Multiple Resistance to *Meloidogyne* spp. and to Bipartite and Monopartite *Begomovirus* spp. in Wild *Solanum* (*Lycopersicon*) Accessions

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ABSTRACT

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The *Ty-1* locus confers tolerance to monopartite and bipartite *Begomovirus* spp. (genus *Begomovirus*, family *Geminiviridae*) and this phenotype is improved in homozygous tomato lines. However, the gene *Mi* (*Meloidogyne* spp. resistance) is in repulsion phase linkage with *Ty-1*, which hampers the large-scale development of multiresistant inbred lines. Seventy-one *Solanum* (section *Lycopersicon*) accessions were whitefly inoculated with the bipartite *Begomovirus* sp. *Tomato rugose mosaic virus* (ToRMV) and simultaneously infested with a mixture of *Meloidogyne incognita* and *M. javanica* under greenhouse conditions in Brazil. Accessions were then transplanted into a nematode-infested field with natural ToRMV infection. A severity index was used to evaluate ToRMV reaction. Nematode evaluation was done by counting the number of galls per root system. Seventeen accessions with *Meloidogyne* spp. and ToRMV resistance were selected and evaluated in Spain against three monopartite *Begomovirus* spp. associated with the tomato yellow leaf curl virus disease, using infectious clones. Systemic infection was monitored by DNA hybridization. Five *S. peruvianum* accessions (PI-306811, PI-365951, LA-1609, LA-2553, and CNPH-1194) displayed nematode and broad-spectrum resistance to all *Begomovirus* spp. tested in both continents. From the breeding standpoint, accessions combining resistance to *Meloidogyne* spp. and to bipartite and monopartite *Begomovirus* spp. would be useful for the development of elite lines expressing all traits in homozygous condition.

The *Geminiviridae* family is composed of viruses with either one or two genomic components of circular, single-stranded DNA encapsidated into icosahedral particles (16). The family *Geminiviridae* contains four genera (*Mastrevirus*, *Curtovirus*, *Topocuvirus*, and *Begomovirus*) based upon their genome organization, insect vectors, and host range. The genus *Begomovirus* has the largest number of characterized species within the family (16). These viruses are considered one of the major threats for tomato (*Solanum lycopersicum* L. = *Lycopersicon esculentum* Mill.) production in tropical and subtropical areas (41). *Begomovirus* spp. have been reported in all continents infecting a wide range of dicot host plants, including tomato (2,16,27,39,41,52,57). Outbreaks of

Begomovirus spp. are mainly associated with population increase of their insect vector, the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), which is well adapted to warm climates (11–13,39,52,57).

In the Old World, notably in the Mediterranean Basin, several monopartite *Begomovirus* spp. causing the tomato yellow leaf curl disease (TYLCD) are prevalent and cause severe damage in tomato (2,31,37). The TYLCD-associated *Begomovirus* spp. complex currently consists of several tentative and 11 fully characterized virus species, among which is *Tomato yellow leaf curl virus* (TYLCV). Monopartite species associated with the TYLCD have also spread to the New World, causing severe yield losses in North America (26,56). TYLCD was also recently reported in Central America, the Caribbean region, and the northern part of South America (44,45).

In Brazil, thus far, only bipartite *Begomovirus* spp. have been reported (9,15,17,22,27,46,47), with dramatically increased incidence after the introduction of *B. tabaci* biotype B in the country (19). Field

surveys in Brazil showed that infection by *Begomovirus* spp. in tomato might cause up to 100% yield losses (6,22,46,47). The rapid spread of the biotype B and its wide host range may have contributed to the natural transfer of new *Begomovirus* spp. from weeds and native solanaceous species to cultivated tomato, thus resulting in the significant increase in the number of new tomato-infecting *Begomovirus* spp. (17,27,46,47).

