Molecular Mapping of *Ty-4*, a New Tomato Yellow Leaf Curl Virus Resistance Locus on Chromosome 3 of Tomato

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ABSTRACT. Resistance to begomoviruses, including bipartite tomato mottle virus (ToMoV) and monopartite tomato yellow leaf curl virus (TYLCV), has been introgressed to cultivated tomato (*Solanum lycopersicum*) from *Solanum chilense* accessions LA1932 and LA2779. A major gene, *Ty-3*, responsible for resistance to ToMoV and TYLCV was previously mapped on the long arm of chromosome 6. In the present study, we identified a 14-cM *S. chilense* introgression on the long arm of chromosome 3 in some resistant breeding lines derived from LA1932. A new begomovirus resistance locus, *Ty-4*, was mapped to the 2.3-cM marker interval between C2_At4g17300 and C2_At5g60160 in the introgression. Analysis of a population segregating for *Ty-3* and *Ty-4* demonstrated that *Ty-3* accounted for 59.6% of the variance, while *Ty-4* only accounted for 15.7%, suggesting that *Ty-4* confers a lesser effect on TYLCV resistance. Recombinant inbred lines (RILs) with *Ty-3* and *Ty-4* had the highest level of TYLCV resistance. The PCR-based markers tightly linked to the *Ty-4* locus as well as the *Ty-3* locus have been recently used in our breeding program for efficient selection of high-levels of begomovirus resistance and now allow for efficient breeding by marker-assisted selection.

Tomato-infecting begomoviruses, including monopartite tomato yellow leaf curl virus (TYLCV) and numerous bipartite viruses including tomato mottle virus (ToMoV), are transmitted by the sweetpotato whitefly (*Bemisia tabaci*), the B biotype of which is also known as the silverleaf whitefly (*B. argentifolii*). These viruses have caused serious losses to tomato production in many tropical and subtropical regions in the world (Polston and Anderson, 1997). Although the use of insecticides for whitefly control can limit disease, epidemics can still occur, and whitefly resistance to the chemicals has been reported (Omer et al., 1993). Therefore, breeding resistant cultivars would be an effective method for control of this disease. Thus far, TYLCV resistance has not been found in *Solanum lycopersicum* germplasm, but has been discovered in several tomato wild species, including *S. pimpinellifolium*, *S. peruvianum*, *S. chilense*, *S. habrochaites*, and *S. cheesmaniae* (Ji et al., 2007b; Pico et al., 1996; Scott, 2007). Early breeding efforts involved the introgression of resistance genes from the wild accessions into cultivated tomato using traditional breeding approaches (Scott et al., 1996), a process that usually takes many years to breed a resistant cultivar with acceptable horticultural characteristics. Marker-assisted selection (MAS) provides a tool to improve the

efficiency of cultivar development. There has been significant progress in the development of markers for important tomato resistance genes (Foolad and Sharma, 2005), including numerous TYLCV resistance genes (Ji et al., 2007b). The first such gene, *Ty-1*, which originated from *S. chilense* accession LA1969, was mapped near a molecular marker (TG97) on chromosome 6 of tomato (Zamir et al., 1994). PCR-based markers closely linked to the *Ty-1* gene have been developed and can be used for MAS (de Castro et al., 2007; Ji et al., 2007a). A second TYLCV resistance gene, *Ty-2* (Hanson et al., 2006), which originated from *S. habrochaites* (Kalloo and Banerjee, 1990), was mapped to the long arm of chromosome 11, delimited by restriction fragment length polymorphism markers TG393 and TG36 (Hanson et al., 2000). This gene was further delimited to a 4.5-cM marker interval (Ji et al., 2009). We recently identified a third begomovirus resistance gene, *Ty-3*, in *S. chilense* accessions LA1932- and LA2779-derived resistant lines, the former carrying a 6-cM introgression and the latter ≈ 27 -cM on the long arm of chromosome 6. *Ty-3* was mapped to the same marker interval in the two introgressions, ≈ 15 cM away from *Ty-1* (Ji et al., 2007a). *Ty-3* differs from the first two genes mainly in two aspects. First, unlike *Ty-1* and *Ty-2*, which are effective against only monopartite TYLCV, *Ty-3* is effective against TYLCV and the bipartite begomovirus ToMoV (Agrama and Scott, 2006; Ji et al., 2007a). In addition, *Ty-1* and *Ty-2* genes express complete or nearly complete dominance (Hanson et al., 2000; Ji et al., 2009; Zamir et al., 1994), while *Ty-3* is more additive (Ji et al., 2007a). *Ty-3* is a major locus explaining a high degree of the observed phenotypic variance from the two accessions, but additional resistance loci are required to obtain the highest levels of resistance

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(Ji et al., 2007a). In a previous study of TYLCV and ToMoV resistance in the backcross progenies derived from various *S. chilense* accessions, Scott et al. (1996) found multigenic control of resistance derived from *S. chilense* accessions. Inheritance studies and QTL analysis also demonstrated involvement of at least two genetic loci on chromosome 6 for TYLCV or ToMoV resistance (Agrama and Scott, 2006; Griffiths, 1998; Griffiths and Scott, 2001). Besides *Ty-3*, we have also searched for additional begomovirus resistance loci in the tomato genome by screening advanced resistant breeding lines derived from various *S. chilense* accessions with molecular markers on all 12 tomato chromosomes. Herein, we report the presence of a new TYLCV resistance locus, *Ty-4*, in a *S. chilense* introgression on the long arm of chromosome 3, and the construction of a molecular linkage map of the *Ty-4* locus using a population segregating for *Ty-3* and *Ty-4* loci.

