Solanum habrochaites Accession LA1777 Recombinant Inbred Lines Are Not Resistant to Tomato Yellow Leaf Curl Virus or Tomato Mottle Virus

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Abstract. Cultivated tomato (Solanum lycopersicum L.) accessions have been susceptible to the whitely-transmitted begomoviruses Tomato yellow leaf curl virus (TYLCV) and Tomato mottle virus (ToMoV) that can cause serious crop damage. S. habrochaites accession LA1777 has been reported to be resistant to TYLCV. To locate putative virus resistance genes, 89 recombinant inbred lines (RILs) previously developed from LA1777 in a tomato background, LA1777 and the susceptible RIL parent E6203, were screened against the begomoviruses TYLCV and ToMoV. An initial study showed 18 RILs had less disease severity to TYLCV or ToMoV. Eight RILs had S. habrochaites alleles at TG27 (restriction fragment length polymorphism marker) on chromosome 1, three RILs had S. habrochaites alleles at TG202 on chromosome 7, and one RIL had S. habrochaites alleles at both marker loci. The RILs with these regions were intercrossed in 10 different cross combinations and F2 seeds were then obtained. The F2 progenies were inoculated separately with both viruses and then evaluated in the field. The F2 plants with less disease severity were selected, but most did not have the markers from the hypothetical resistance regions. The F3 progenies were then inoculated and rated for disease severity to both viruses. None of the F3s demonstrated any increased level of resistance, even if derived from F2s homozygous for the target regions from both chromosomes. All plants from every cross combination were susceptible for both TYLCV and ToMoV, suggesting that there is no begomovirus resistance in the LA1777 RIL population. Some limitations of capturing all genes in an RIL population derived from an outcrossing accession are discussed.

The begomoviruses, Tomato yellow leaf curl virus (TYLCV) and Tomato mottle virus (ToMoV), vectored by the silverleaf whitefly (Bemisia argentifolii Bellows & Perring) can cause severe losses in tomato production. Most cultivated tomatoes are extremely susceptible to these diseases, and yield losses can reach 100% if plants are infected in early growth stages, especially by TYLCV (Green and Kallo, 1994; Picó et al., 1996). Accessions of several wild Solanum species, including S. habrochaites S. Knapp & D.M. Spooner., S. chilense (Dunal) Reiche., S. pimpinellifolium (Jusl.) Mill., S. cheesemani (L. Riley), and S. peruvianum (L.) Mill., have been reported by many authors to have TYLCV resistance, and intensive breeding efforts have been undertaken to develop cultivars with TYLCV resistance (Scott, 2006). Several accessions of S. habrochaites have been reported to be resistant to TYLCV (Ji et al., 2007), and some of these accessions have been used in breeding programs for developing TYLCV-resistant lines. The accession of S. habrochaites B6013 has demonstrated excellent resistance to TYLCV from Taiwan to south India (Kallo and Banerjee, 1999), and resistance in this accession was reported to be controlled by two epistatic genes (Banerjee and Kallo, 1987). One resistant line, H24, has been developed from this accession and a gene controlling TYLCV (Tyl-2) in it has been mapped to chromosome 11 (Hanson et al., 2000, 2006). The accession LA1777 was reported to be highly resistant to TYLCV (Fargette, 1991; Hanson, 1999; Ioannou, 1985; Moustafa, 1991) and also to ToMoV (Hanson, 1999). Another accession of S. habrochaites, LA0386, has also been reported as resistant to TYLCV with dominant control by more than one gene (Hassan et al., 1984). Picó et al. (2000) reported that LA0386 had a higher level of resistance than LA1777 when using agroinoculation. Vidavsky and Czosnek (1998) developed a TYLCV-resistant line named Li902 from a cross between S. habrochaites accessions LA1777 and LA0386 followed by crossing to S. lycopersicum and selfing resistant, symptomless individuals. The authors suggested that the resistance in this line is controlled by one major dominant gene with some minor genes. The line Li902 has been evaluated and used as a broad-based source of resistance for begomovirus resistance breeding programs in Guatemala, where seven bipartite begomoviruses have been reported (Mejía et al., 2005; Nakhlak et al., 2005), and for TYLCV resistance breeding programs in Middle Eastern countries (Maruthi et al., 2003). However, there is no report specifically describing the origin and chromosomal location of resistance gene(s) in line Li902.