Breeding strategies intending to introgress genetic resistance or tolerance to *Begomovirus* spp. from wild *Solanum* spp. (section *Lycopersicon*) (40) to cultivated *S. lycopersicum* has been considered to be the best control measure (33), especially due to the difficulty of controlling virus epidemics via the chemical control of the vector. Distinct sources of resistance to either TYLCD-associated viruses or Brazilian *Begomovirus* spp. have been reported in accessions of the following wild species: *S. pimpinellifolium* L., *S. habrochaites* Knapp & Spooner, *S. peruvianum* L., *S. cheesmaniae* (Riley) Fosberg, and *S. chilense* (Dunal) Reiche (18,41–43,51). Thus far, the most important loci employed in tomato breeding programs for *Begomovirus* spp. resistance are *Ty-1* (36,61,62), *Ty-2* (24,25), *Ty-3* (28,29), *tcm-1* (20,23), and *tgr-1* (7). These loci are currently being used either alone or in pyramiding strategies (59). The *Ty-1* locus was introgressed from the wild species *S. chilense* (36,61,62), whereas the *Ty-2* locus was introgressed from *S. habrochaites* (24,25). The recessive resistance to *Begomovirus* spp. found in the Brazilian line TX-468-RG is conferred by the *tcm-1* gene, which was characterized in a *S. lycopersicum* inbred line derived from selfing the hybrid Tyking (20,23). The locus *tgr-1*, present in the tomato inbred line FLA653, was derived from a cross between *S. chilense* (LA-2779) and Tyking (7). More recently, the partially dominant *Ty-3* resistance gene (which confers resistance to TYLCV as well as tolerance to bipartite *Begomovirus* spp. infecting tomato in North America) was introgressed from crosses derived from *S. chilense* LA-2779 (28,29).

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Tomato plants are also affected by nematode species of the genus *Meloidogyne* (family *Meloidogynidae*) (55), the causal agents of the root-knot disease. This soilborne disease is especially severe in tropical and subtropical areas of the world (58,60). Genetic resistance is the best strategy to limit damage caused by the root-knot disease (60). Resistance to *Meloidogyne* spp. due to the single dominant gene *Mi* were identified in *S. peruvianum* accessions (5,50,60), and this gene was successfully introgressed into cultivated tomato (54). The introgression of the *Mi* gene into commercial tomato cultivars was an important breeding achievement, minimizing yield and quality losses (10). The *Mi* gene is located in chromosome 6 and confers broad-spectrum resistance to isolates and races of three root-knot nematode species (*Meloidogyne incognita*, *M. javanica*, and *M. arenaria*) but not to *M. hapla* (3,21,35). However, the *Mi* resistance expression is not stable with soil temperatures above 28°C (4) and it is also ineffective against some resistance-breaking isolates of *M. incognita*, *M. javanica*, and *M. arenaria* (10,30). More recently, other resistance genes or alleles (named from *Mi-2* to *Mi-9*) have been reported in either chromosome 6 or chromosome 12 (60). Some of these genes or alleles conferred resistance to *M. hapla* and to *Mi*-breaking isolates, being also effective under high soil temperature (4,60).

The *Mi* and *Ty-1* loci have been introgressed into cultivated tomato from different wild *Solanum* sources and have been mapped in close repulsion phase linkage (i.e., linkage between one desirable and one undesirable trait) in chromosome 6 (14,36,62). The initial efforts to combine homozygous resistance to *Begomovirus* spp. and nematodes in single inbred lines or cultivars were unsuccessful. Genetic studies revealed that, in the accession *S. peruvianum* PI-128657, resistance to *Meloidogyne* spp. (*Mi/Mi*) is linked with susceptibility to *Begomovirus* spp. (*ty-1/ty-1*), whereas, in *S. chilense* LA-1969, resistance to *Begomovirus* spp. (*Ty-1/Ty-1*) is linked with susceptibility to *Meloidogyne* spp. (*mi/mi*) (14,62). In addition, there is experimental evidence showing that improved levels of resistance can be obtained when the *Ty-1* locus is in the homozygous condition (9). Therefore, this repulsion-phase linkage hampers the large-scale development of stable and multiresistant inbred lines and hybrids. In this context, the identification of broad resistance sources to both groups of pathogens is highly desirable, especially for tropical regions where tomato is cultivated under high inoculum pressure from both groups of pathogens. The present work aimed to search in a large *Solanum* (section *Lycopersicon*) collection for new breeding sources combining resistance to both mo-

nopartite and bipartite *Begomovirus* spp. as well as to *Meloidogyne* spp.

