Ty-6, a major begomovirus resistance gene on chromosome 10, is effective against Tomato yellow leaf curl virus and Tomato mottle virus

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Abstract

Ty-6 is a major resistance gene on chromosome 10 of tomato that provides resistance against monopartite and bipartite begomoviruses and complements resistance conferred by the known Ty-3 and ty-5 genes.

Resistance to monopartite and bipartite begomoviruses is an important breeding objective for cultivated tomato. Several begomovirus resistance genes have been introgressed from related Solanum species and are available for breeding purposes. In the present study, we mapped an additional locus, Ty-6, to chromosome 10 of tomato. Ty-6 is effective against both monopartite Tomato yellow leaf curl virus (TYLCV) and bipartite Tomato mottle virus (ToMoV). Gene action is incomplete dominance, with an intermediate resistance response when Ty-6 is heterozygous. Analysis of populations segregating for Ty-6 along with Ty-3 or ty-5 indicates that the highest level of resistance against TYLCV is attained when Ty-6 is combined with an additional resistance allele. Our results also demonstrate that ty-5 is ineffective against ToMoV. Although multiple SNPs linked to Ty-6 were identified and can be used for breeding purposes, none of these were consistently polymorphic between Ty-6 and ty-6 breeding lines. Further research is underway to generate resequencing data for several Ty-6 inbred lines for the discovery of additional sequence polymorphisms that can be used for fine mapping and characterizing the Ty-6 locus.

Introduction

Begomoviruses, from the family Geminiviridae, cause economically significant diseases of major vegetable crops in the world. Two begomoviruses, Tomato yellow leaf curl virus (TYLCV) and African cassava mosaic virus (ACMV), were recently listed among top 10 crop viruses due to their scientific importance and global economic impact (Rybicki 2015). The Begomovirus genus is the largest of family Geminiviridae and contains more than 200 species (Fauquet et al. 2008). Begomoviruses have either a monopartite or bipartite genome and are transmitted mainly through the insect vector, sweet potato whitefly (Bemisia tabaci) (Zhou 2013; Rojas et al. 2005). The begomoviruses that infect cultivated tomato (Solanum lycopersicum L.) in the USA and other tropical and subtropical regions include monopartite and bipartite viruses such as TYLCV, Tomato yellow leaf curl Thailand virus (TYLCTHV), Tomato mottle virus (ToMoV), and two newly identified viruses, Tomato leaf deformation virus (ToDeV) and Tomato leaf curl purple vein virus (ToLCPVV) (Polston and Anderson 1997; Moriones and Navas-Castillo 2000; Macedo et al. 2018; Melgarejo et al. 2018).
Management strategies for begomoviruses rely heavily on insecticide treatments to control whiteflies, but such measures can be ineffective because of the development of resistance against insecticides in the vector (Moriones and Navas-Castillo 2000; Omer et al. 1993).

Use of genetic resistance against begomoviruses has proved successful in tomato. Tomato breeding strategies have primarily focused on the introgression of resistance alleles from related wild germplasm. Resistance has been identified in a number of *Solanum* species, including *S. pimpinellifolium, S. peruvianum, S. chilense, S. habrochaites* and *S. cheesmaniae* (Ji et al. 2007; Picó et al. 1996; Scott 2006), and several resistance genes have been introgressed and genetically characterized in tomato. *Ty-1* was introgressed from *S. chilense* accession LA1969 and mapped to chromosome 6 of tomato (Zamir et al. 1994; Verlaan et al. 2011). Another TYLCV resistance gene, *Ty-3*, was introgressed from *S. chilense* accessions, LA1932/LA2779/LA1938, and also mapped to chromosome 6 (Ji et al. 2007).