Materials and Methods

PLANT MATERIALS. Four advanced breeding lines derived from each *S. chilense* accessions LA2779, LA1932, and the intercroses of lines derived from these two sources (LA2779/LA1932) that displayed a high level of resistance to TYLCV and ToMoV were screened with over 400 PCR-based markers on the 12 tomato chromosomes to identify *S. chilense* introgressions in tomato genome. These lines were produced from interspecific crosses between tomato and the wild accessions, followed by four to six generations of backcrossing to tomato and six to 10 cycles of self-pollination. Selection for TYLCV and/or ToMoV resistance for over 15 years was done phenotypically by inoculation with one or the other of these pathogens, without any assistance from molecular markers before 2005. In addition to the advanced breeding lines, we also used several early breeding lines (less than five backcrosses) in the screening process, including 960719, 960729, and 960744, which were used as resistant parents in previous studies (Agrama and Scott, 2006; Griffiths, 1998; Griffiths and Scott, 2001). All three were the same for the first three backcrosses (BC₃); the latter had a different parent in the fourth BC (BC₄), while the former two had the same BC₄ parent but diverged in the BC₄F₂ generation. *S. lycopersicum* cv. Horizon (Scott et al., 1985) was used as the susceptible control (mean disease severity ≈ 3.5 ; see "Inoculation and Disease Evaluation" below), and *S. chilense* accessions LA2779 and LA1932 [mean disease severity ≈ 0 (Scott et al., 1996)] were included as the resistant controls for all PCR experiments. An F₇ line, 040980, derived from a cross between susceptible *S. lycopersicum* line 7655B and a begomovirus resistant line 000529, has accessions LA2779 and LA1932 in its pedigree. A population of 201 progeny from selection 040980–3 segregating for TYLCV resistance was employed to map a new TYLCV resistance locus *Ty-4*.

Plant materials used in a replicated trial to analyze the effects on TYLCV resistance by combining *Ty-3* and *Ty-4* loci included RILs derived from LA2779/LA1932 containing *Ty-3* alone or both *Ty-3* and *Ty-4*, susceptible control 'Horizon', and resistant controls including one LA2779-derived breeding line 8602 and two commercial F₁ hybrid cultivars Tygress (Semini, St. Louis, MO) and SecuriTY 28 (Harris Moran, Modesto, CA). Both of these hybrids are heterozygous for the *Ty-1* gene. The wild *S. chilense* accessions were obtained from Tomato Genetics Resource Center at University of California, Davis.

INOCULATION AND DISEASE EVALUATION. The inoculation procedure was the same as described by Griffiths and Scott (2001). In this procedure, seedlings are inoculated with whiteflies from a colony maintained on tomato plants that are infected with TYLCV and thus the whiteflies are viruliferous for this virus. All individual plants were rated for TYLCV disease severity three times according to the method described by Ji et al. (2007a). The third rating was used for statistical analysis to record the most accurate resistance levels of plants and to minimize the possibility of escapes (Griffiths, 1998). The rating scale was from 0 to 4, as documented by Scott et al. (1996), where 0 = no symptoms, 1 = slight symptoms visible only on close inspection, 2 = intermediate symptoms visible on part of the plant, 3 = severe symptoms over the entire plant, and 4 = severe symptoms and stunting. Intermediate scores (0.5, 1.5, etc.) were incorporated to allow for more precise disease severity ratings.

FIELD EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS. Following inoculation, seedlings were transplanted to field plots at Gulf Coast Research and Education Center, University of Florida, Wimauma. The 201 progeny from selection 040980–3 were transplanted in one continuous block. The seven homozygous RILs and susceptible and resistant control were transplanted to the field in a randomized complete block design with three blocks and 12 plant plots. The soil in the field was Myakka, Haplaquents, and St. Johns sandy soil. The beds were raised 25 cm high, 71 cm wide at the top, and 81 cm wide at the base. Plants were spaced 46 cm apart within plots that were 92 cm apart in rows with 152 cm between rows. The beds had been fumigated with 67% methyl bromide: 33% chloropicrin at 197 kg·ha⁻¹ and the beds were covered with white plastic mulch. The plants were fertilized with a total of 169 kg·ha⁻¹ of nitrogen, 37 kg·ha⁻¹ of phosphorus, and 210 kg·ha⁻¹ of potassium throughout the season through drip irrigation. The plants were staked and tied, and sprayed with pesticides as needed throughout the season according to Olson et al. (2008).

