Identification of Resistance to *Potato yellow mosaic virus*-Trinidad Isolate (PYMV-TT) Among *Lycopersicon* Species

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ABSTRACT

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Three studies were carried out with the objective of identifying resistance to *Potato yellow mosaic virus*-Trinidad isolate (PYMV-TT) among *Lycopersicon* species through field screening and using field-inoculated infector rows. The presence of PYMV-TT was confirmed using dot blot hybridization assays. In the first study, eight commercial cultivars of tomato were tested for resistance. In a subsequent study, 11 breeding lines showing high levels of resistance to *Tomato mottle virus* (ToMoV-Florida) and six lines resistant to *Tomato yellow leaf curl virus* (TYLCV-Old World Begomovirus) were screened for resistance to PYMV-TT. All breeding lines and the commercial cultivars tested were susceptible to PYMV-TT infection. The third study involved screening 92 accessions from a representative core collection of wild *Lycopersicon* germ plasm. PYMV-TT was not detected in individuals of 22 accessions.

The geminivirus disease (*Potato yellow mosaic virus*, PYMV-TT) of tomato (*Lycopersicon esculentum* Mill.) is a major impediment to profitable large-scale cultivation of tomato in Trinidad and Tobago (41). PYMV-TT belongs to the genus *Begomovirus*, a group of bipartite geminiviruses that infects certain dicotyledonous plants and is transmitted throughout the tomato crop in a pervasive manner via the voracious feeding of the sweet potato whitefly, *Bemisia tabaci*-Biotype B (41). The high fecundity rates of *B. tabaci* contribute to maintaining a high inoculum potential throughout the tomato-growing season (5,6). Typical symptoms of PYMV-TT infection include the presence of yellow mottling of the lamina, leaf distortion (crinkling, curling, leaf size reduction), and whole-plant stunting in severe cases (41). Although yield losses in tomato due to begomovirus infection have not been estimated, observable incidence of the disease in large cultivations is near 100% (41). PYMV and strains of PYMV have also been reported in Guadeloupe (33), Martinique (33), Panama (41), and Venezuela (12).

Despite the importation of tomato cultivars that are believed to have increased tolerance/resistance levels to Old World begomoviruses such as *Tomato yellow leaf curl virus* (TYLCV), disease incidence

remains relatively high. The adoption of several cultural practices such as controlled insecticide use aimed at managing vector activity is ineffective in large-scale tomato cultivation (32,43). Genetic resistance (23,30,32) and supplemental disease management tactics (32) are important in the long-term management of geminivirus diseases.

In instances where genes for resistance cannot be obtained from commercial cultivars, it becomes necessary to screen wild species of *Lycopersicon*. The center/origin of tomato domestication may provide the main contributors to genetic resistance (14,40). Most breeding programs have used resistant sources identified from wild accessions of *Lycopersicon* (11,21,27). However, no studies on identifying resistance to PYMV have been done.

While a number of breeding programs have been initiated to develop resistance to begomoviruses, it is unclear what level of protection will be afforded against begomoviruses or whether resistance will be general or specific to certain viruses within the group. Such information would provide better understanding of disease management against new virus introductions and recombinant viruses (29,35,38).

The objective of this study was to identify resistance to PYMV-TT among commercial tomato cultivars, tomato breeding lines with resistance to *Tomato mottle virus* (ToMoV-Florida) or TYLCV, and accessions of wild *Lycopersicon* species.

MATERIALS AND METHODS

Three separate field experiments were conducted with the objective of identifying resistance to PYMV-TT among *Lycopersicon* spp. using dot blot hybridization to

detect the presence of PYMV-TT and using symptom development. In the first experiment, four commercial cultivars ('Cascade', 'Nema 1400', 'Kada', and 'Tropic Boy') popularly grown in Trinidad and four hybrids (F1 Fiona, F1 Tyking, F1 Topcross 21, and Lignon c8-6) that have been reported to have some levels of resistance to geminiviruses (19,20,36) were tested for their levels of resistance to PYMV-TT. In the second experiment, 11 breeding lines from Florida (E592-6 to EI 925-2) with resistance to ToMoV (New World Begomovirus) and six breeding lines from France (Chil 93 lines and TxLA1969) considered to have resistance to TYLCV (Old World Begomovirus) were tested for their resistance to PYMV-TT. In the third experiment, 92 wild accessions representing five *Lycopersicon* spp. (*L. cheesmanii*, *L. chilense*, *L. hirsutum*, *L. peruvianum*, and *L. pimpinellifolium*) were screened for resistance to PYMV-TT infection after field inoculation along with a susceptible tomato cultivar, 'Topic Boy', as a positive control.