Later, *Ty-1* and *Ty-3* were determined to be allelic and to code for an RNA-dependent RNA polymerase, which imparts resistance by increasing cytosine methylation of viral genomes (Verlaan et al. 2013; Butterbach et al. 2014). The second TYLCV resistance gene discovered was *Ty-2*, which was introgressed on chromosome 11 from *S. habrochaites* (Hanson et al. 2006; Yang et al. 2014; Yamaguchi et al. 2018). Yamaguchi et al. (2018) recently determined that *Ty-2* is a nucleotide-binding domain and leucine-rich repeat-containing (NB-LRR) gene (Yamaguchi et al. 2018). In addition to *Ty-1* and *Ty-3*, tomato lines derived from *S. chilense* accessions exhibited multi-genic control of TYLCV resistance, indicating the presence of additional resistance loci in studied introgressed lines (Scott et al. 1996). Later, another TYLCV resistance gene, *Ty-4*, was identified from *S. chilense* accession, LA1932, and mapped on chromosome 3 (Ji et al. 2009). Compared to other *Ty* genes, *Ty-4* is less effective against TYLCV (Kadirvel et al. 2013). A recessive resistance to TYLCV derived from the cultivar ‘Tyking’ was also observed in some of the tomato breeding lines (Hutton et al. 2012). This recessive resistance was mapped on chromosome 4 as *ty-5* locus (Hutton et al. 2012; Anbinder et al. 2009). *Ty-5* encodes a messenger RNA surveillance factor Pelota (Pelo) which is involved in ribosome recycling phase of protein synthesis (Lapidot et al. 2015).

Besides the above-mentioned genes, recent studies provide evidence for the presence of another, unidentified, resistance gene(s). For example, Hutton et al. (2012) found that TYLCV resistance in the *ty-5* parental lines, Fla. 8753 and Fla. 344, was significantly greater than that of F₂ progeny which were homozygous for *ty-5*, and F₂ progeny lines derived from Fla. 8753 also demonstrated segregation for resistance that was not due to *ty-5* (Hutton et al. 2012). Verlaan et al. (2013) likewise indicated the presence of additional resistance allele in Fla. 8680 (Verlaan et al. 2013). It is likely that the putative resistance allele reported by Hutton et al. (Hutton et al. 2012) in Fla. 8383 and in Fla. 8638B is the same as that in Fla. 8680, since Fla. 8680 is the resistant parent in a cross from which Fla. 8383 is derived (S. Hutton, Unpublished). This resistance allele is presumably inherited from *S. chilense* and was recently designated as *Ty-6* (Scott et al. 2015). Here, we report the genetic mapping of *Ty-6*, and we characterize the effect of *Ty-6* alone and in combination with other resistance genes.

### Materials and methods

#### Plant materials and experimental design

Six large-fruited, fresh market tomato breeding lines with moderate or high levels of begomovirus resistance were used as donor parents to develop F₂ populations segregating for resistance. Breeding lines with resistance derived from *S. chilense* accession LA2779 (LA2779) included Fla. 8680, Fla. 8383 and Fla. 8503C. Fla. 8680 expresses a high level of resistance that is based on *Ty-3* and on another locus (loci) (Verlaan et al. 2013). Fla. 8383 and Fla. 8503C were each selected from a cross between Fla. 8680 and a susceptible breeding line; each expresses a moderate level of resistance that is not based on *Ty-3*. Fla. 8472B, Fla. 8638B and Fla. 8382B have high levels of resistance derived from *S. chilense* accession LA1938 (LA1938) and from ‘Tyking’; Fla. 8638B and Fla. 8382B are full-sib lines, and resistance in both lines is conferred, in part, by *ty-5* from ‘Tyking’ (Hutton et al. 2012). Breeding lines Fla. 7776, Fla. 7060, Fla. 7987, Fla. 8059, Fla. 8044 and Fla. 7781 were used as susceptible parents for the development of F₂ populations for mapping and characterization of the *Ty-6* locus. Each of these lines is further described in Supplementary Table S1.

Fla. 8383 was crossed to Fla. 7776, and an F₂ population was developed for the initial genetic mapping of *Ty-6*; subsequently, Fla. 8503C was crossed to Fla. 7060 to develop a population for *Ty-6* location confirmation and for genetic mapping of ToMoV resistance. Fla. 8382B was crossed to Fla. 8059 and Fla. 8638B was crossed to Fla. 7987 to study the effect of *Ty-6* in combination with *ty-5* on TYLCV resistance; Fla. 8472B was crossed to Fla. 8044 to study the effect of *Ty-6* in combination with *ty-5* on ToMoV resistance. Fla. 8680 was crossed to Fla. 7781 to study the effect of *Ty-6* in combination with *Ty-3* on TYLCV resistance. All experiments were conducted in the field using randomized complete block design with three blocks and with six plant plots for each parent and ~40 plant plots for F₂ populations.