MATERIALS AND METHODS

Simultaneous evaluation of *Solanum* (section *Lycopersicon*) accessions for resistance to Tomato rugose mosaic virus and to *Meloidogyne* spp. in Brazil. This evaluation was conducted at the experimental station of the National Center for Vegetable Crops Research (CNPV)/ EMBRAPA (Brasília-DF, Brazil). In total, 71 *Solanum* (section *Lycopersicon*) accessions were evaluated (46 *S. peruvianum*, 9 *S. pimpinellifolium*, 6 *S. lycopersicum*, 5 *S. habrochaites*, 2 *S. chilense*, 1 *S. corneliomuelleri*, and 2 accessions derived from interspecific crosses between *S. lycopersicum* and *S. peruvianum*; Table 1). Plants from each accession were inoculated in a greenhouse at 20 days after sowing (two- to three-leaf growth stage) by mixing equal amounts of eggs obtained from populations of *M. incognita* race 1 and *M. javanica* (6,000 eggs per plant). Simultaneously, plants were also exposed during 10 days to a population of viruliferous *B. tabaci* reared on Tomato rugose mosaic

virus (ToRMV) infected source plants. The inoculated plants were then transplanted (34 days after sowing) into field plots naturally infested with a mixture of *M. incognita* race 1 and *M. javanica*. Natural *Begomovirus* spp. infections also occurred and, although other *Begomovirus* spp. have been reported in Brazil, ToRMV was the predominant virus under the open field conditions during this study. The experimental field was divided into nine blocks with seven accessions and one block with eight accessions in an augmented block design. The internal controls (standards) included in each augmented block were plots with 10 plants of *S. lycopersicum* cv. Viradoro (susceptible to ToRMV and resistant to *Meloidogyne* spp. due to the presence of the *Mi* gene) and 10 plants of *S. lycopersicum* TX-468-RG (resistant to ToRMV due to the presence of the *tcM-1* locus and susceptible to *Meloidogyne* spp.; 20,23). The number of plants tested per accession varied because seed germination of the wild accessions was not uniform. The evaluations for ToRMV resistance were carried out at 30 days after field transplanting using the scale from 0 to 4

Table 1. Reaction of *Solanum* (section *Lycopersicon*) accessions to Tomato rugose mosaic virus (ToRMV) and to a mixed population of *Meloidogyne incognita* and *M. javanica* in Brasília-DF, Brazil

Species of <i>Solanum</i> (section <i>Lycopersicon</i>)	Accession no. or name	SSI means (ToRMV) ^a	No. of plants free of <i>Meloidogyne</i> galls/total tested plants
<i>S. lycopersicum</i>	TX-468-RG ^b	0.35	00/34
<i>S. lycopersicum</i>	Viradoro ^c	2.78	43/44
<i>S. lycopersicum</i>	Kada	2.59	00/11
<i>S. lycopersicum</i>	Angela Hiper	2.43	00/14
<i>S. lycopersicum</i>	CNPV-0095	1.51	00/17
<i>S. lycopersicum</i>	CNPV-0202	2.00	00/16
<i>S. lycopersicum</i>	CNPV-0457	0.97	00/07
<i>S. lycopersicum</i>	CNPV-1478	1.88	00/01
<i>S. chilense</i>	LA-1967	0.15	01/24
<i>S. chilense</i>	CNPV-0943	0.66	00/08
<i>S. pimpinellifolium</i>	PI-126436	2.38	00/03
<i>S. pimpinellifolium</i>	PI-126925	2.25	00/05
<i>S. pimpinellifolium</i>	PI-126931	1.13	00/05
<i>S. pimpinellifolium</i>	CNPV-0597	1.43	00/05
<i>S. pimpinellifolium</i>	CNPV-0769	0.75	04/04
<i>S. pimpinellifolium</i>	LA-1341	0.75	02/18
<i>S. pimpinellifolium</i>	LA-1342	0.75	01/01
<i>S. pimpinellifolium</i>	LA-1584	0.32	00/06
<i>S. pimpinellifolium</i>	CNPV-1195	0.57	08/08
<i>S. corneliomuelleri</i>	CNPV-1033	0.00	01/01
<i>S. habrochaites</i>	LM-041	0.14	02/20
<i>S. habrochaites</i>	PI-127826	0.00	00/05
<i>S. habrochaites</i>	PI-127827	0.17	00/03
<i>S. habrochaites</i>	PI-134417	0.19	00/05
<i>S. habrochaites</i>	CNPV-1034	0.17	05/05
<i>S. peruvianum</i>	PI-306811	0.05	08/08
<i>S. peruvianum</i>	CNPV-0782	0.75	06/06
<i>S. peruvianum</i>	CNPV-0783	0.28	16/16
<i>S. peruvianum</i>	CNPV-0785	0.13	07/07
<i>S. peruvianum</i>	CNPV-0786	0.46	06/06
<i>S. peruvianum</i>	CNPV-0787	0.23	08/08
<i>S. peruvianum</i>	LA-1616	0.27	28/28

(continued on next page)

^a Symptom severity index (33), where 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaves; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing and curling, with some reduction in size, yet plants continue to develop; and 4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stops.