PCR, LINKAGE ANALYSIS, AND QTL MAPPING. All markers used in this study were PCR-based, including sequence-characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) markers taken from the public domain or designed from public sequences (Table 1). PCR, linkage analysis, and QTL mapping followed the procedures described by Ji et al. (2007a). The program MAPMAKER/QTL 1.1 (Lincoln et al., 1993) was applied to identify and map the resistance loci and also obtain estimates of the percentage of phenotypic variation explained by each locus as well as components of variance for each resistance locus. Analysis of variance and/or Duncan's multiple range test in SAS (version 9; SAS Institute, Cary, NC) were used to analyze the association of molecular markers with disease resistance in the population segregating for TYLCV resistance, and the TYLCV resistance in the replicated trial of RILs.

Results

***S. CHILENSE* INTROGRESSIONS IN TYLCV-RESISTANT BREEDING LINES.** TYLCV-resistant breeding lines derived from *S. chilense* accessions LA1932, LA2779, and their intercroses (LA2779/LA1932) were screened with over 400 molecular markers from the tomato genome to identify introgressions that may carry other TYLCV resistance loci in addition to the *Ty-3* locus reported previously (Ji et al., 2007a). Prior information indicated possible *S. chilense* introgressions on tomato chromosomes 11,

Table 1. Sequence-characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) markers on tomato chromosome 3 used to map the *Ty-4* locus and near the *sp* gene on chromosome 6.