For haplotyping, in addition to above listed lines, additional resistant and susceptible lines were included. Resistant lines Fla. 8753, Fla. 344, Fla. 8624 and Fla. 8062 were
derived from ‘Tyking’ and LA1938 (Hutton et al. 2012; Scott et al. 2015). Fla. 8753, Fla. 344 and Fla. 8062 each have a high level of resistance due to the presence of ty-5 and Ty-6. Fla. 8624 contains only Ty-6 and has a moderate level of resistance to TYLCV. Fla. 7804 and Fla. 8022 are large-fruited susceptible inbreds.

Inoculation and disease evaluation

All inoculations were performed using whiteflies viruliferous for TYLCV or ToMoV according to the method developed by Griffiths and Scott (Griffiths and Scott 2001) with some modifications. Briefly, seedlings 4 weeks past the cotyledon stage (three to four leaves) were exposed to viruliferous whiteflies for 2 weeks in growth chambers. For these purposes, separate viruliferous whitefly colonies were maintained in temperature-controlled growth rooms on TYLCV- or ToMoV-infected tomato plants, and whitefly-infested plants were transferred to separate growth rooms for each inoculation. Growth rooms were maintained at 25 °C with a 14 h photoperiod. On average, 20 whiteflies per plant were used during inoculation of tomato breeding lines and F2 populations. Following inoculation, the whiteflies were killed by treating plants with an insecticidal soap and with imidacloprid [Admire (Bayer CropScience, Research Triangle Park, NC)], prior to transplanting to the field. Plants were rated for disease severity in the field approximately 40 days after exposure to whiteflies on a 0–4 disease severity index (DSI) scale as described previously (Scott et al. 1996), where 0 = no symptoms and 4 = severe symptoms and stunting. Intermediate scores such as 1.5 and 2.5 were incorporated to allow for more precise disease severity ratings.

DNA extraction and molecular marker genotyping

DNA was extracted from young leaves of individual plants using a modified cetyltrimethylammonium bromide (CTAB) procedure (Fulton et al. 1995). Leaf samples for DNA isolation were collected from plants at seedling stage before transplanting to field.

For genotyping, single-nucleotide polymorphism (SNP) information generated by the Solanaceae Coordinated Agricultural Project (SoLCAP) was used to identify a subset of 384 SNP markers optimized for genotyping fresh market tomatoes (Hamilton et al. 2012; Sim et al. 2012a, b). SNP markers were optimized by filtering data generated from the SoLCAP Infinium Array (Illumina, Foster City, CA) based on allele frequency for 140 tomatoes annotated as contemporary fresh market varieties (Sim et al. 2012b). Markers with high polymorphic information content (PIC) were retained. Genetic positions based on Sim et al. (2012a) were used to select SNPs distributed across all 12 chromosomes. Finally, gaps in genome coverage were filled in by selecting high PIC markers based on physical position. For the initial mapping, the 384-SNP panel was used to genotype 203 TYLCV-inoculated F2 plants and their parents, Fla. 7776 and Fla. 8383, using competitive allele-specific PCR genotyping chemistry (Supplementary Table S2; KASP, www.lgcgroup.com).

For confirmation of TYLCV resistance QTL detected in the Fla. 7776 x Fla. 8383 population and for mapping ToMoV resistance, an independent F2 population from the cross between Fla. 7060 and Fla. 8503C was genotyped with chromosome 10 specific markers. Prior to inoculation, transplants of this population were separated into two groups: one for phenotyping with TYLCV and the other for phenotyping with ToMoV. Both groups were genotyped in cooperation with Nunhems USA, Inc. (www.nunhemsusa.com) and Bejo Seeds, Inc. (www.bejoseeds.com) using proprietary markers corresponding to the Ty-6 interval on chromosome 10 (Supplementary Table S3).

To study the effect of combinations of different Ty genes on TYLCV or ToMoV resistance in other listed populations, individual plants were genotyped with one or more of the following: the proprietary marker, B_04 for Ty-6; UF_10.61192 for Ty-6 which corresponds to the solcap_snps_61192 (Sim et al. 2012a); SNAC1 (Anbinder et al. 2009) and TY5.2 markers for ty-5; and TY3-5 for Ty-3 (Supplementary Table S4).