^b *Begomovirus* spp.-resistant and *Meloidogyne* spp.-susceptible control.

^c *Begomovirus* spp.-susceptible and *Meloidogyne* spp.-resistant control.

^d *S. lycopersicum* × *S. peruvianum*.

proposed by Lapidot et al. (34) with minor modifications, where 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaves; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing and curling, with some reduction in size, yet plants continue to develop; and 4 = very severe plant stunting and yellowing and pronounced leaf cupping and curling. Nematode evaluation was done at the end of the plant's vegetative cycle (approximately 100 days after field transplanting) by visual scoring for the presence or absence as well as the number of nematode galls in the root system.

Young terminal leaflets (0.5 g of tissue) from accessions scoring between 0 and 1 were sampled at 30 days after field transplanting and analyzed for the presence of ToRMV infection via polymerase chain reaction (PCR). Symptomatic leaf samples of Viradoro were also included as internal controls. Total DNA was extracted from each plant according to a modified cetyltrimethylammonium bromide protocol (8). The DNA quality was confirmed by electrophoresis on 1.2% agarose gels stained

with ethidium bromide. PCR amplification was conducted using the primer pair PAL1v1978/PARc715 broadly used for universal detection of *Begomovirus* spp. (49), including ToRMV (17). The reactions were performed using a Gene Amp PCR System 9700 thermocycler in a total volume of 12.5 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.4 mM MgCl₂, 250 µM each deoxiribonucleotide (dATP, dTTP, dGTP, and dCTP), 0.8 M each primer, and one unit of *Taq* polymerase with 30 ng of DNA template. Tomato DNA purified from healthy plants and from ToRMV-infected plants were used as negative and positive PCR controls, respectively. The PCR-amplified products were analyzed in 1.2% agarose gels.

Evaluation of *Solanum* (section *Lycopersicon*) accessions selected in Brazil for resistance to monopartite *Begomovirus* spp. associated with the TYLCD in Spain. This evaluation was carried out at the Experimental Station of La Mayora/CSIC (Málaga, Spain) under insect-proof greenhouse conditions. Seventeen accessions previously selected for resistance to the bipartite *Begomovirus* sp.

ToRMV and *Meloidogyne* spp. in Brazil (Table 2) were further assayed for resistance or tolerance to monopartite *Begomovirus* spp. associated with TYLCD in Spain. The infectious clone of one isolate of the ES strain of *Tomato yellow leaf curl Sardinia virus* (TYLCSV-ES [ES:Mur1:92], GenBank accession number Z25751) and of isolates of the strains IL and Mld of *Tomato yellow leaf curl virus* (TYLCV-IL [ES:ALm:99] and TYLCV-Mld [ES:72:97], GenBank accession numbers AJ489258 and AF071228, respectively) were used as inoculum. In all, 30 plants of each accession (10 plants per virus tested) were agro-inoculated (20,38) at the three- to four-leaf growth stage. The experimental design was a randomized complete block with two replications of five plants each. The inoculated plants were scored for symptoms at 17 and 30 days post inoculation (dpi) using the scale from 0 to 4 proposed by Lapidot et al. (34) (see above). The presence of viral DNA in young, noninoculated tissues was also analyzed via tissue-blot molecular hybridization (see below). The accession TX-468-RG (resistant to monopartite and bipartite *Begomovirus* spp.) and cv. Moneymaker (susceptible to all known *Begomovirus* spp.) were included as controls. Either the second or the third leaf (from the plant tip) was removed and cross-sections of leaf petioles were squash blotted (tissue printed) on a positively charged nylon membrane (Nylon⁺, Roche Diagnostics). Two replicated tissue prints were done per plant. Samples from healthy Moneymaker plants and from Moneymaker plants infected with known viruses were used as negative and positive controls, respectively. Virus detection was conducted by molecular hybridization as described (1), using a mixture of digoxigenin-labeled DNA probes able to detect all the TYLCD-associated *Begomovirus* spp. under evaluation (38). The accession TX-468-RG, resistant to monopartite and bipartite *Begomovirus* spp. (20,23), and cv. Moneymaker, susceptible to all known *Begomovirus* spp., were included as controls.