Marker	Chromosome no.	Position (cM) ²	Matched unigene ³	Forward primer (5'-3')	Reverse primer (5'-3')	Restriction enzyme	Reference source of primer ⁴
CLEZ-17-15B	3	67	U316643	GCTTAAGTGGAGCTTTGCCACCAA	GCACGAGTTCACTTGCTCTTT	<i>RsaI</i>	Present study
CLET-24-12B	3	70		CAACCATCTAGCAATGAATCT	GAGGATTCACCTCTCTCGATAC	<i>KpnI</i>	SGN, 2008
C2_At1g02140	3	71		TCCGTTATGCTAACAATCCAAC	TGTGTTCATTTCCCATCACAATCTC	<i>HhaI</i>	SGN, 2008
C2_At3g02420	3	72.2		ACGAGAATGATCCGAGATGGGC	AGAAAGTGTGAGGACGATGATG	<i>HaeIII</i>	SGN, 2008
TG74	3	74.2		CATGCTTGAAAGCAGTGGA	GATTATACGAGGCTCAAGGA	<i>TaqI</i>	SGN, 2008
C2_At5g62390	3	75.6		TGCTACTAAGTGTGATGCCATTGAG	TTGGGGTCGATAACATCAAGC	<i>EcoRV</i>	SGN, 2008
C2_At5g11110	3	75.7		TGGTGAAGGCACAGGGCAC	TCTTTAGCTGATCTATTTAGCAGC	<i>TaqI</i>	SGN, 2008
C2_At5g38880	3	76		TGGTAATGCTAAACACTGCCTTGACG	ATGCTGGTGTTCACCACTTCATC	<i>EcoRV</i>	SGN, 2008
C2_At3g10220	3	76.9		TGGCTTCTCAGTTACAGATTCAAGG	AACTCCGGAGTCACTGACG	<i>RsaI</i>	SGN, 2008
FEY	3	79		ACCGCTTCTCTATCAAGCA	ATGCCGAATAACCAAGCAAC	<i>MspI</i>	SGN, 2008
C2_At4g17300	3	81	U216063	ATTAAACCGTGTCTGGCAACTC	GCTCACTTTCGAAATCACATCCCC	<i>AflI</i>	Maxwell, 2009
				AATGG	ATTTCACC		
C2_At5g60160	3	83.3		TTCTCGGGGCTTTTCTCCTC	TCGTGATCGCAACATATATCTCG	<i>NlaIII</i>	SGN, 2008
C2_At5g63460	3	83.3		TTCTCGGGGCTTTTCTCCTC	TCGTGATCGCAACATATATCTCG	<i>HinfI</i>	SGN, 2008
C2_At1g05350	3	84.5		TGAACGAACCTAAAGCTGAAAG	TCCGAACCTTCAACAAGTACTTCAATGTG	<i>MseI</i>	SGN, 2008
TG599	3	85		TGTTGGATCTTGTCTGT	TTGTATGGTGCAACTTCCC	<i>HindIII</i>	Present study
C2_At5g52820	3	90		TGGGATCTAAATACCCAGACACC	ACAGAAAGAACCAATTTCTGTGC	<i>RsaI</i>	SGN
P6-25	5	≈25		GTAGTGAATGATGCTGCTC	GCTCTGCCTATTGTCCATATATAACC	None	Ji et al., 2007 ^c
TG472	6	44		GCCTTGCTCCAAACCTGATGG	CTGAAACTACAGCCGTAATAGC	<i>HinfI</i>	Present study
C2_At1g5220	6	44		ACATTGGACAAATAGCAAGTIC	TGAGACGACAGCAGGCATCATC	<i>TaqI</i>	SGN, 2008
C2_At4g10030	6	44		TCGGTCAGCTGCTCTTGATGTC	TTTCTCCAAAGCTGTGACCAAC	<i>EcoRV</i>	SGN, 2008
CT184	6	N/A		TTTCCGTGATTGCCAACAA	ACCAAAGAGTCAATGGATGG	<i>DdeI</i>	Present study
C2_At1g22850	6	46		ATCATTTGTTCCATTGGTGAACG	TGCAAGAAATTTCTTGTCCCTTC	<i>HpyCH4IV</i>	SGN, 2008
C2_At1g71950	6	46.5		TGAGCAACCTAAAGATCAAGAACCTG	TAACAATGCCTCTTTTGAGGCTC	<i>DdeI</i>	SGN, 2008
TG365	6	50		GTCTGATTTCACTGGATATGTTGTG	CCCAACTCTTCGCCAATTTGCTAAC	<i>HpyCH4IV</i>	Present study
C2_At5g07960	6	52.3		AAGATCTCTCTATAGATTACTCCG	TGAATATAATAGCAAGCCACGAGC	<i>HpyCH4IV</i>	SGN, 2008
C2_At5g62530	6	55.5		TTGCACTGGAAGATCCTTGGG	TGATTCAGCTGAACACTTCTGACC	<i>TaqI</i>	SGN, 2008
T1639	6	57	U216282	AACGTAGCCCAATCAACTTCCCA	CCGCCATTGACACGAATGATCTGT	<i>HaeIII</i>	Present study
C2_At4g24690	6	62.5		TTGCCCTTCAGGGGAGAAAGTTTG	TCAACTAATTCATGGCAGGTTTG	None	SGN, 2008
C2_At1g24360	6	62.7		TTGCCCTTCAGGGGAGAAAGTTTG	TCAACTAATTCATGGCAGGTTTG	<i>DdeI</i>	SGN, 2008
TG292	6	64		CAG CCT ATG CAC CAT GAA CCA TCA	TAA GCA GGC AAC TAC CGC CGA ATA	<i>DdeI</i>	Present study
U144275	6	67		ATGGCAGTTAACGGTGCTTC	TGCCTTCATATGTGCTGCTC	<i>TaqI</i>	SGN, 2008
C2_At2g43360	6	67		TCGATCTCTCTTTTCATGGCG	TTGAGGACAATACGAACAATCTTC	None	SGN, 2008
C2_At1g09340	6	67.3		ATCCTGTGAAGAGTGTCTTCTCC	TGGCGCAAGCCTTAGCTAATC	<i>HpyCH4IV</i>	SGN, 2008
T1462	6	69	U234831	GAGCCATGTGGAATGCTTCT	GTGTGGACTTATGGAATCTGCCA	<i>DdeI</i>	Present study
TG579	6	73		TGCTTCCGAACCTGTACTCCATA	CGCATTTACTGGGTGTGTGAAA	<i>MnlI</i>	Present study
TG578	6	75.5		CATGCCGTGCAAGTGTTCACAAAG	ACCTTACTATCATGCCAGCCCAA	None	Present study
<i>sp</i>	6	81.5		AGGGTTGAAGTTTCATGGTGG	GATGTTCCCTGAGATATGGA	<i>MvaI</i>	Francis, 2009
TG275	6	N/A		CTGCAGCTTCTGTTTTGTG	ATCAGCTCAATGCTGCAAGT	<i>RsaI</i>	Present study
TG279	6	80		GTAGAATCCGCTGTCGCTTC	TATGTCCACGAAGTCGGTGA	<i>DraI</i>	Present study
C2_At1g29900	6	81.7		TGATACATGTGCTGCAAAATTTGAGGC	AAATTCAATGGGTTTGACCTCC	<i>DdeI</i>	SGN, 2008
cLEX-2-F13	6	85		GGTGAACGATGCGAACTTTTGATTG	CCTCTTCTATCTTAGGTGCCAGGT	<i>DdeI</i>	Present study

²From Tomato-EXPEN 2000 map (Fulton et al., 2002; Sol Genomics Network, 2008).

³Primers were designed from the matched unigene sequences.

⁴SGN = Sol Genomics Network.