Genetic mapping, QTL analysis and statistical analysis

Genotyping data of 156 SNP markers tested on the (Fla. 8383 x Fla. 7776) F2 population were used for linkage map construction using JoinMap 4.1 (Van Ooijen 2006). A threshold recombination frequency of <0.25 was used for grouping loci into linkage groups. For QTL detection, linkage map information generated by JoinMap 4.1 was used for analysis in QTL Cartographer version 2.5 (Wang et al. 2012). QTLs were identified by single-locus QTL analysis using interval mapping. The threshold LOD scores were calculated using 1000 permutations as given in the software (Churchill and Doerge 1994). A minimum LOD score of > 3.0 was used to declare a QTL.

Disease severity data were analyzed using a Wald-type statistics (WTS) procedure for nonparametric ordinal data (Shah and Madden 2004). The overall effect of Ty-6 alone and in combination with Ty-3 or ty-5 on TYLCV and/or ToMoV resistance was calculated by WTS and analysis of variance type statistics (ATS) on ranked data using PROC MIXED procedure in SAS (version 9.4; SAS Institute, Cary, NC, USA). Relative marginal effects (RME)
and 95% confidence intervals were calculated according to the procedure given previously (Shah and Madden 2004).

Results

The TYLCV resistance locus, Ty-6, maps to chromosome 10 of tomato

Fla. 8383 is a fresh market breeding line with moderate resistance to TYLCV derived from S. chilense accession LA2779. As this line does not contain any previously known TYLCV resistance genes, we crossed Fla. 8383 with the susceptible parent, Fla. 7776, to develop an F2 mapping population. Resistance in the F1 was intermediate to the parents, indicating incomplete dominance (Table 1). Additionally, segregation among F2 individuals generally followed three phenotypic levels, similar to that observed for the parents and the F1 (Fig. 1). The F2 population of 203 individuals was genotyped using a 384 SNP array, of which 158 SNPs were confirmed polymorphic between the parents and segregating in the population. Linkage analysis generated a total map distance of 878.4 cM for all chromosomes except chromosome 11 (which had no polymorphic SNPs). Interval mapping identified Ty-6 as a single QTL on chromosome 10 explaining 59.4% of the phenotypic variance with LOD score of 41.1 (Fig. 2). The SNP marker solcap_snp_sl_61192 showed the strongest association with the phenotype. No evidence of any other QTL associated with TYLCV resistance was found on other chromosomes (Supplementary Fig. S1).

Ty-6 confers resistance against ToMoV

In addition to TYLCV, breeding lines carrying Ty-6 are also resistant to ToMoV. To further confirm the Ty-6 locus for resistance to TYLCV and to test for an effect of this gene against a bipartite begomovirus, an F2 population developed from the cross between Fla. 7060 and Fla. 8503C was used. F2 plants were divided into two groups: one (110 F2 plants) of which was inoculated with TYLCV and the other (114 F2 plants) inoculated with ToMoV. Both sets of F2 plants were genotyped with same set of chromosome 10 specific molecular markers. QTL analyses identified the same locus on chromosome 10 in both groups of F2 plants with LOD scores of 33.19 for TYLCV and 19.36 for ToMoV, demonstrating the efficacy of Ty-6 against both TYLCV and ToMoV (Fig. 3). The markers N_18 (and B_02) explained 97.57% of phenotypic variance for TYLCV and 99.44% of phenotypic variance for ToMoV. Resistance conferred by Ty-6 against TYLCV and ToMoV was additive in nature, confirming incompletely dominant inheritance (Tables 2, 3).

Table 1 Mean disease severity index (DSI) of TYLCV-resistant parent, Fla. 8383, TYLCV-susceptible parent, Fla. 7776, and F1 plants

| Population | Ty-6 | N  | Mean DSIa | RME  | 95% CI          |
|------------|------|----|-----------|------|----------------|
| Fla. 7776  | –    | 12 | 3.9a      | 0.81944 | 0.74263–0.83142 |
| F1 (Fla. 8383 × Fla. 7776) | ++  | 12 | 3.0b      | 0.51389 | 0.48664–0.54096 |
| Fla. 8383  | ++   | 12 | 2.0c      | 0.16667 | 0.16667          |

aDSI based on a 0–4 scale where higher numbers indicate more severe virus symptoms; mean separations within either viral trial are based on 95% confidence intervals (CI) of relative marginal effects (RME) on ranked DSI data.

Fig. 1 TYLCV disease severity on the susceptible parent, Fla. 7804; the Ty-6 resistant parent, Fla. 8624 (containing Ty-6); and F2 plants with different Ty-6 genotypes.
produced a comparable level of resistance to heterozygosity for both Ty-3 and Ty-6, but otherwise, greater levels of resistance were achieved with two-gene combinations than with either gene alone. The highest level of resistance was observed in plants homozygous for both Ty-3 and Ty-6.