Of the eight varieties of tomato (*L. esculentum*) tested, 'Cascade', 'Nema 1400', 'Kada', and 'Tropic Boy' were obtained locally from commercial suppliers, while F1 Fiona, F1 Tyking, F1 Topcross-21, and Lignon c8-6 were supplied by H. Laterrot (INRA-France). The 11 breeding lines considered to have high levels of resistance to ToMoV (Florida) and six breeding lines showing resistance to TYLCV were supplied by J. A. Scott (University of Florida, Gainesville) and H. Laterrot (INRA-France), respectively. The breeding lines from Florida were derived from ToMoV-resistant plants from BC2 generations, five of the six breeding lines (Chil 93-2 – Chil 93-21) from France were derived from TYLCV-resistant plants from BC3, and the other (TxLA1969) was derived from TYLCV-resistant plants from BC1. Ninety-two wild accessions of *L. chilense*, *L. hirsutum*, *L. peruvianum*, *L. pimpinellifolium*, and *L. cheesmanii*, representing the core collection of *Lycopersicon* spp., were provided by C. M. Rick (Tomato Genetic Resources Center, University of California, Davis).

The field experiments were conducted at the University of the West Indies Field Station at Champ Fleurs, Trinidad, located in the River Estate Loam Series. It is situated between the major tomato cultivating areas of Aranguez and Pasea, where epi-

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phytotics of the geminivirus disease are seen on a yearly basis (41). All the experiments were conducted during September 1999 to February 2000.

Infecter rows. Infecter rows were established 2 weeks in advance of transplanting the test plants and were used to maintain a high inoculum potential throughout the tests. The highly susceptible commercial cultivar Kada was used in infecter rows surrounding each replicate in experiments 1 and 2, while 'Tropic Boy', another PYMV-TT-susceptible commercial variety was used in infecter rows planted after every 10 test rows in experiment 3, since 'Kada' became unavailable through local suppliers. Plants used in infecter rows were field-inoculated (subjected to PYMV-TT infection through natural disease pressure in the field). Random samples were tested using polymerase chain reaction (PCR) amplification to confirm the presence of PYMV-TT.

Experimental design. The three experiments were carried out separately, each in a randomized complete block design. In the first experiment, the eight tomato cultivars were evaluated in 5.4 m² plots (3 rows, 3 m long, 21 plants) with four replications, at a spacing of 0.90 × 0.50 m. The 17 breeding lines in experiment 2 were evaluated in 7.2 m² plots (3 rows, 4 m long, 27 plants) with two replications at spacing of 0.90 × 0.50 m. In experiment 3, the 92 wild *Lycopersicon* accessions along with 'Tropic Boy' (control) were evaluated in single-row plots (7 plants per row), with two replications. Test plants were germinated in PROMIX in seedling trays and transplanted between 24 and 30 days post-germination into the field.

The experiments were carried out on cambered beds to ensure adequate drainage and were sprayed with a soil fungicide (Rhizolex 600 liters/ha, Sumitomo, Japan. Caribbean Chemicals Ltd., local distributors) before transplanting. The replications were kept weed-free throughout the experiments by manual weeding. Plants were fertilized (350 kg/ha, 13:13:17:2 N-P-K-Mg) and staked 1 month after transplanting. Aphids were controlled (plants sprayed from an emulsifiable concentrate of Fastac 0.25 to 2.5 liters product/ha, BASF, Australia) to prevent potyviral infections.