RESULTS

Reaction of *Solanum* (section *Lycopersicon*) accessions to ToRMV and *Meloidogyne* spp. in Brazil. Plants of the control cv. Viradoro were fully susceptible to ToRMV but were resistant to *Meloidogyne* spp. (Table 1). In contrast, the line TX-468-RG exhibited resistance to ToRMV but was susceptible to *Meloidogyne* spp. (Table 1). The results of the control plants indicated that a uniform inoculation pressure was obtained for both pathogens and that suitable environmental conditions occurred for symptoms expression during the trial. Forty-one accessions (from the wild species *S. chilense*, *S. pimpinellifolium*, *S. habrochaites*, and *S. peruvianum*) exhibited a resistance reaction to ToRMV, similar to that of the resistant

Table 1. (continued from preceding page)

Species of <i>Solanum</i> (section <i>Lycopersicon</i>)	Accession no. or name	SSI means (ToRMV) ^a	No. of plants free of <i>Meloidogyne</i> galls/total tested plants
<i>S. peruvianum</i>	LA-1270	0.45	10/10
<i>S. peruvianum</i>	LA-1677	0.38	26/26
<i>S. peruvianum</i>	CNPH-0934	0.25	01/01
<i>S. peruvianum</i>	CNPH-0936	0.80	30/30
<i>S. peruvianum</i>	LA-1113-1	0.00	03/04
<i>S. peruvianum</i>	CNPH-0939	0.73	05/05
<i>S. peruvianum</i>	CNPH-1194	0.04	07/07
<i>S. peruvianum</i>	PI-128660	0.05	04/04
<i>S. peruvianum</i>	CNPH-1434	0.07	24/38
<i>S. peruvianum</i>	CNPH-1435	0.67	09/09
<i>S. peruvianum</i>	CNPH-1436	0.66	10/10
<i>S. peruvianum</i>	CNPH-1438	0.23	33/33
<i>S. peruvianum</i>	LA-0454	0.13	33/36
<i>S. peruvianum</i>	CNPH-1440	0.20	22/42
<i>S. peruvianum</i>	CNPH-1443	0.33	02/06
<i>S. peruvianum</i>	CNPH-1444	0.50	25/26
<i>S. peruvianum</i>	PI-365951	0.11	23/23
<i>S. peruvianum</i>	PI-365952	0.00	18/18
<i>S. peruvianum</i>	CNPH-1447	0.63	01/01
<i>S. peruvianum</i>	PI-365953	0.13	13/13
<i>S. peruvianum</i>	CNPH-1449	0.30	00/05
<i>S. peruvianum</i>	CNPH-1450	0.17	02/02
<i>S. peruvianum</i>	LA-1609	0.10	21/25
<i>S. peruvianum</i>	LA-1626	0.06	21/21
<i>S. peruvianum</i>	CNPH-1456	0.50	02/02
<i>S. peruvianum</i>	CNPH-1459	0.35	04/33
<i>S. peruvianum</i>	LA-1973	0.00	01/01
<i>S. peruvianum</i>	CNPH-1461	0.31	12/21
<i>S. peruvianum</i>	CNPH-1462	0.13	12/14
<i>S. peruvianum</i>	LA-2152	0.00	13/13
<i>S. peruvianum</i>	CNPH-1464	0.27	13/13
<i>S. peruvianum</i>	CNPH-1465	0.33	18/20
<i>S. peruvianum</i>	CNPH-1466	0.40	07/07
<i>S. peruvianum</i>	LA-2185	0.03	03/03
<i>S. peruvianum</i>	CNPH-1468	0.32	20/23
<i>S. peruvianum</i>	LA-2553	0.05	12/12
<i>S. peruvianum</i>	CNPH-1470	0.48	12/12
<i>S. peruvianum</i>	LA-2744	0.08	27/27
<i>S. peruvianum</i>	CNPH-1472	0.63	02/09
S4 ^d	CNPH-0513	0.25	00/04
S4 ^d	CNPH-0515	0.42	07/07

control TX-468-RG (i.e., with severity index values below 0.35). ToRMV was detected via PCR in all leaf samples of the susceptible control Viradoro. On the other hand, ToRMV was not detected by PCR analysis in samples of *S. habrochaites* PI-127826. For *S. chilense* LA-1967, only 1 plant was PCR positive out of 25 analyzed. In the accessions *S. peruvianum* LA-1609 and LA-1626, only a few plants were positive. For nematode evaluation, 37 accessions displayed either no or very few tiny galls in their root systems. This resistant reaction was mainly observed in accessions belonging to the wild species *S. peruvianum* (Table 1).