Recombinant inbred lines (RILs) derived from LA1777 in S. lycopersicum (Monforte and Tanksley, 2000), that cover ≥83% of the S. habrochaites genome, are available and provide a means to locate genes with resistance to TYLCV and other begomoviruses. Therefore, we screened 89 of these RILs for resistance to TYLCV and ToMoV. In an initial study, none of the RILs demonstrated a high level of resistance to either disease (Momotaz et al., 2005). However, 18 lines displayed potentially low levels of resistance to ToMoV or TYLCV. Nine of these lines had S. habrochaites alleles at TG27 (restriction fragment length polymorphism marker) on chromosome 1, and four of the lines had S. habrochaites alleles at TG202 on chromosome 7 (Momotaz et al., 2005). We hypothesized that minor genes in these regions on chromosomes 1 and 7 might provide better resistance when combined. Thus, the objective of this work was to combine RILs expressing low levels of resistance to determine if higher levels of resistance could be obtained and, thus, identify the location of genes from LA1777 resistant to TYLCV and ToMoV.

Materials and Methods

Spring and Fall 2004
In the Spring of 2004, crosses were made between RILs with markers that were correlated with the low levels of resistance from 2003 testing (Table 1). The developed hybrids were designated 401 to 410. Seeds were bulked from 10 plants of each F1 hybrid grown in the field to obtain F2 seeds in the Fall of 2004.

Spring 2005
In the Spring of 2005, seeds of the 10 F2 lines, the susceptible control ‘Horizon’, and the resistant control line 927 (advanced breeding line derived from S. chilense accession LA1932) were sown in flats containing Black Beauty spent coal (Reed Minerals Div., Highland, IN) on 25 Jan. Seedlings of these lines were transplanted at the cotyledon stage into two sets of Speedling trays (3.8-cm2 cell size; Speedling, Sun City, FL) in the greenhouse on 7 Feb. On 25 Feb., trays were moved into whitely-proof greenhouses for virus inoculation.
Inoculation. Inoculation was done according to the method described by Griffiths and Scott (2001). Two B. argentinofoli colonies viruliferous for TYLCV or ToMoV were maintained separately on plants of the dwarf tomato ‘Florida Lanai’. Plants at the three leaf stage were inoculated separately for 16 d by placing them in growth chambers with TYLCV- or ToMoV-infested ‘Florida Lanai’ plants. There were two virus-infected source plants per ≈550 test plants. Each inoculation plant had eight to 12 leaves and each was heavily infested with whitely adults and immature life stages. The source plants were shaken daily to disperse whitely adults onto the test plants. On 14 Mar., whiteflies were killed with a foliar application of a 1% v/v solution of M-Pede® (potassium salts of fatty acids; Dow AgroSciences LLC, Indianapolis, IN). The trays were then drenched with Admire 2F® (imidaclopid; Bayer CropScience LP, Research Triangle Park, NC) at the rate of 3 mL/1000 plants. On 21 and 22 Mar., the inoculated seedlings of TYLCV and ToMoV, respectively, were transplanted into separate field plots for each disease.

Twenty plants per F2 were planted and spaced 46 cm apart within rows with 152 cm between rows. The beds had been fumigated with 67% methyl bromide:33% chloropicrin at 197 kg ha⁻¹ and covered with white plastic mulch. The plants were fertilized with a total of 293 kg ha⁻¹ of nitrogen and 486 kg ha⁻¹ of phosphorus and potash over the growing season. The plants were staked and tied at 197 kg ha⁻¹ of nitrogen and 67% methyl bromide:33% chloropicrin with 67% methyl bromide:33% chloropicrin. The plants were irrigated by seepage ditches adjacent to the rows.

Fertilizer. The plants were staked and tied at 197 kg ha⁻¹ of nitrogen and 67% methyl bromide:33% chloropicrin with 67% methyl bromide:33% chloropicrin. The plants were irrigated by seepage ditches adjacent to the rows.

Resistance assessment was begun 45, 60, and 70 d after inoculation (DAI) in the field. Disease severity was rated using a 0 to 4 scale of Scott et al. (1996) in which 0 = no symptoms, 1 = slight symptoms visible only on close inspection, 2 = mild symptoms on part of the plant, 3 = severe symptoms over the entire plant, and 4 = severe symptoms and stunting. The plants were also subjectively rated for general vigor. A plant was considered tolerant if it had a lower virus disease severity rating with greater vigor. Data from the rating 70 DAI were used for analysis.

Results

The resistant control 927 was highly resistant to both TYLCV and ToMoV (Table 2). A very high percentage of the F2 plants were considered tolerant if it had a lower virus disease severity rating with greater vigor. Data from the rating 70 DAI were used for analysis.