Dot blot hybridization. After 6 to 8 weeks in the field, newly developing flush leaves were collected on an individual plant basis and pooled on a replicate basis for experiment 1; and for experiments 2 and 3, a subsample of five randomly selected plants per replicate were used individually for dot blot hybridization assays (1,21,34,37). The leaf samples were washed, ground 1:5 wt/vol in extraction buffer (Bioreba AG, Switzerland: Tris, 2.4 g; NaCl, 8.0 g; PVP, 20 g; Tween 20, 0.5 g; KCl, 0.20 g; Na₂S₂O₃, 0.20 g per liter, pH 7.4), filtered through miracloth (Calbio-

chem-Novabiochem Corporation, La Jolla, CA) and blotted in triplicate on a nylon membrane (Amersham International plc, England). The DNA was fixed to the membrane in a UV Cross Linker (Stratagene Inc., Cedar Creek, TX), then subjected to southern hybridization at high stringency using a DIG-labeled (DIG DNA labeling and detection kit—Roche Diagnostics/Boehringer-Mannheim Inc., Germany) fragment of the PYMV-TT (DNA-A). Leaf extracts prepared from PYMV-TT-infected leaves from infecter rows of 'Kada' or 'Tropic Boy' were used as positive controls and healthy tomato leaf extracts as negative controls. Data were recorded as positive, negative, or segregating.

Symptoms. Symptoms were recorded on five random plants per replicate for the commercial varieties and on an individual plant basis for the breeding lines and wild accessions 6 to 8 weeks after transplanting. Recording was based on the following scoring system: 0, no yellowing, leaf curling or leaf size reduction; 1, specks of yellow and no leaf distortion, no leaf size reduction; 2, patches of yellowing, slight leaf distortion, no leaf size reduction; 3, yellowing, leaf rolling or curling, moderate leaf size reduction; 4, overall yellowing, severe leaf distortion, severe leaf size reduction, stunting of plant. Plants that were consistently negative in dot blot assays (over replications), with a symptom score of 0, were classified as immune. Individuals that showed mild symptoms (range 0 to 1 or 1 to 2), with a positive test in dot blot assays, were classified as resistant. Plants that were considered to be susceptible exhibited moderate scores (2 to 3) but were consistently positive in dot blot tests. Test plants that showed positive dot blot assays and a mean symptom score of 3 or higher were categorized as highly susceptible. Individuals that did not give uniform re-

sults in dot blot assays were classified as segregating.

Data presentation. Results were described for experiment 1, while results were presented as mean symptom score ± standard error and symptom range for experiment 2. Results were presented as positive, negative, or segregating based on dot blot assays for experiment 3.

RESULTS

Infecter plots. The infecter rows and the positive control plots (experiment 3) showed 100% infection with PYMV-TT, based on dot blot tests, indicating that the inoculum level was uniformly high throughout the field in all the experiments.

Resistance to PYMV-TT among commercial cultivars. The commercial cultivars tested (experiment 1) were all infected with PYMV-TT, based on dot blot assays. However, symptom severity scores varied among the cultivars. In this study, 'Cascade', 'Nema 1400', 'Kada', 'Tropic Boy', and Lignon c8-6 recorded symptom scores above 3.0 and may be considered highly susceptible to PYMV-TT. F1 Fiona and F1 Tyking showed attenuated symptoms varying between scores 1.5 and 2.0 and may be considered resistant to PYMV-TT. F1 Topcross 21, with intermediary symptom scores (2 to 3), may be considered susceptible to PYMV-TT. Symptoms were manifested as yellow mottling and reduction of leaf size with whole plant stunting and gross distortion of leaves in severe cases of infection.

Resistance to PYMV-TT among breeding lines. There was a wide variation in symptom severity scores within the breeding lines evaluated in experiment 2, although all the replicates (pooled samples) tested positive for PYMV-TT in dot blot assays (Table 1). The breeding lines E790-1, E II 794-9, and E 854-8 were the most promising, with lower mean symptom

Table 1. Tomato breeding lines, sources of resistance, mean symptom scores, percentages of non-symptomatic plants (NSP), and reaction of the lines in dot blot assays following infection by Trinidad strain of *Potato yellow mosaic virus* (PYMV-TT)

Breeding lines	Resistance source	Mean score	NSP	Symptom range	Dot blot assay ^a
E592-6	LA 1961	3.3 ± 0.75	0	2-4	+
E608-2	LA 1968	3.1 ± 0.74	0	2-4	+
E659-2	LA 1938	3.2 ± 0.86	0	1-4	+
E672-15	LA 1938	2.2 ± 0.86	0	1-4	+
EI 701-4	LA 1959	2.4 ± 1.18	4	0-4	+
E790-1	LA 1968	1.5 ± 0.99	9	0-3	+
EII 794-9	LA 2779	1.5 ± 1.26	25	0-3	+
E 854-8	LA 1932	1.2 ± 0.82	50	0-3	+
EI 856-4	LA 1932	1.8 ± 0.77	0	1-4	+
EI 898-9	LA 1938	2.1 ± 0.87	0	1-4	+
EI 925-2	LA 1938	2.6 ± 0.84	0	1-4	+
Chil 93-21	LA 1969	2.5 ± 0.80	0	1-4	+
Chil 93-19	LA 1969	2.9 ± 0.88	0	1-4	+
Chil 93-16	LA 1969	2.6 ± 0.89	0	1-4	+
Chil 93-6	LA 1969	2.7 ± 0.96	0	1-4	+
Chil 93-2	LA 1969	2.1 ± 1.11	0	2-4	+
TxLA1969	LA 1969	2.7 ± 0.91	0	1-4	+