Two F2 populations derived from crosses Fla. 8059 × Fla. 8382B and Fla. 7987 × Fla. 8638B were analyzed to characterize the resistance response of Ty-6 in combination with ry-5 against TYLCV. In both populations, resistance was significantly associated with Ty-6 (P < 0.0001) and ry-5 (P < 0.0001); interactions were also significant between Ty-6 and ry-5 in the tested populations probably due to the recessive nature of ry-5 (Table 6). As with the Ty-3 and Ty-6 combination, Ty-6 and ry-5 likewise provided complementary resistance, and results were similar for both populations (Table 7). Plants homozygous for Ty-6 were equally resistant as plants homozygous for ry-5, and heterozygosity at the ry-5 locus provided no control, which is consistent with it being a recessive gene. Again, the highest level of disease control was observed in plants with two-gene combinations, and plants homozygous for both genes were more resistant than those homozygous for ry-5 and heterozygous for Ty-6.

The effect of Ty-6 in combination with ry-5 was also evaluated against ToMoV using an F2 population from the cross Fla. 8044 × Fla. 8472. Results demonstrated that Ty-6 has a significant effect toward reducing ToMoV disease severity (Table 3). However, neither ry-5 nor its interaction with Ty-6 had any significant effect on TYLCV disease severity, demonstrating that ry-5 is ineffective against ToMoV (Table 8). Similar to the population from a cross Fla. 7060 × Fla. 8503C, the current population also indicated incomplete dominant inheritance of Ty-6 in resistance against ToMoV (Table 9).

**Effect of Ty-6 in combination with Ty-3 or ry-5**

In order to understand interactions among Ty resistance genes, we studied F2 breeding populations segregating for Ty-6 with Ty-3 or ry-5, the latter of which are both effective against TYLCV (Verlaan et al. 2013; Lapidot et al. 2015). The Fla. 8680 × Fla. 7781 population was genotyped for Ty-3 and Ty-6 and phenotyped for TYLCV. Results indicated significant associations of TYLCV resistance with both Ty-6 (P < 0.0001) and Ty-3 genes (P < 0.0001) (Table 4). The interactions between Ty-6 and Ty-3 were nonsignificant indicating no involvement of epistatic effects. Segregation in the population indicated complementary action of Ty-3 and Ty-6 for resistance to TYLCV (Table 5). Although both genes effectively reduced TYLCV disease severity, Ty-3 provided a stronger resistance than Ty-6 (Table 5). Homozygosity for Ty-3

**Haplotype differences among TYLCV-resistant and TYLCV-susceptible tomato germplasm**

Tomato breeding programs regularly rely on marker-assisted selections in order to identify resistant plants without conducting virus inoculations or performing evaluations under conditions of high disease pressure. With the goal of identifying molecular markers that consistently distinguish between Ty-6 and susceptible genotypes, nine resistant and seven susceptible breeding lines were surveyed genotypically with 19 SNP markers corresponding to the Ty-6 physical region (Fig. 4). None of the SNP markers, however, consistently distinguished between resistant and susceptible genotypes. Although the SNP markers solcap_snp_sl_61131 and solcap_snp_sl_61108 correctly identified the Ty-6 genotype among most of the breeding lines surveyed, these markers did not detect the resistant
allele in Fla. 8472B, and they also failed to consistently distinguish between all susceptible and resistant lines in a broader panel of germplasm that was tested (data not shown). Thus, although these markers are useful within specific populations, they may have limited utility for consistently tracking Ty-6 across diverse germplasm.

**Discussion**

Disease resistance against begomoviruses is one of the most important breeding objectives in tomato breeding programs across the world. The University of Florida,
Institute of Food and Agricultural Sciences (UF/IFAS) tomato breeding program initiated its begomovirus resistance project in the early 1990s, after ToMoV emerged and caused significant crop losses in the state (Scott et al. 1996; Scott and Schuster 1991). Early disease screens identified several ToMoV resistant *S. chilense* accessions.
Table 7  Mean disease severity index (DSI) and relative marginal effect (RME) for TYLCV disease severity on genotypes segregating for the $ty$-5 and the $Ty$-6 loci in $F_2$ populations from the crosses between Fla. 8059 and Fla. 8382B, and between Fla. 7987 and Fla. 8638B