12, 3, and 7 (Y. Ji and J. Scott, unpublished data; Zamir et al. 1994). Therefore, we initially screened the resistant breeding lines with molecular markers on these four chromosomes. The early LA1932-derived line 960719 was shown to carry two additional *S. chilense* introgressions in addition to the introgression in the *Ty-3* region reported previously (Ji et al., 2007a): one is ≈ 35 cM, spanning markers from TG472 to the *sp* gene and TG275 on the long arm of chromosome 6, and the other is ≈ 14 cM, spanning markers from C2_At1g02140 to TG599 on the long arm of chromosome 3 (Fig. 1). Molecular marker analysis also indicated that the early LA1932-derived line 960729 carries only the latter additional introgression besides the *Ty-3* introgression. Advanced breeding lines derived from LA2779/LA1932, including line 040980 used for segregation analysis, carry the same introgressions on chromosomes 3 and 6 as did their early ancestor line 090729. Molecular marker analysis in the present study indicated that both introgressed segments in these lines originated from LA1932. Other regions of the tomato genome were also screened with molecular markers, with an average of one marker every 5 cM, but no *S. chilense* introgression was detected in these regions (data not shown).

MAPPING OF THE TYLCV RESISTANCE LOCI. Analysis of variance for the population segregating for introgressions on chromosomes 3 and 6 (*Ty-3* region) indicated that all the four molecular markers contained within the *S. chilense* introgression on chromosome 6 were significantly associated with disease severity ratings. For example, the SCAR marker P6–25 designed from bacterial artificial chromosome (BAC) clone 56B23 displayed the highest association with the TYLCV disease severity ratings (Table 2). Similarly, all 12 markers contained within the *S. chilense* introgression on chromosome 3, such as C2_At4g17300, a CAPS marker derived from a *cosII* marker, were also significantly associated with disease severity ratings, but to a lesser degree compared with markers from chromosome 6 (Table 2). There was no significant interaction between the genotypes of the two markers P6–25 and C2_At4g17300 (Table 2). However, genotypes with *S. chilense* alleles at both marker loci displayed the highest level of resistance, while genotypes with *S. lycopersicum* alleles at both loci showed the severest TYLCV disease (Table 3).

Ty-3 was mapped to the marker interval between cLEG-31-P16 (20 cM) and C2_At5g41480 (26 cM) on the long arm of chromosome 6 (Fig. 2). A new TYLCV resistance locus, *Ty-4*, was mapped in the present study to the marker interval between C2_At4g17300 (81 cM) and C2_At5g60160 (83.3 cM) on the long arm of chromosome 3. *Ty-3* and *Ty-4* loci had more dominance than additivity, with dominance-to-additive ratios of 0.70 and 1.06, respectively (Table 4). About 60% of the variance in the TYLCV resistance in the population was explained by the *Ty-3* locus, while the *Ty-4* locus accounted for only $\approx 16\%$ of the variance, suggesting *Ty-3* had a major effect on resistance, while *Ty-4* had a lesser effect.

Fig. 1. One introgressed segment and two separate introgressed segments were identified on chromosomes 3 and 6 of tomato, respectively, in the early breeding lines derived from *Solanum chilense* accession LA1932, such as lines 960719 (shown) and 960744. The upper smaller introgression (6 cM in length) on chromosome 6 was also present in the advanced resistant lines derived from LA1932, while the lower larger introgression (≈ 35 cM in length) was not found in the advanced lines for a limited number of markers tested. The introgression (≈ 14 cM) on chromosome 3 was also present in the advanced resistant breeding lines derived from LA1932 combined with LA2779 (LA2779/LA1932).

Table 2. Analysis of variance for the association between markers C2_At4g17300 (linked to *Ty-4*) on tomato chromosome 3 or P6–25 (linked to *Ty-3*) on chromosome 6 and the average disease severity of progenies from a plant heterozygous for both *Solanum chilense* introgressions on chromosome 3 and 6, respectively, which were inoculated with tomato yellow leaf curl virus (TYLCV).

Genotype ^z			Disease severity (0–4 scale) ^y			
C2_At4g17300 (<i>Ty-4</i>)	P6–25 (<i>Ty-3</i>)	Plants (no.)	Mean	Range	F value	P > F ^x
S	S/H/R	48	1.48	0–3.5	19.3 ^w	<0.0001
H	S/H/R	106	0.63	0–3.0		
R	S/H/R	47	0.78	0–2.5		
S/H/R	S	50	2.1	0–3.5	129.4 ^v	<0.0001
S/H/R	H	110	0.53	0–2.0		
S/H/R	R	41	0.26	0–1.5		
S/H/R	S/H/R	201	0.87	0–3.5	0.49 ^u	0.7420

^zR = homozygous for the *Solanum chilense* allele, S = homozygous for the *Solanum lycopersicum* allele, H = heterozygous, S/H/R = all three different genotypes were observed for the marker in a given group of plants.

^y0 = no symptoms, 1 = slight symptoms visible only on close inspection, 2 = intermediate symptoms visible on part of the plant, 3 = severe symptoms over the entire plant, 4 = severe symptoms and stunting; intermediate scores (0.5, 1.5, etc.) were incorporated to allow for more precise disease severity ratings.

^xProbability that the F value is not significant; a probability ≤ 0.05 indicates a significant F value.

^wFor marker C2_At4g17300.

^vFor marker P6–25.

^uFor interaction between C2_At4g17300 and P6–25.

