. This inbred line may be particularly a source of ToCV tolerance for breeders, based on its high and stable levels of ToCV tolerance and due to the fact that it is a cultivated tomato (*S. lycopersicum*) which will facilitate its employment in genetic improvement programs. Future studies would be necessary to investigate the genetic basis of the ToCV tolerance in 'LT05' and the expected impact of this trait on tomato fruit yields.

In the ToCV trial #3 carried out in Brazil, two *S. lycopersicum* accessions 'Kumato' and 'Gostomiel' were identified with low to medium levels of symptom severity (Table 4). Coincidently, these two cultivars have the presence (in homozygous condition) of the *green-flesh* (*gf*) mutation (Kerr 1956), which is located on the long arm of the tomato chromosome 8 (Kerr 1958). Its major effect includes the inhibition of chlorophyll degradation since affects the normal function of a STAY-GREEN (SGR) protein (Akhtar et al. 1999). Therefore, it would be interesting to further investigate if the *gf* mutation could be responsible for the attenuation of the ToCV-induced symptoms by limiting the severe manifestation of foliar chlorosis, which is typically associated with this virus infection in susceptible tomatoes.

In wild tomato species, some stable phenotypes with low symptom severity were identified in *S. habrochaites* PI 127827 and PI 126445. In the case of *S. habrochaites* PI 127827, individual plants with contrasting responses were identified after analyses with RT-PCR and dot-blot hybridization assays. RT-PCR indicated the prevalent systemic presence of ToCV, but some plants display very low signals in the hybridization assay (Fig. 2a, b). Therefore, it would be interesting to investigate if some mechanism of virus resistance could still be segregating in this accession as it is an allogamous species that can display a high variability as well as heterozygosity. Further studies from inbred lines generated by self-pollination of resistant plants (i.e. the ones with low viral accumulation) should be conducted to evaluate this aspect. The potential involvement of vector-resistance mechanisms was discarded, since *S. habrochaites* PI 127827 was susceptible to *T. vaporariorum* colonization in WF trial #1 (not different to other accessions) and *B. tabaci* MEAM1 colonization in WF trial #2 (not different to the sensitive subgroup of accessions), whereas *S. habrochaites* PI 126445 was susceptible to *B. tabaci* MEAM1 colonization based upon the results of the WF trial #2.

Other wild species accessions that displayed low symptom severity were *S. peruvianum* 'CGO 6711' (in the ToCV trial #1), *S. chilense* LA1967, and *S. pennellii* LA0716 (in the ToCV trial #3). In the case of *S. peruvianum* 'CGO 6711', it is interesting to note that the analysis of individual plants under evaluation by standardized dot-blot hybridization indicated very low hybridization signals in all five samples (Fig. 2b). Again, this could be related to some mechanism of resistance to virus accumulation (multiplication and/or movement within the plant). In this case, we cannot yet discard the potential involvement of vector resistance mechanisms, as *S. peruvianum* 'CGO 6711' could not be included in the WF trials. Therefore, further studies are needed with this accession. No standardized viral titer evaluations were performed for *S. chilense* LA1967 and *S. pennellii* LA0716. However, *S. chilense* LA1967 displayed high levels of *B. tabaci* MEAM1 susceptibility based upon oviposition values (Table 4). Thus, its low level of ToCV symptom severity is likely unrelated to vector resistance. *Solanum pennellii* LA0716 was the only accession that showed a clear-cut resistance for *B. tabaci* MEAM1 infestation (Table 4). Therefore, it is probably that the mild ToCV symptoms observed *S. pennellii* LA0716 could be related, at least in part, to extreme resistance to *B. tabaci* MEAM1. It is also important to point out the light green leaf color of *S. pennellii* LA0716, which makes difficult a precise assessment of ToCV symptoms in plants derived from

this germplasm. In fact, ToCV symptoms in *S. pennellii* LA0716 is manifested, in general, by an even lighter greenish leaf color rather than the typical chlorosis and leaf patches observed in other *Solanum* species. This is the first report of *S. pennellii* as possible source of tolerance to ToCV, although it has already been reported as displaying a significant delay of TICV symptom expression, which was also associated with its low whitefly preference (Mutschler and Wintermantel 2006).