| Population     | $ty$-5 | $Ty$-6 | N  | Mean DSI$^b$ | RME    | 95% CI          |
|----------------|--------|--------|----|--------------|--------|-----------------|
| 8059 × 8382B   |        |        | 16 | 3.6a         | 0.85270| 0.74898–0.90865 |
|                | –      | –      | 14 | 2.9b         | 0.59487| 0.48097–0.69795 |
|                | –      | ++     | 12 | 2.1c         | 0.34549| 0.26532–0.43726 |
|                | ++     | –      | 25 | 3.4a         | 0.76563| 0.68183–0.82655 |
|                | ++     | ++     | 47 | 2.9b         | 0.61209| 0.56035–0.65959 |
|                | –      | ++     | 33 | 2.2c         | 0.39915| 0.34840–0.45333 |
|                | ++     | –      | 10 | 2.1c         | 0.36823| 0.28538–0.46083 |
|                | ++     | ++     | 26 | 1.4d         | 0.15805| 0.12912–0.19839 |
| 7987 × 8638B   | –      | –      | 21 | 3.7a         | 0.82960| 0.75382–0.87932 |
|                | –      | ++     | 33 | 2.9b         | 0.62861| 0.58829–0.66653 |
|                | –      | ++     | 17 | 2.1c         | 0.37829| 0.31494–0.44699 |
|                | ++     | –      | 28 | 3.7a         | 0.84126| 0.79790–0.87214 |
|                | ++     | ++     | 27 | 2.8b         | 0.59908| 0.53552–0.65852 |
|                | ++     | +      | 19 | 2.3c         | 0.40893| 0.35758–0.46279 |
|                | ++     | –      | 22 | 2.1c         | 0.36741| 0.28618–0.45939 |
|                | ++     | +      | 30 | 1.4d         | 0.20914| 0.16873–0.26180 |
|                | ++     | +      | 20 | 0.9e         | 0.10230| 0.07905–0.14019 |

$^a$ $ty$-5 and Ty-6 genotypes are based on markers, TY5.2 and proprietary marker B_04, respectively, in cross 8059 × 8382B. In cross 7987 × 8638B, $ty$-5 and Ty-6 genotypes are based on marker SlNAC1 and UF_10.61192, respectively

$^b$ DSI based on a 0–4 scale where higher numbers indicate more severe virus symptoms; mean separations within either viral trial are based on 95% confidence intervals (CI) of relative marginal effects (RME) on ranked DSI data. TYLCV DSI for Fla. 8059 = 4.0, Fla. 8382B = 0.5, Fla. 7987 = 4.0, Fla. 8638B = 0.5

Table 8 Statistical analysis for effects of $ty$-5 and Ty-6 toward ToMoV resistance in $F_2$ population developed from the cross between Fla. 8044 and Fla. 8472

| Population | Source | Num DF | Den DF | $\chi^2$ | $F$ value | $Pr > \chi^2$ |
|------------|--------|--------|--------|---------|----------|---------------|
| 8044 × 8472| $ty$-5 | 2      | 134    | 0.19    | 0.10     | 0.9081        |
|            | Ty-6   | 2      | 134    | 15.52   | 7.76     | 0.0004***     |
|            | $ty$-5 × Ty-6 | 4      | 134    | 3.75    | 0.94     | 0.4404        |

Table 9 Mean disease severity index (DSI) and relative marginal effect (RME) for ToMoV disease severity on genotypes segregating for the $ty$-5 and the $Ty$-6 loci in $F_2$ population from the cross between Fla. 8044 and Fla. 8472

| Population | $ty$-5$^a$ | $Ty$-6$^a$ | N  | Mean DSI$^b$ | RME    | 95% CI          |
|------------|------------|------------|----|--------------|--------|-----------------|
| 8044 × 8472| –          | –          | 11 | 2.6a         | 0.63724| 0.40941–0.81161 |
|            | –          | ++         | 15 | 1.9ab        | 0.50452| 0.38414–0.62424 |
|            | –          | ++         | 10 | 1.4b         | 0.39371| 0.26406–0.54350 |
|            | ++         | –          | 22 | 2.2a         | 0.56294| 0.45312–0.66472 |
|            | ++         | +          | 32 | 1.7ab        | 0.51814| 0.43916–0.59564 |
|            | ++         | +          | 18 | 1.4ab        | 0.42347| 0.32013–0.53645 |
|            | ++         | –          | 8  | 2.7a         | 0.71933| 0.48409–0.86402 |
|            | ++         | +          | 17 | 1.4ab        | 0.43007| 0.29219–0.58305 |
|            | ++         | +          | 10 | 0.7b         | 0.29371| 0.17496–0.45910 |