Table 1. Crosses of recombinant inbred lines (RILs) that had partial begomovirus resistance from previous testing and restriction fragment length polymorphism markers (RFLPs) associated with the RILs.

| Designation | RIL crosses | RFLP markers |
|-------------|-------------|--------------|
| 401         | LA3963 × LA3993 | U1* × TG27 (1)* |
| 402         | LA3986 × LA3993 | TG202 × TG27 (1) |
| 403         | LA3968 × LA3993 | TG180 (12) × TG27 (1) |
| 404         | LA4001 × LA3993 | TG27, TG202 × TG27 (1) |
| 405         | LA3913 × LA3950 | TG27 × TG202 (7) |
| 406         | LA3915 × LA3950 | TG27 × TG202 (7) |
| 407         | LA3970 × LA3950 | TG27 × TG202 (7) |
| 408         | LA3993 × LA3950 | TG27 × TG202 (7) |
| 409         | LA3963 × LA3950 | U1 × TG202 (7) |
| 410         | LA4001 × LA3950 | TG27, TG202 × TG202 (7) |

*Unidentified introgressed region in RIL LA3963.

Table 2. TYLCV and ToMoV disease severity frequency distributions and means at 70 d after inoculation began for F2 genotypes in the Spring of 2005.

| Genotypes | Disease | 0 | 1 | 2 | 2.5 | 3 | 4 | Mean ± |
|-----------|---------|---|---|---|-----|---|---|-------|
| 401       | TYLCV   | 0 | 0 | 0 | 0   | 0 | 1 | 18.0 ± |
| 402       | ToMoV   | 0 | 0 | 0 | 0   | 0 | 20| 39.0 ± |
| 403       | TYLCV   | 0 | 0 | 0 | 0   | 5 | 15| 3.6 ± |
| 404       | ToMoV   | 0 | 1 | 0 | 1   | 1 | 17| 3.6 ± |
| 405       | TYLCV   | 0 | 0 | 0 | 2   | 4 | 14| 3.5 ± |
| 406       | ToMoV   | 0 | 0 | 1 | 2   | 17| 3.6 ± |
| 407       | TYLCV   | 0 | 0 | 0 | 1   | 5 | 14| 3.7 ± |
| 408       | ToMoV   | 0 | 0 | 0 | 1   | 19| 4.0 ± |
| 409       | TYLCV   | 0 | 0 | 0 | 0   | 0 | 20| 3.7 ± |
| 410       | ToMoV   | 0 | 0 | 0 | 0   | 0 | 19| 3.8 ± |
| 401       | TYLCV   | 0 | 0 | 0 | 0   | 5 | 15| 3.5 ± |
| 402       | ToMoV   | 0 | 0 | 0 | 0   | 0 | 18| 4.0 ± |
| 403       | TYLCV   | 0 | 0 | 0 | 2   | 17| 3.8 ± |
| 404       | ToMoV   | 0 | 0 | 0 | 0   | 0 | 19| 3.9 ± |
| 405       | TYLCV   | 0 | 0 | 0 | 2   | 14| 3.5 ± |
| 406       | ToMoV   | 0 | 0 | 0 | 0   | 0 | 19| 3.8 ± |
| 407       | TYLCV   | 0 | 0 | 0 | 0   | 5 | 15| 3.5 ± |
| 408       | ToMoV   | 0 | 0 | 0 | 0   | 0 | 18| 4.0 ± |
| 409       | TYLCV   | 0 | 0 | 0 | 2   | 17| 3.8 ± |
| 410       | TYLCV   | 0 | 0 | 0 | 0   | 0 | 19| 3.9 ± |
| Horizon   | TYLCV   | 2 | 0 | 4 | 1   | 3 | 25| 3.3 ± |
| Horizon   | ToMoV   | 1 | 7 | 2 | 0   | 17| 2.3 ± |
| 927       | TYLCV   | 1 | 8 | 2 | 0   | 0 | 0 | 0.1 ± |
| 927       | ToMoV   | 1 | 4 | 0 | 0   | 0 | 0 | 0.4 ± |

*Based on 20 plants per line per disease.

Horizon is the susceptible control and 927 is the resistant control.