^a + = positive in dot blot assay for infection by PYMV-TT.

scores and larger numbers of symptomless plants (9, 25, and 50%, respectively), and may be considered to have some levels of resistance to PYMV-TT. In addition, while breeding lines E 854-8 and EI 856-4 both had the same source of resistance (LA1932), there was marked difference in symptom range. This was also seen with lines E608-2 and E790-1, whose source of resistance was LA1968. The Chil 93 lines had mean symptom scores over 2 and may be regarded as susceptible to PYMV-TT (Table 1). Symptomless plants were not tested individually.

Screening of *Lycopersicon* germplasm for resistance. Ninety-two accessions from five *Lycopersicon* species varied in the numbers of accessions resistant to infection by PYMV-TT (Table 2). *L. cheesmanii* had no resistant accessions, *L. chilense* had 11, *L. hirsutum* had 7, *L. peruvianum* had 3, and *L. pimpinellifolium* had 1. Plants from susceptible *L. cheesmanii* accessions had mottling and crinkling of leaves, while others were symptomless but were positive in dot blot assays. Symptoms incited by PYMV-TT infection of susceptible *L. chilense* accessions were more severe and included yellow mottling, curling, and crinkling of leaves with accompanying severe leaf reduction. Susceptible *L. hirsutum* accessions contained plants with severe whole-plant stunting, distortion, mottling, curling, and crinkling of leaves. Interestingly, one accession of *L. hirsutum* (LA 1624) contained only symptomless plants that tested positive in dot blot assays. Plants of susceptible accessions of *L. peruvianum* displayed moderate symptoms, with the exception of three accessions (LA2732, LA2185, and LA0153), which had severe symptoms of whole-plant stunting, gross leaf distortion and reduction with yellow mottling. Accessions of *L. pimpinellifolium* contained plants that exhibited mild symptoms of disease, while plants of one accession (LA 0722) displayed only mild symptoms and were negative for dot blot assays and may be considered resistant to PYMV-TT infection.

DISCUSSION

All commercial cultivars tested positive for PYMV-TT, but there was variation in symptom severity. F1 Fiona and F1 Tyking showed the lowest symptom severity scores among the commercial cultivars tested and were considered to have some levels of resistance to PYMV-TT based on these scores and positive dot blot signals obtained. These lines have differing levels of resistance to a number of begomoviruses from Cuba, Costa Rica, Martinique, and Israel (19,20,36). In Martinique, although the infecting geminivirus was not stated in that study, resistance of F1 Fiona and F1 Tyking varieties was reported to be "high" based solely on symptom development, while it was reported that results of tests

carried out on these two lines in Costa Rica showed "no evidence of resistance," but the method of evaluation was not stated (36). In Cuba, F1 Fiona, F1 Tyking, and Lignon c8-6 were screened based on the number of healthy plants and impact on yield after exposure to TYLCV (19). F1 Tyking had the largest number of healthy plants and highest mean fruit weight, followed by F1 Fiona and Lignon c8-6. However, in Israel, F1 Fiona and F1 Tyking were not reported to have high levels of "resistance" to TYLCV based on symptom severity, total and average fruit yield, and fresh plant weight assessments (20).