$^a$ $ty$-5 genotype based on marker TY5.2 and Ty-6 genotype based on marker B_16

$^b$ DSI based on a 0–4 scale where higher numbers indicate more severe virus symptoms; mean separations within either viral trial are based on 95% confidence intervals (CI) of relative marginal effects (RME) on ranked DSI data. ToMoV DSI for Fla. 8044 = 3.2, Fla. 8472 = 0.0
(i.e., LA1932, LA1938, LA1961, LA1968, and LA2779), which were used for introgression breeding (Scott and Schuster 1991; Scott et al. 1996). Throughout subsequent selection cycles, these breeding materials were screened with ToMoV, and once TYLCV was discovered in Florida, lines were screened separately with the two viruses. These efforts led to the genetic mapping and cloning of Ty-3, a major TYLCV resistance gene on chromosome 6 derived independently from two S. chilense accessions, LA1932 and LA2779 (Ji et al. 2007; Verlaan et al. 2013). Ty-3 was thought to contribute partial resistance to ToMoV, and a minor role for this gene against ToMoV has been confirmed (Ji et al. 2007). Further analysis of breeding lines with resistance derived from LA1932 led to the identification of the Ty-4 resistance locus on chromosome 3 (Ji et al. 2009), and screening of breeding material carrying Ty-3 alone or in combination with Ty-4 against multiple bipartite begomoviruses indicated greater resistance with the two-gene combination (Nakhla et al. 2004). However, it was not clear if other bipartite resistance loci besides Ty-4 may be present in advanced breeding materials.

More recently, several studies have provided supporting evidence for an additional locus from S. chilense. For example, phenotypic analysis of selected Ty-3 recombinant inbred lines derived from a cross with Fla. 8680 indicated partial TYLCV resistance in several RILs that lacked Ty-3 (Verlaan et al. 2013). Variation for resistance that was not due to Ty-5 or to any other known locus was also described in Fla. 8753 and Fla. 344, both of which derive their resistance from the hybrid, ‘Tyking’ and from S. chilense accession LA1938 (Hutton et al. 2012). Additionally, Hutton et al. (2012) described genetic resistance in the inbred lines Fla. 8638B and Fla. 8383 that was likewise not due to any known resistance locus (Hutton et al. 2012). Scott et al. (2015) recently designated the new resistance locus in Fla. 8638B, Fla. 8624 and Fla. 8680 as Ty-6, and efforts have been ongoing to map this locus in tomato (Scott et al. 2015).

In the present study, an F2 population derived from Fla. 8383 and inoculated with TYLCV was used to map Ty-6 to the distal end of chromosome 10. This locus was then confirmed in multiple populations for its effect against TYLCV as well as ToMoV. Although the exclusive use of whitely-mediated inoculations in the present study could suggest an effect of Ty-6 against the insect vector, Caro et al. (2015) showed that Fla. 8383 is also resistant against Agrobacterium-mediated TYLCV inoculation, demonstrating that the resistance conferred by Ty-6 is not against the vector but against begomoviruses. Using a RIL population developed from the cross between Fla. 456 and a susceptible line, CLN1621L, Kadirvel et al. (2013) identified four QTLs on chromosomes 4, 6, 10 and 11 for resistance to Tomato yellow leaf curl Thailand virus Taiwan strain (TYLC-CTHV-TW). Of these, the QTLs on chromosomes 4 and 10 explained the greatest amount of phenotypic variation.