Table 3. Tomato yellow leaf curl virus disease severity for different combinations of genotypes for markers C2_At4g17300 (linked to *Ty-4*) on tomato chromosome 3 or P6-25 (linked to *Ty-3*) on chromosome 6.

Genotype ^z			Disease severity (0–4 scale) ^y	
C2_At4g17300 (<i>Ty-4</i>)	P6-25 (<i>Ty-3</i>)	Plants (no.)	Mean	Range
S	S	16	2.66	2–3.5
S	H	23	0.96	0–2.0
S	R	9	0.72	0–1.5
H	S	21	1.83	0–3.0
H	H	64	0.41	0–2.0
H	R	21	0.07	0–0.5
R	S	13	1.85	1–2.5
R	H	23	0.43	0–1.5
R	R	11	0.23	0–1.5

^zR = homozygous for the *Solanum chilense* allele, S = homozygous for the *Solanum lycopersicum* allele, H = heterozygous.

^y0 = no symptoms, 1 = slight symptoms visible only on close inspection, 2 = intermediate symptoms visible on part of the plant, 3 = severe symptoms over the entire plant, 4 = severe symptoms and stunting; intermediate scores (0.5, 1.5, etc.) were incorporated to allow for more precise disease severity ratings.

REDUCED RECOMBINATION IN THE *S. CHILENSE* INTROGRESSED SEGMENT. The recombination length for the whole *S. chilense* introgressed segment from C2_At1g02140 to TG599 on chromosome 3 in the LA2779/LA1932-derived advanced breeding lines was estimated to be 4.2 cM (Fig. 2), about a 2.5-fold reduction compared with 14 cM in length from the tomato reference map (Fulton et al., 2002; Sol Genomics Network, 2008). Similarly, recombination was greatly reduced in the introgressed segment on chromosome 6, where only one recombinant was found among 201 progeny, giving an estimation of 0.25 cM for the introgression from cLET-31-P16 to C2_At5g41480, which was nearly a 24-fold reduction compared with the same region of 6 cM in length from the tomato reference map.

ENHANCING TYLCV RESISTANCE BY PYRAMIDING *Ty-3* AND *Ty-4*. Analysis of variance for RILs containing *Ty-3* alone or in combination with *Ty-4* showed significant differences for TYLCV resistance among these lines (Table 5) with no significant difference among the replicates (data not shown). All RILs containing *Ty-3* had significantly higher levels of TYLCV resistance than the susceptible controls, as well as the F₁ hybrids heterozygous for *Ty-1*. The two RILs containing *Ty-3* and *Ty-4* (074766-Y3 and 024525-2) were significantly more resistant than those with *Ty-3* alone. Furthermore, plants with homozygous and/or heterozygous *Ty-3* and *Ty-4* had lower mean disease severity scores than did plants with only *Ty-3* in the mapping population (Tables 2 and 3). The level of TYLCV resistance also varied significantly among the four RILs containing *Ty-3* without *Ty-4* (Table 5), suggesting that additional TYLCV resistance loci besides *Ty-3* and *Ty-4* may exist in these lines.

Discussion

***Ty-4*, A MINOR TYLCV RESISTANCE LOCUS.** The *Ty-3* locus was mapped to the marker interval between cLEG-31-P16 (20 cM) and C2_At5g41480 (26 cM) on the long arm of chromosome 6 from the present investigation, which is consis-

tent with the previous report (Ji et al., 2007a). About 60% of the variance in the TYLCV resistance in the segregating population can be explained by *Ty-3* locus, suggesting that *Ty-3* is a major locus responsible for TYLCV resistance, a finding also consistent with the previous report (Ji et al., 2007a). The *Ty-3* locus was shown to be effective against monopartite TYLCV and bipartite ToMoV (Ji et al., 2007a). In the present study, we showed that *Ty-4* accounted for about 16% of the variation and thus would be considered a minor locus compared with *Ty-3*. An early report also suggested a minor association between an introgression from LA1969 on chromosome 3 and the TYLCV tolerance (Zamir et al., 1994). *Ty-4* was effective against TYLCV, but we did not test bipartite begomoviruses. However, inbreds with *Ty-3* and *Ty-4* have had greater resistance than lines with *Ty-3* alone in Guatemala (D.P. Maxwell, unpublished data), suggesting that *Ty-4* is effective against multiple (up to seven) bipartite begomoviruses that were reported to be present there (Nakhla et al., 2005).