TYLCV = Tomato yellow leaf curl virus; ToMoV = Tomato mottle virus.
Table 3. Marker analysis of some selected F₂ plants with their individual disease severity ratings for TYLCV and ToMoV.

| Line     | Possible marker(s) | TG27 | TG202 | TG180 | Disease severity* |
|----------|--------------------|------|-------|-------|-------------------|
| TG27 only|                    |      |       |       |                   |
| 401-Y10* |                   | —    | —     | —     | 1.5 2.0 3.0       |
| 409-6    |                    | —    | —     | —     | 0.5 2.5 3.5       |
| 409-19   |                    | —    | —     | —     | 2.0 3.0 3.5       |
| TG202 only|                   |      |       |       |                   |
| 402-Y4   |                    | h    | e     | 1.0   | 1.0 1.0           |
| 402-Y10  |                    | h    | e     | 2.0   | 2.0 2.5           |
| 403-Y3   |                    | h    | —     | —     | 2.0 2.0 3.0       |
| 403-Y18  |                    | he   | —     | —     | 2.0 2.0 3.0       |
| 403-Y19  |                    | he   | —     | —     | 1.5 2.0 3.0       |
| 403-7    |                    | he   | —     | —     | 0.5 1.5 2.5       |
| 403-8    |                    | he   | —     | —     | 0.5 2.0 3.0       |
| 403-14   |                    | he   | —     | —     | 0.5 0.5 1.0       |
| TG180 × TG27|                |      |       |       |                   |
| 402-Y4   |                    | h    | e     | 1.0   | 1.0 1.0           |
| 402-Y10  |                    | h    | e     | 2.0   | 2.0 2.5           |
| 403-Y11  |                    | he   | e     | 2.5   | 2.5 3.0           |
| 403-3    |                    | he   | e     | 0.0   | 0.0 2.0           |
| 405-Y4   |                    | —    | —     | —     | 1.5 1.5 2.5       |
| 405-7    |                    | e    | e     | 0.0   | 0.0 2.0           |
| 406-14   |                    | e    | e     | 1.0   | 1.0 1.0           |
| TG27, TG202 × TG27|          |      |       |       |                   |
| 404-Y3   |                    | h    | he    | —     | 2.0 2.0 2.5       |
| 404-Y20  |                    | h    | he    | —     | 1.0 1.5 2.5       |
| 404-3    |                    | h    | e     | —     | 0.5 2.0 3.0       |
| 404-13   |                    | h    | he    | —     | 1.0 1.5 2.0       |
| TG202 × TG202|              |      |       |       |                   |
| 410-Y4   |                    | —    | —     | —     | 1.5 1.5 2.0       |
| 410-Y18  |                    | h    | h     | —     | 1.5 1.5 2.0       |
| 410-2    |                    | h    | h     | —     | 0.0 2.0 3.0       |

*See Table 1.

Discussion

We found no evidence for resistance to TYLCV or ToMoV in lines derived from RILs that were combined based on their vigorous growth after inoculation in an earlier study (Momotaz et al., 2005). Further evidence that regions on chromosomes 1 and 83% of the S. habrochaites genome covered. The present study along with our previous one (Momotaz et al., 2005) strongly indicate that resistance genes for TYLCV or ToMoV are not present in the 83% of the S. habrochaites genome covered by the RIL population used in our experiments. Resistance gene(s) derived by Vidavsky and Czosnek (1998) may be introgressed from LA386 or from part of the LA1777 genome not present in the RILs. This could include the 17% not present in RILs or other genes not represented by the RILs. The latter is possible because S. habrochaites accession LA1777 is an outcrossing species, in which resistance alleles may not be fixed. The RILs were derived from a single plant (Bernacchi and Tankels, 1997) that likely did not have all of the genetic variation of LA1777. Further evidence for this possibility emerged from our LA1777 whitefly resistance work. We detected regions that may be associated with silverleaf whitefly resistance based on CAPS markers and an interspecific F₂ population. However, three of the RILs that should have had a CAPS marker did not have the polymorphism (data not shown). At present, the source(s) of resistance reported
by Vidavsky and Czosnek (1998) remain unknown. Picó et al. (2000) provided evidence that LA0386 had better TYLCV resistance than LA1777 and it may be that the resistance was derived from LA0386. In this work, agroinoculation was used, which would circumvent infection by the whitefly. This is important because LA1777 has a high density of type IV glandular trichomes and is not attractive to the whitefly (see Muigai et al., 2003). Some previous studies reporting resistance to TYLCV from LA1777 have not addressed this issue. However, resistance from LA1777 cannot be ruled out unequivocally, although we have shown that the resistance is not present in the LA1777 RILs. Other researchers should be aware of this before they attempt to locate resistance in the LA1777 RIL population.

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