There is difficulty in comparing the results of these studies to that of this study

due to differing evaluation methods used to assess tomato lines for resistance to begomovirus disease. It also seems that terminology for resistance and tolerance are used interchangeably. For instance, many studies have identified varying levels of tolerance to geminivirus diseases, using symptom expression alone as an indicator (16,26,36) where mild mosaic or plant malformations indicate tolerance. Tolerance is considered by other researchers as the absence of observable symptoms of disease in spite of the confirmed detection of geminiviral DNA in an individual (42,43). Still others have used a more relaxed definition and indicate tolerance where symptoms are mild such that they

Table 2. *Lycopersicon* species, accession number, origin, and disease reaction of *L. cheesmanii*, *L. chilense*, *L. hirsutum*, *L. peruvianum*, and *L. pimpinellifolium* to infection by a Trinidad isolate of Potato yellow mosaic virus (PYMV-TT)

Species	Accession	Origin	Dot blot assay		
			Negative	Positive	Segregating
<i>L. cheesmanii</i>	LA166	Galapagos Islands	N ⁿ ^a	N ^p	+/- ^b
<i>L. cheesmanii</i>	LA0317	Ecuador	N ⁿ	N ^p	+/-
<i>L. chilense</i>	LA2750	Chile	N ⁿ	+	N ^s
<i>L. chilense</i>	LA2748	Chile	N ⁿ	N ^p	+/-
<i>L. chilense</i>	LA1971	Peru	N ⁿ	N ^p	+/-
<i>L. chilense</i>	LA1969	Peru	-	N ^p	N ^s
<i>L. chilense</i>	LA1965	Peru	N ⁿ	N ^p	+/-
<i>L. chilense</i>	LA1967	Peru	-	N ^p	N ^s
<i>L. chilense</i>	LA1963	Peru	-	N ^p	N ^s
<i>L. chilense</i>	LA1960	Peru	-	N ^p	N ^s
<i>L. chilense</i>	LA1958	Peru	N ⁿ	+	N ^s
<i>L. chilense</i>	LA1938	Peru	-	N ^p	N ^s
<i>L. chilense</i>	LA1932	Peru	N ⁿ	+	N ^s
<i>L. chilense</i>	LA2755	Peru	-	N ^p	N ^s
<i>L. chilense</i>	LA2931	Chile	-	N ^p	N ^s
<i>L. chilense</i>	LA2930	Chile	N ⁿ	+	N ^s
<i>L. chilense</i>	LA2884	Chile	-	N ^p	N ^s
<i>L. chilense</i>	LA2779	Chile	-	N ^p	N ^s
<i>L. chilense</i>	LA2778	Chile	-	N ^p	N ^s
<i>L. chilense</i>	LA2773	Chile	-	N ^p	N ^s
<i>L. chilense</i>	LA2767	Chile	N ⁿ	N ^p	+/-
<i>L. chilense</i>	LA2759	Chile	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA1361	Peru	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA0407	Ecuador	N ⁿ	+	N ^s
<i>L. hirsutum</i>	LA1266	Ecuador	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA1223	Ecuador	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA1361	Peru	-	N ^p	N ^s
<i>L. hirsutum</i>	LA1353	Peru	-	N ^p	N ^s
<i>L. hirsutum</i>	LA2329	Peru	-	N ^p	N ^s
<i>L. hirsutum</i>	LA2680	Ecuador	N ⁿ	+	N ^s
<i>L. hirsutum</i>	LA2650	Peru	N ⁿ	+	N ^s
<i>L. hirsutum</i>	LA2409	Peru	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA2204	Peru	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA2174	Peru	N ⁿ	+	N ^s
<i>L. hirsutum</i>	LA2167	Peru	N ⁿ	+	N ^s
<i>L. hirsutum</i>	LA2158	Peru	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA2155	Peru	-	N ^p	N ^s
<i>L. hirsutum</i>	LA2128	Ecuador	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA2119	Ecuador	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA2109	Ecuador	N ⁿ	+	N ^s
<i>L. hirsutum</i>	LA2103	Ecuador	N ⁿ	+	N ^s
<i>L. hirsutum</i>	LA2098	Ecuador	N ⁿ	+	N ^s
<i>L. hirsutum</i>	LA1928	Peru	-	N ^p	N ^s

(continued on next page)

^a Nⁿ = none of the plants tested gave negative hybridization signals for both replicates for PYMV-TT infection; N^p = none of the plants tested gave positive hybridization signals for both replicates for PYMV-TT infection; N^s = none of the plants tested were found to be segregating.