Identification of accessions with simultaneous resistance to bipartite *Begomovirus* and *Meloidogyne* spp. in Brazil. Accessions exhibiting no *Meloidogyne* root galls and displaying a disease severity index lower than 0.10 to ToRMV infection were considered as resistant to both groups of pathogens. In all, 18 *S. peruvianum* accessions and 1 *S. corneliomuelleri* did comply with these selection criteria (Table 1). It is worth noting that CNPH-0515 (derived from the interspecific cross *S. lycopersicum* × *S. peruvianum*) also exhibited a significant resistant reaction to the *Meloidogyne* spp. and to ToRMV.

Evaluation of *Solanum* (section *Lycopersicon*) accessions selected in Brazil against monopartite *Begomovirus* spp. in Spain. Seventeen accessions that exhibited resistance to both nematodes and ToRMV in Brazil were evaluated for resistance to TYLCD-associated *Begomovirus* spp. in assays conducted in Spain. All of

the accessions evaluated exhibited either no or very mild symptoms at 17 or 30 dpi when challenged via agroinoculation with the three monopartite *Begomovirus* spp. tested (Table 2). According to the disease severity index scale (scores 0 to 4), plants of the accessions under evaluation displayed maximum scores of 2.0. At 30 dpi, the susceptible control Moneymaker had symptom severity index scores of 3.0 for TYLCSEV-ES, 3.0 for TYLCV-IL, and 2.5 for TYLCV-Mld. Only 1 of 12 Moneymaker plants tested escaped infection and did not exhibit symptoms after agroinoculation (Table 2). At the formal sampling date, plants of the resistant control TX-468-RG had an average score of 0.0 for TYLCSEV-ES, 0.5 for TYLCV-IL, and 0.0 for TYLCV-Mld, with all plants being free of systemic virus infection as shown in the hybridization assays (Table 2). In this experiment, the *S. peruvianum* accessions PI-365951, LA-1609, and PI-306811 exhibited no symptoms after TYLCV-Mld inoculation and no virus accumulation. In contrast, the *S. peruvianum* accessions LA-0454, LA-2152, LA-1113-1, and LA-2185 displayed plants with symptoms (scores ranging from 1 and 2) and exhibiting positive hybridization signals, indicating systemic virus accumulation. Three plants of CNPH 1194 accumulated virus at 17 dpi; however, no virus was detected at 30 dpi, similar to previous reports for the resistant control TX-468-RG (20,23), suggesting recovery from virus infection. The remaining accessions showed no clear visual symptoms but they present some plants with positive reactions for systemic virus

infection (Table 2). None of the plants from the accessions *S. peruvianum* PI-365951 and LA-2152 accumulated detectable amount of virus when they were challenged with TYLCSEV-ES. Two plants of *S. peruvianum* LA-0454 exhibited symptoms (severity index ranging from 1 to 1.5), whereas the remaining plants exhibiting a positive hybridization signal remained asymptomatic. Similarly to what was observed for TYLCV-Mld, two CNPH-1194 plants exhibited TYLCSEV-ES accumulation by molecular hybridization at 17 dpi; however, at 30 dpi, all the hybridization tests were negative, again suggesting recovery from virus infection. Evaluations for TYLCV-IL resistance indicated that five *S. peruvianum* accessions (PI-365951, CNPH-1194, PI-306811, LA-2553, and LA-1626) exhibited no detectable amount of virus at 30 dpi. Disease symptoms were only observed in *S. peruvianum* LA-1113-1, whereas the other accessions were asymptomatic, although they exhibited a variable number of positive plants by molecular hybridization (Table 2). Among the accessions resistant to bipartite *Begomovirus* and *Meloidogyne* spp., two of them (PI-365951 and CNPH-1194) exhibited no symptoms and no detectable virus accumulation when challenged with each of the three TYLCD-associated *Begomovirus* spp. tested. Moreover, three additional lines (LA-1609, LA-2553, and PI-306811) exhibited no symptoms and sporadic virus infection, and all plants of the line LA-1626, which was challenged only with TYLCV-IL, were free of both symptoms and systemic infection.