The wild species *S. peruvianum*, *S. chilense*, and *S. habrochaites* have been reported as sources of ToCV resistance in previous works (García-Cano et al. 2010; Mansilla-Córdova et al. 2018). In fact, the accession 'IAC-CN-RT5' (hybrid from *S. lycopersicum* cv. 'Angela Gigante' × *S. peruvianum* LA0444-1) and the line '802-11-1' (derived from two cycles of selfing and selection of 'IAC-CN-RT5') displayed some levels of resistance (low systemic infection and reduced virus multiplication), coincident with our preliminary results for *S. peruvianum* 'CGO 6711' in ToCV trial #1.

The accessions of *S. pimpinellifolium* evaluated here have consistently shown high levels of ToCV symptom severity, in particular *S. pimpinellifolium* 'TO-937', the material chosen as our sensitive reference. However, standardized dot-blot hybridization analysis showed low hybridization signals in some of *S. pimpinellifolium* 'TO-937' plants (Fig. 2b), which could be related to levels of resistance to virus infection (multiplication and/or movement within the plant) or some mechanism of interference with the vector, which in our case was confirmed for *B. tabaci* MEAM1 (Table 4), corroborating previously results obtained for *B. tabaci* MED in Spain (Rodríguez-López et al. 2011) and for *B. tabaci* MEAM1 in Brazil (Silva et al. 2014). Thus, *S. pimpinellifolium* 'TO-937' could have an interesting case of extremely high ToCV sensitivity, with higher levels of symptom severity even with low viral titers, which might merit further investigation.

Mansilla-Córdova et al. (2018), studying the tolerance to ToCV in a set of commercial tomato cultivars, found that most of the evaluated materials did not display significant yield and quality losses. However, some cultivars were highly sensitive, showing reductions up to 58.1 and 71.9% in total fresh weight and fruit yield, respectively. Perhaps, this cultivar-specific trait of high sensitivity to ToCV infection (as observed for *S. pimpinellifolium* 'TO-937') might be also present in some of the currently available commercial tomato hybrids. The unaware use of these highly sensitive accessions could be an important factor in the emergence of ToCV for many tomato-producing regions around the globe. A similar phenomenon was observed in the epidemiology of *Tomato torrado virus* (ToTV), another whitefly-transmitted RNA virus, which is emerging in some regions of the world (Navas-Castillo et al. 2014).

In conclusion, our work allowed the identification of a subset of stable sources of ToCV tolerance and some novel promising sources of virus resistance (*sensu* Cooper and Jones (1983). Among the stable sources, we highlight the *S. lycopersicum* breeding line 'LT05' (with high level tolerance to ToCV-induced symptoms even displaying high viral titers) and *S. habrochaites* accessions PI 127827 and PI 126445 (whose reactions may involve some mechanisms of restriction to virus accumulation). We also documented *S. peruvianum* 'CGO 6711' as a potential source of high resistance levels, which was associated with low viral titers. However, we were not able to exclude the potential involvement of vector resistance mechanisms of *S. peruvianum* 'CGO 6711' since it was not included in our WF trails due to the low amount of seeds available. Other tolerant accession, *S. chilense* LA1967, may have more likely resistance mechanism to the virus since it displayed high levels of *B. tabaci* MEAM1 infestation. In the other hand, *S. pennellii* LA0716 might have its tolerance to ToCV associated with interference with the vectors, in agreement with previous works with TICV (Mutschler and Wintermantel 2006). It will be of breeding interest to carry out additional inheritance and genomic mapping studies with these promising sources aiming to elucidate if they share similar tolerance/resistance mechanisms with previously identified sources or if they are under a less complex genetic control.

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