^b - = Negative hybridization signals obtained for both replicates for PYMV-TT infection; + = positive hybridization signals obtained for both replicates for PYMV-TT infection; +/- = positive hybridization signals obtained for one replicate and negative hybridization signals obtained for the other replicate for PYMV-TT infection.

are expected to have minimal impact on crop yield (16,30). We understand tolerance to refer to the ability of a plant genotype to produce acceptable fruit quality and quantity (yield) in spite of pathogenic or abiotic influence (7,25). In this study, yield loss assessments were not carried out on these commercial cultivars since they were uniformly infected with PYMV-TT, and hence tolerance to PYMV-TT cannot be determined and compared for these lines. Given the difficulty observed with respect to assigning resistant, immune, tolerant, and susceptible disease reactions to *Lycopersicon* spp., it is suggested that a standardized and reliable system of evaluation be proposed so that data from different sources can be more effectively compared.

By using symptom development supported by molecular methods for detecting geminiviruses (9,24,35), the *Lycopersicon* germplasm can be more effectively characterized with the objective of identifying sources of resistance to geminivirus diseases (1,31,43), especially when using field inoculation trials to screen large numbers of breeding lines. Symptom expression can be affected by several growth variables such as soil conditions, temperature, insect damage, and secondary infections by other viruses (13). As such, using methods of detecting geminiviruses in conjunction with symptom recording would provide more efficient means of identifying resistance to geminiviruses.

The breeding lines (E592-6 to EI 925-2) mass selected for resistance to ToMoV,

derived from various accessions of *L. chilense*, showed varying degrees of symptom severity to infection by PYMV-TT in experiment 2 of this study. At least three lines (E790-1, EII 794-9, and E 854-8) may be considered potential resistant sources, as evidenced by the relatively high number of symptomless plants and attenuated mean symptom scores below 2 obtained. The low mean scores may suggest that most genes governing resistance to ToMoV and PYMV-TT may be common in the *L. chilense* lines LA 1968 and LA 2779. Although LA 1932 was a common source of resistance for breeding lines E 854-8 and EI 856-4, there was a marked variation in symptom range. This variation could be a result of secondary potyviral infection or, more importantly, due to backcrossing the parental types (the parental lines were backcrossed at least twice) from which these two lines were obtained. The resulting lines E 854-8 and EI 856-4 may not be genetically uniform or may be considered to be heterogeneous. This phenomenon of heterogeneity in the progeny of backcrossed parents has been reported (30).

ToMoV (Florida) and PYMV-TT are closely related begomoviruses that belong to the same genetic clade (41). This study may show that genetic relatedness between begomoviruses may correspond to certain levels of shared resistance profiles since three lines considered resistant to ToMoV (Florida) (E790-1, EII 794-9, and E 854-8) did show some level of resistance to PYMV-TT, as demonstrated by the high percentage of symptomless plants and low mean symptom scores for those plants that exhibited symptoms of PYMV-TT infection (Table 1). It has been speculated that shared resistance among closely related geminiviruses could form the basis for achieving broad-spectrum resistance (31). If the large degree of segregation for symptom severity (1 to 4) in Chil 93 breeding lines has a genetic basis, then there may be opportunities to breed resistance to even divergent begomoviruses by selecting a complementary genetic system through shuttle breeding.

The Chil 93 lines screened derive their resistance to TYLCV from LA 1969, and similarly, accessions of wild *L. chilense* were found to have moderate levels of resistance to PYMV-TT in this study. The Chil 93 lines were developed by backcrossing LA 1969-derived resistance lines at least twice with F1 Tyking, a commercial cultivar that showed moderate levels of resistance to PYMV-TT in this study. The Chil 93 lines infected with PYMV-TT showed a wide variation of symptom severity (range 1 to 4). This suggests that the genes responsible for resistance to TYLCV and PYMV-TT may be different, at least in LA 1969. LA 1969 is reported to have resistance against a number of begomoviruses, both from the New World (31) and

Table 2. (continued from previous page)