Table 2. Reaction of *Solanum* (section *Lycopersicon*) accessions challenged with the monopartite *Begomovirus* spp. *Tomato yellow leaf curl virus*-Mld (TYLCV-Mld), *Tomato yellow leaf curl Sardinia virus*-ES (TYLCSEV-ES), and *Tomato yellow leaf curl virus*-IL (TYLCV-IL) inoculated via agroinoculation using infectious clones^a

Accessions	TYLCV-Mld		TYLCSEV-ES		TYLCV-IL	
	NSP/TNP	NIP/TIP	NSP/TNP	NIP/TIP	NSP/TNP	NIP/TIP
LM-041	NT	NT	NT	NT	00/10	00/10
PI-365951	00/08	00/08	00/07	00/07	00/09	00/09
CNPH-1033	00/10	02/10	00/07	05/07	00/10	09/10
LA-1609	00/08	00/08	00/07	01/07	00/10	02/10
LA-0454	02/09	06/09	02/10	06/10	00/10	07/10
LA-2553	00/09	01/09	00/10	01/10	00/10	00/10
CNPH-0785	00/06	01/06	00/10	01/10	00/09	01/09
PI-128660	00/10	04/10	00/05	01/05	00/10	04/10
LA-2152	01/09	01/09	00/10	00/10	00/09	01/09
LA-1113-1	02/06	05/06	00/08	03/08	04/10	06/10
CNPH-1194	00/10	00/10	00/10	00/10	00/10	00/10
PI-306811	00/09	00/09	00/07	01/07	00/10	00/10
LA-2185	01/09	06/09	00/06	02/06	00/09	09/09
LA-2744	00/09	05/09	00/10	03/10	00/10	05/10
LA-1973	00/09	06/09	00/03	01/03	00/09	06/09
PI-365952	00/10	06/10	00/07	05/07	00/09	06/09
PI-365953	00/08	03/08	00/06	01/06	00/09	06/09
LA-1626	NT	NT	NT	NT	00/09	00/09
Moneymaker ^b	03/04	03/04	03/03	03/03	05/05	05/05
TX-468-RG ^c	00/04	00/04	00/04	00/04	00/04	00/04

^a Resistance reaction was scored at 30 days after inoculation. Accessions tested were *Solanum peruvianum*, except the accessions CNPH 1033 (*S. corneliomuelleri*) and LM 041 (*S. habrochaites*). NSP/TNP= number of symptomatic plants/total number of inoculated plants and NIP/TIP= number of infected plants/total number of inoculated plants. Virus detection was done by molecular hybridization on tissue print blots, at 30 days post inoculation; NT = not tested (seed not available for all tests).

^b *Begomovirus* spp.-susceptible control.

^c *Begomovirus* spp.-resistant control.

DISCUSSION

The diseases caused by *Begomovirus* spp. are currently considered one of the most serious constraints for tomato cultivation worldwide (31). Also, nematodes of the genus *Meloidogyne* are significant problems, being responsible for severe tomato yield losses (55,58,60). Due to their economic importance, many breeding programs are now focused on development of cultivars resistant to these pathogens. The main tomato breeding strategy for controlling pathogens as diverse as *Begomovirus* and *Meloidogyne* spp. has been the introgression into cultivated accessions of resistance genes with broad-spectrum resistance or the pyramiding of different genes into elite genetic materials (59,60).

Therefore, the identification of new sources combining resistance to both *Begomovirus* and *Meloidogyne* spp., such as those reported here, is of great practical interest for tomato breeding programs worldwide. Multiresistant accessions were initially found by screening a collection of 71 *Solanum* (section *Lycopersicon*) accessions that were simultaneously inoculated with a mixture of *M. incognita* and *M. javanica* eggs and whiteflies inoculated with ToRMV under greenhouse and field conditions in Brazil. For ToRMV, 41 accessions (from the wild species *S. pimpinellifolium*, *S. chilense*, *S. habrochaites*, and *S. peruvianum*) exhibited resistance, with many of them being reported for the first time as sources of resistance. For *Meloidogyne* spp., several accessions, mainly belonging to *S. peruvianum* (Table 1), exhibited resistance similar to that previously reported for distinct accessions of this wild tomato species (3,60). These results suggest that *Mi* alleles or new *Meloidogyne* resistance genes (60) might be present in these accessions, which should be further studied. Interestingly, multiresistant sources (i.e., to both groups of pathogens) were found in 18 accessions, comprising the species *S. peruvianum*, *S. corneliomuelleri*, and an S4 line derived from the interspecific cross *S. lycopersicum* × *S. peruvianum* (Table 1).