![Ty-6 marker haplotyping of select University of Florida breeding lines exhibiting resistance (green text) and susceptibility (red text) to TYLCV. All breeding lines were tested for the presence and absence of Ty-6. The physical location (in Mega bases) of molecular markers is based on version SL3.0 of the tomato genome. (Abbreviations: sl = solcap_snp_sl; B = Bejo proprietary marker; snp417 = SGN-U317657_C2_At3g47930_snp417)
Whereas the chromosome 4 QTL likely corresponded to the ty-5 locus, the other QTL mapped between 61 and 63 Mb on chromosome 10 (Kadirvel et al. 2013). Several markers corresponding to the same interval and used in the present study (e.g., B_02 and N_18) demonstrated very strong linkage with Ty-6. Fla. 456 is a UF/IFAS breeding line with resistance derived from S. chilense accession LA2779 and from ‘Tyking’ (Bian et al. 2007). Based on this pedigree and the current genetic mapping information, it is likely that the QTL on chromosome 10 in Fla. 456 is due to the presence of Ty-6. This line has consistently displayed high levels of resistance against TYLCV, Tomato leaf curl Taiwan virus (ToLCTWV) and other predominant begomoviruses in Taiwan, Senegal, Mali, south India, Indonesia, the Philippines and El Salvador (Kadirvel et al. 2013; Chomdej et al. 2007). It is likely that Ty-6 is involved in each of these resistance responses, but further research is needed to understand whether these responses are due to ty-5, to Ty-6 or to the combination of the two genes.

The identification of Ty-6 is an important finding, not only because it expands the toolkit of Ty genes available to breeders, but also because it confers resistance to both monopartite and bipartite begomoviruses. Our results clearly demonstrate that Ty-6 is effective against both TYLCV and ToMoV. Although Ty-3 was once considered to be highly effective against ToMoV, our findings, along with those presented by Scott et al. (2015), suggest that ToMoV resistance in lines such as Fla. 8680 is due primarily to the contribution of Ty-6 (Agrama and Scott 2006; Ji et al. 2007; Scott et al. 2015). Likewise, although ty-5 and Ty-6 collectively contribute to TYLCV in lines such as Fla. 8638B and Fla. 8472, we found that ty-5 is completely ineffective against ToMoV, and the bipartite resistance in such lines is due rather to the presence of Ty-6 (Table 8). This, however, does not imply that ty-5 is ineffective against all bipartite begomoviruses. It is very likely that the recessive resistance locus, tcm-1, derived from ‘Tyking’ is the same as ty-5, and this locus conferred resistance to the bipartite Tomato chlorotic mottle virus (Giordano et al. 2005). Similarly, the chromosome 4 QTL identified by Kadirvel et al. (2013) conferred resistance to the bipartite TYLCV-TW. Thus, although ty-5 is useful against TYLCV and many other viruses, it does not have efficacy against all bipartite begomoviruses.

Scott et al. (2015) reported S. chilense as the source of Ty-6 in Fla. 8624 and Fla. 8638B, and this is likely true, considering that this species is in the pedigree of all Ty-6-containing UF/IFAS lines tested so far. Our results cannot verify this, however, since none of the markers tested consistently distinguishes resistant and susceptible haplotypes, and there is no evidence supporting the presence of a large introgression. Wild species introgressions are often accompanied by linkage with genes that negatively affect horticultural performance (termed, linkage drag). Such was the case with the S. chilense introgressions for Ty-1/Ty-3 and Ty-4, which hampered cultivar development for many years (Hutton et al. 2015; Verlaan et al. 2011). Interestingly, there is no apparent linkage drag associated with Ty-6, and recent surveys of UF/IFAS begomovirus-resistant breeding lines indicate that this allele was maintained through many cycles of horticultural selection (S. Hutton, unpublished data). These data suggest that if S. chilense is in fact the source of Ty-6, the introgression may be very small, and it may be contained within a region that is highly syntenic to cultivated tomato and suffers no homeologous suppression of recombination. Another possibility is that the Ty-6 resistance is derived from sources other than S. chilense such as cv. ‘Tyking.’

Considering the broad range efficacy Ty-6 confers against mono- and bipartite begomoviruses, as well as the complementary resistance it provides in combination with other genes, Ty-6 will likely prove extremely useful for many tomato breeding programs throughout the world. Although some of the markers used in this study were associated with Ty-6, none of those can be broadly applied in marker-assisted breeding, for reasons mentioned earlier. Further research is currently underway to generate whole genome resequencing data for several Ty-6 inbred lines. These results should lead to the discovery of additional sequence polymorphisms than can be used for developing improved markers for use in breeding, in identifying the origin of the gene and in fine mapping the locus.

Author contribution statement JWS, SFH, HS and RS contributed to conception of experiments and acquired phenotypic data. EO, CS, DMF and S-CS designed genotyping assays and produced genotypic data. UG and SFH were responsible for data analysis.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests.

Human and animal right statement This article does not contain any studies with human or animal subjects.
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