Species	Accession	Origin	Dot blot assay		
			Negative	Positive	Segregating
<i>L. hirsutum</i>	LA1777	Peru	—	N ^p	N ^s
<i>L. hirsutum</i>	LA1740	Peru	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA1918	Peru	N ⁿ	+	N ^s
<i>L. hirsutum</i>	LA1731	Peru	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA1721	Peru	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA1624	Ecuador	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA1560	Peru	N ⁿ	N ^p	+/-
<i>L. hirsutum</i>	LA1347	Peru	—	N ^p	N ^s
<i>L. hirsutum</i>	LA1363	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1395	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1379	Peru	—	N ^p	N ^s
<i>L. peruvianum</i>	LA1365	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1364	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1360	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1351	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1339	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1331	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1929	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1910	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1626	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1609	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1474	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1984	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1982	Peru	—	N ^p	N ^s
<i>L. peruvianum</i>	LA1973	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1954	Peru	N ⁿ	+	N ^s
<i>L. peruvianum</i>	LA1951	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1945	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1937	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2964	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2981	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2744	Chile	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2732	Chile	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2553	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2328	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2185	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2172	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2163	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2157	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA2152	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA0103	Peru	—	N ^p	N ^s
<i>L. peruvianum</i>	LA0153	Peru	N ⁿ	+	N ^s
<i>L. peruvianum</i>	LA0441	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA0444	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA0454	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1274	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1292	Peru	N ⁿ	N ^p	+/-
<i>L. peruvianum</i>	LA1305	Peru	N ⁿ	N ^p	+/-
<i>L. pimpinellifolium</i>	LA1261	Ecuador	N ⁿ	N ^p	+/-
<i>L. pimpinellifolium</i>	LA0722	Peru	—	N ^p	N ^s
<i>L. pimpinellifolium</i>	LA2184	Peru	N ⁿ	N ^p	+/-

the Old World (43), which is supported by our results.

Accessions of wild species of *Lycopersicon* have been used since the 1940s by breeders as sources of disease resistance in tomato (23,40). Many studies have similarly identified resistance to geminiviruses (8,10,11,15,20,22,27,30,43) from these wild species. The importance of this type of screening is exemplified by the number of breeding programs geared toward developing resistant lines through recovery from wild *Lycopersicon* species that have been established in Brazil (10,11,28), Florida (39), Lebanon (1), India (2,3,4,27), and Guatemala (25) using lines of *L. peruvianum* (8), *L. hirsutum* (42), and *L. pimpinellifolium* × *L. peruvianum* and *L. cheesmanii* × *L. peruvianum*.

We screened the core collections of five *Lycopersicon* species for resistance to PYMV-TT. A number of the wild *Lycopersicon* accessions were segregating for resistance, which seems to conform to observations of others (31). This segregation for resistance may indicate either that some of the plants escaped infection or the presence of some genetic heterogeneity within the accessions. This genetic heterogeneity may be explained by the mass-crossed system of selection that was used to maintain the germplasm. The progeny derived from mass-crossed or open pollinated populations would not be genetically homogeneous, and it is possible that genes conferring resistance may be segregating in these populations (42).

Resistance was found in one accession of *L. pimpinellifolium* (LA 0722). Other research has indicated the usefulness of screening accessions of *L. pimpinellifolium*, and it was reported that plants of accession LA 121 were assessed to be a potential source of resistance to TYLCV infection (15). This accession was not available for our study.

The only other extensive screening study for resistance to a begomovirus (TLCV) among wild accessions of *Lycopersicon* species identified only four accessions from a total of 601 wild accessions screened (27). Using symptom development as the only indicator of resistance, the results of this and other studies showed that two accessions, LA 1777 of species *L. hirsutum* and LA 1969 of species *L. chilense*, were considered to be resistant to TLCV (27) and TYLCV (43), respectively. In this study, plants of these two accessions were also found to be resistant to PYMV-TT. Using disease incidence, disease severity, and back-indexing as indicators of resistance, one study identified five accessions of *L. peruvianum* that were TYLCV-free after graft-inoculation (16). Of these five accessions, only LA 1274 was included in experiment 3 of this study and contained segregating individuals.

Resistance to begomoviruses may exist at two levels, pathogen entry (negligible

efficiency of whitefly transmission) and/or at the level of systemic spread (31). The field screening technique employed in this study does not discriminate between these levels but can serve as a first step to identifying accessions for further resistance studies, by mechanical inoculation (sap, agro-bacterium, or graft-inoculation) and finally through rigorous and controlled whitefly inoculation studies.

While strategies to overcome disease outbreaks have focused mainly on developing tomato lines that are resistant to the virus, breeding efforts may be expanded to include lines that are not predisposed to whitefly feeding. While the potential of using insect-resistant tomato cultivars has been discussed (17,18,23), due to numerous limitations associated with this approach, it is believed that a combined source of resistance and an integrated approach to disease management may increase the success of large-scale tomato cultivation while reducing the need for pesticide use and its associated economic and environmental costs.

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