COMBINING *Ty-3* AND *Ty-4* LOCI BY MAS. Plants carrying *Ty-3* and *Ty-4* loci from the mapping population and RILs showed excellent levels of TYLCV resistance (Tables 2, 3, and 5). These findings support multigenic control of TYLCV resistance in the *S. chilense* accessions, as observed previously in a study of TYLCV and ToMoV resistance in the backcross progenies derived from various *S. chilense* accessions in which segregation ratios could not be explained by a single dominant gene (Scott et al., 1996). Inheritance and QTL studies also suggested involvement of at least two loci for TYLCV or ToMoV resistance (Agrama and Scott, 2006; Griffiths and Scott, 2001). The resistance of genotypes homozygous for *Ty-3* and *Ty-4* was significantly higher than that of genotypes with *Ty-3* alone, which in turn were significantly higher than commercial hybrids heterozygous for *Ty-1* (Table 5). However, it has been easier to develop commercially acceptable hybrid cultivars using *Ty-1* or *Ty-2* because they are single dominant genes with high levels of resistance as opposed to using resistance from *Ty-3* and/or *Ty-4* from *S. chilense* accessions LA1932 and LA2779, which require more than one gene for the best resistance (Griffiths and Scott, 2001). However, with the discovery of *Ty-4* and the development of markers tightly linked to *Ty-3* and *Ty-4*, it is now possible for the first time to use MAS to rapidly incorporate high resistance levels into recurrent parent lines with nearly the same ease as incorporating *Ty-1* or *Ty-2*. One approach would be to put *Ty-3* and *Ty-4* into both parents of a hybrid. The data in Tables 2 and 3 indicate it might also be possible to have one parent homozygous for both loci while the other could have only *Ty-3* or perhaps only *Ty-4*. Differences in disease severity among the four RILs containing *Ty-3* alone suggested additional resistance gene(s) may exist in some LA1932-derived breeding lines. This claim was supported by analysis of TYLCV resistance among the F₂ progeny of a LA1932-derived line containing neither *Ty-3* nor *Ty-4*, which segregated for the TYLCV resistance (Y. Ji and J. Scott, unpublished data).

REDUCED RECOMBINATION IN THE *S. CHILENSE* INTROGRESSIONS CARRYING THE TYLCV RESISTANCE LOCI. A nearly 2.5-fold reduction of recombination relative to the tomato reference map (Fulton et al., 2002) was observed in the introgressed segments carrying the *Ty-4* locus, which is most likely due to sequence divergence between *S. chilense* and the cultivated tomato. Reduction of recombination between homeologous chromosomes due to nucleotide divergence was also observed in introgressions from many other tomato wild species (Canada

et al., 2006). A genome-wide reduction in recombination frequencies was observed for the introgressed *S. lycopersicoides* segments in the background of cultivated tomato, often to as low as 0% to 10% of the normal levels (Canady et al., 2006), while recombination within introgressed segments derived from *S. pennellii* or *S. habrochaitesis* typically are about 15% to 30% of the normal levels (Alpert and Tanksley, 1996; Monforte and Tanksley, 2000). In the present study, we observed

a much greater reduction of recombination (≈ 24 -fold) in the introgressed segments carrying the *Ty-3* locus than the 2.5-fold reduction in the introgression carrying the *Ty-4* locus. This greater reduction of recombination may be due to the relatively smaller introgressed segment on chromosome 6, which is ≈ 6 cM in length compared with the 14-cM *Ty-4* introgression. This assertion is in agreement with the previous study of a library of *S. lycopersicoides* introgression lines in which shorter introgressions usually demonstrated greater reductions in homeologous recombination than larger introgressions (Canady et al., 2006).

Reduced recombination within the alien introgressed segments carrying genes of interest may pose potential disadvantages for plant breeders. Plant breeders usually have to make extra efforts (such as more time and larger selecting populations) to break down linkage drag and remove the excessive alien chromatin that causes detrimental effects on the horticultural features of cultivars. Additionally, reduced recombination within introgressed segments may make it difficult to combine tightly linked resistance genes from different sources (in trans configuration) into a single inbred parent (in cis orientation). This is the case for the root-knot nematode resistance *Mi* gene and the TYLCV resistance gene *Ty-1* (Ji et al., 2007b). Numerous tomato breeding programs around the world tried for many years to bring these two genes together in coupling phase, but this was achieved only recently by Seminis Inc. with the assistance of molecular markers (Hoogstraten and Braun, 2005).

PYRAMIDING RESISTANCE GENES OF VARIOUS ORIGINS TO EXPAND THE LEVEL AND RANGE OF TYLCV RESISTANCE. The linkage drag situation, however, could be significantly improved using molecular markers tightly linked to genes of interest in the introgressed segments because these markers could facilitate the identification of recombinants with shorter introgressions but still carrying the target gene(s) in succeeding generations. Similarly, with the assistance of molecular markers, it is

Fig. 2. Genetic map of the begomovirus resistance locus *Ty-4* on tomato chromosome 3 and the previously mapped begomovirus resistance locus *Ty-3* on chromosome 6. Linkage maps were constructed from a mapping population derived from a genotype heterozygous for introgressions on chromosomes 3 and 6 (in the middle). Included on the left is a linkage map for chromosome 3 (designated as Chr. 3) and on the right for chromosome 6 (designated as Chr. 6), which were simplified from the Tomato-EXPEN 2000 map (SGN, 2008). All markers are PCR-based, including sequence-characterized amplified region (SCAR) markers and cleaved amplified polymorphic sequence (CAPS) markers taken from the public domain or designed from public sequences. Shaded regions represent introgressions from *Solanum chilense*. The markers in nonintrogression regions are not drawn to scale. The numbers on the left of each map represent the published map locations (cM) for the markers (in the Chr. 6 or Chr. 3 map) or linkage distances (cM) between markers in the introgressions obtained from the mapping population.