Seventeen accessions found to be resistant to *Meloidogyne* spp. and the bipartite *Begomovirus* sp. ToRMV in Brazil were also evaluated against three monopartite *Begomovirus* spp. associated with TYLCD in Spain. The majority of the plants from these accessions displayed only mild or no symptoms to each of the three monopartite *Begomovirus* spp., even though systemic virus accumulation was observed in some plants. Six accessions exhibited significant resistance to all monopartite *Begomovirus* spp. tested, demonstrating an expanded range of resistance compared with parental sources (Table 2). The *S. peruvianum* accessions CNPH-1194 and PI-365951 exhibited the best performance, with no symptoms and no detectable systemic virus accumulation. The accessions LA-

2152, LA-2553, LA-1609, and PI-306811 were also considered to be promising sources of resistance. The accession LA-1626 was challenged only with TYLCV-IL and it displayed an immune-like resistance to this virus. Therefore, further studies are needed to determine whether it can have a similar resistant reaction to other monopartite *Begomovirus* spp.

It should be taken into account that the ToRMV evaluation in Brazil was done only via whitefly inoculation procedures. Therefore, the ToRMV resistance observed for the accessions reported here could be related to either virus transmission (vector resistance) or virus infection (virus resistance). Our results cannot discriminate between these two resistance mechanisms. Therefore, further studies with distinct inoculation techniques (e.g., whitefly, biolistic inoculation, graft inoculation, and agroinoculation) should be conducted with ToRMV in order to elucidate which resistance mechanisms (53) are operative against ToRMV.

According to De Castro et al. (14), some accessions could contain *Mi* and *Ty-1* in a repulsion-phase linkage as observed for the accessions *S. peruvianum* PI-128657 and *S. chilense* LA-1969. The repulsion-phase linkage of the *Ty-1* and *Mi* loci (14,62) has hampered the large-scale development of stable and multiresistant inbred lines and hybrids. It is interesting to note that similar combinations of resistance and susceptibility to both pathogens were also observed in our work. Thus, for example, *S. chilense* LA-1967 displayed resistance to ToRMV but was susceptible to *Meloidogyne* spp. in the assay conducted in Brazil. The same reaction was observed in plants of the *S. habrochaites* accessions PI-127826 and PI-127827. In contrast, resistance to *Meloidogyne* spp. and susceptibility to ToRMV was found in the *S. peruvianum* accessions CNPH-0782, CNPH-1435 (= LA-0107), and CNPH-1436 (= PI-126944). However, additional genetic testing will be necessary to confirm the set of genes involved in these accessions, because there is also a possibility that the resistance to *Begomovirus* and *Meloidogyne* spp. in these sources might not be related to *Ty-1* or *Mi*.

The results reported here demonstrate promising possibilities of discovering new resistance sources in the genus *Solanum* (section *Lycopersicon*) with resistance to both monopartite and bipartite *Begomovirus* spp. and to nematodes. In terms of expression of the resistance, the identification of sources combining resistance to both groups of pathogens is very important because there are some indications that gene dosage is important for expression of both *Ty-1* (9) and *Mi* (60) loci. The species *S. peruvianum* showed a relatively high frequency of accessions resistant to *Begomovirus* spp. and also to *M. javanica* and *M. incognita*. Our study pointed out the *S.*

peruvianum accessions PI-306811, CNPH-1194, and PI-365951 were among the ones that displayed the best performance against all the pathogens tested in both continents. Due to this broad resistance spectrum, these accessions are considered potential multiresistance sources for breeding programs. The important limitation of these wild species is the natural genetic isolation in relation to the cultivated species, *S. lycopersicum* (42,43). This characteristic will demand the employment of embryo-rescue technique after interspecific crossings (54), as was done to introgress the *Mi* gene from *S. peruvianum* PI-128657 into *S. lycopersicum*.

Several biochemical and molecular markers were identified in close linkage with the genomic regions encompassing the *Mi* (32,35,48,60) and *Ty-1* (14) loci in the tomato chromosome 6. This genomic information and the development of segregating populations could be used to test whether the resistance factors described here also reside in this particular region of the tomato genome. Additional studies should be carried out using individual plants from the accession CNPH-0515 (S₄ generation derived from interspecific crossing between *S. lycopersicum* and *S. peruvianum*) that exhibited resistance to ToRMV and against two *Meloidogyne* spp. In this case, the natural crossing barrier between the two host species was already overcome and the generation of pure breeding lines for resistance to both groups of pathogens would be suitable for inheritance, allelism, and linkage studies with other resistance sources available for tomato breeding.

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