Table 4. Components of variance for the resistance loci in the mapping population segregating for tomato *Ty-3* and *Ty-4* loci.

Locus	Marker interval	Mean disease severity	Additive variance	Dominance variance	Dominance-to-additive ratio	Log-likelihood	Variance explained (%)
<i>Ty-3</i>	CLEG-31-P16 – C2_At5g41480	2.05	–0.9310	–0.6481	0.70	39.6	59.6
<i>Ty-4</i>	C2_At4g17300 – C2_At5g60160	1.46	–0.4258	–0.4526	1.06	7.4	15.7

Table 5. Tomato yellow leaf curl virus (TYLCV) disease severity for tomato recombinant inbred lines containing *Ty-3* with or without *Ty-4*.

Line	Genotype ^z	Resistance source	Plants (no.)	Mean disease severity (0–4 scale) ^y
'Horizon'	Susceptible	Susceptible	36	2.85 a
'Tygress'	Ty1ty1	LA1969	35	1.87 b
'SecuriTy 28'	Ty1ty1	LA1969	32	1.48 c
074766–7	Ty3Ty3ty4ty4	LA2779/LA1932 ^x	35	1.1 d
074766–Y5	Ty3Ty3ty4ty4	LA2779/LA1932	36	0.93 de
051005	Ty3Ty3ty4ty4	LA2779/LA1932	36	0.78 ef
8602	Ty3Ty3ty4ty4	LA2779	35	0.60 fg
051018–Y6	Ty3Ty3ty4ty4	LA2779/LA1932	35	0.47 g
024525–2	Ty3Ty3Ty4Ty4	LA2779/LA1932	36	0.18 h
074766–Y3	Ty3Ty3Ty4Ty4	LA2779/LA1932	35	0.10 h

^zFor brevity, the '-' is omitted in the name of an allele, for example *Ty1* = *Ty-1*.

^y0 = no symptoms, 1 = slight symptoms visible only on close inspection, 2 = intermediate symptoms visible on part of the plant, 3 = severe symptoms over the entire plant, 4 = severe symptoms and stunting; intermediate scores (0.5, 1.5, etc.) were incorporated to allow for more precise disease severity ratings. Means disease severity with the same letter are not significantly different at $P \leq 0.05$ via Duncan's multiple range test.

^x*Ty-3* and *Ty-4* originated from LA1932 in the lines derived from LA2779/LA1932 (see text for detail).

possible to systematically incorporate multiple resistance genes of different origins into a single elite genotype to enhance the degree of resistance or tolerance to major pathogens such as begomoviruses. Furthermore, pyramiding resistance genes originating from different sources could also expand the resistance against a wider range of begomoviruses (Vidavski, 2007).

Currently, four TYLCV resistance loci (*Ty-1* through *Ty-4*) have been mapped to different regions of the tomato genome, each of which can be tagged with tightly linked PCR-based molecular markers that have been developed in previous and current studies. These resistance loci originate from different wild tomato accessions and possess various modes of TYLCV resistance. *Ty-1*, which originated from *S. chilense* accession LA1969 and is nearly completely dominant to TYLCV, is not effective against ToMoV, a bipartite begomovirus (Ji et al., 2007b; Zamir et al., 1994). *Ty-2*, which originated from *S. habrochaites* (Kalloo and Banerjee, 1990) and showed completely dominant TYLCV inheritance, is also not effective against ToMoV (Hanson et al., 2000; Ji et al., 2007b). Neither gene is effective against all strains of TYLCV. Unlike *Ty-1* and *Ty-2*, the more recently mapped begomovirus resistant locus *Ty-3*, which is found in several *S. chilense* accessions such as LA2779 and LA1932, is more additive in nature and is effective against TYLCV and ToMoV (Agrama and Scott, 2006; Ji et al., 2007a). *Ty-4* originated from *S. chilense* accession LA1932 and has a smaller effect than *Ty-3*. Robust PCR-based markers developed from current and previous studies such as JB-1, T0302, P6–25, and C2_At4g17300 can be used to effectively tag *Ty-1* though *Ty-4* loci, respectively, and thus enhance selection efficiency (de Castro et al., 2007; Garcia et al., 2007; Ji et al., 2007c; Ji et al., 2009). In addition, these PCR-based markers can expedite the process of pyramiding these resistance loci into a single elite genotype, thus elevating the level of resistance as well as broadening the range of begomovirus resistance. Lines with different resistance gene combinations need to be tested against a range of begomoviruses to determine their effects, as they can be unpredictable (Vidavski,

2007). Also, the effects of linkage drag may well increase as genes are pyramided and this will have to be addressed.

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