Identification of Quantitative Trait Loci Conferring Resistance to *Bemisia tabaci* in an F₂ Population of *Solanum lycopersicum* × *Solanum habrochaites* Accession LA1777

Aliya Momotaz¹, Jay W. Scott², and David J. Schuster

Gulf Coast Research and Education Center, IFAS, University of Florida, 14625 CR 672, Wimauma, FL 33598

ADDITIONAL INDEX WORDS. *Lycopersicon esculentum*, *Lycopersicon hirsutum*, molecular marker, sweet potato whitefly, tomato, type IV trichomes

ABSTRACT. *Solanum habrochaites* S. Knapp and D.M. Spooner accession LA1777 have reported resistance to the sweetpotato whitefly (SPWF), *Bemisia tabaci* (Genn.). An interspecific F₂ population of 171 plants between tomato [*Solanum lycopersicum* L. (formerly *Lycopersicon esculentum* Mill.)] and LA1777 was bioassayed against adult SPWF in a greenhouse using clip cages. A selective genotyping analysis was used with 11 resistant and 10 susceptible plants to locate resistance genes by testing them with molecular markers spanning most of the tomato genome at about 10-cM intervals. Markers in four regions were found to be associated with resistance, where three of them showed significantly strong associations and one showed a weak association through chi-square and analyses of variance. However, through quantitative trait locus (QTL) analysis using molecular markers, all four regions were identified as major QTLs with logarithm of odds (LOD) values of 4.87 to 5.95. The four QTLs were identified near the markers TG313 on chromosome 10, C2_At2g41680 on chromosome 9, TG523/T0408 on chromosome 11, and TG400/cLEG-37-G17 on chromosome 11. Multiple regression analysis produced similar results as above with fixed effects of single loci as well as interaction among some of the QTLs.

Tomato is widely grown and economically one of the most important vegetable crops worldwide, with a value of over \$1.4 billion in the United States alone (USDA, 2008). Biotype B of the sweetpotato whitefly (SPWF), also known as the silverleaf whitefly (*Bemisia argentifolii* Bellows & Perring), is one of the most damaging insects pests of tomato. The pest causes significant crop losses through phloem sap feeding and induction of plant disorders, including irregular ripening of tomato (Schuster et al., 1996). SPWF causes damage indirectly through the transmission of plant viruses, primarily begomoviruses, one of the most damaging of which is tomato yellow leaf curl virus (TYLCV) (Polston, 2001; Polston and Anderson, 1997; Zeidan et al., 1999). TYLCV can reduce yield by up to 100%, depending upon time of infection (Saikia and Muniyappa, 1989).

The SPWF is difficult to control with insecticides first because it feeds and oviposits mainly on the abaxial leaf surfaces (Sharaf, 1986), and second because it has developed resistance to most classes of insecticides applied for its control (Byrne et al., 2003; Denholm et al., 1996; Palumbo et al., 2001), including the new systemic neonicotinoid insecticides imidacloprid and thiamethoxam (Elbert and Nauen, 2000; Schuster et al., 2010). Clearly, host plant resistance could provide

economical and environmentally sound management of the pest. However, breeding efforts have been hampered by association of resistance with horticulturally detrimental traits associated with the wild species (linkage drag).

Some work with broad-based virus resistance genes in tomato for whitefly-vectored begomoviruses is in progress (Ji et al., 2007; Vidavsky, 2007). Some breeding lines are available that have TYLCV-specific resistance genes from *S. chilense* (Dunal) Reiche, *S. peruvianum* L., *S. pimpinellifolium* L., and *S. habrochaites* accessions (Ji et al., 2007; Lapidot et al., 1997; Pico et al., 1999; Scott, 2007; Vidavsky and Czosnek, 1998; Zamir et al., 1994) and that provide one method to control losses to TYLCV. However, if SPWF is not controlled, irregular ripening can make fruits unmarketable (Schuster et al., 1996). Thus, breeding SPWF-resistant cultivars would be desirable as adjuncts to TYLCV-resistant cultivars.

Accessions of *S. habrochaites* f. *glabratum* C. H. Mull., *S. habrochaites* f. *typicum* Humb. & Bonpl., *S. pennellii* Correll, and *S. pimpinellifolium* have been reported to be resistant to *B. tabaci* (Alba et al., 2005; Berlinger et al., 1983; Dahan, 1985; Heinz and Zalom, 1995; Liedl et al., 1995; Muigai, 1997; Muigai et al., 2003; Shevach-Urkin, 1983; Snyder et al., 1998; Yorit, 1986). The *S. habrochaites* f. *typicum* accession LA1777 was highly resistant to *B. tabaci*, resulting in fewer numbers of immature life-stages per unit area of leaflet relative to *S. lycopersicum* (Muigai et al., 2003). The whitefly resistance in *S. habrochaites* was related to naturally occurring allelochemicals present in single-lobed glandular trichomes (type IV) that do not occur in cultivated tomato (Muigai et al., 2002). In *S. pennellii*, acylsugars present in type IV trichomes were related to whitefly resistance (Blauth et al., 1998; Lawson et al., 1997). The *S. habrochaites* accession LA1777 contains volatile

Received for publication 2 Oct. 2009. Accepted for publication 2 Feb. 2010. This research was supported in part by USDA T-Star grant no. PL 89-106.

We wish to thank Jose Diaz for assistance with the molecular marker work, Cathy Provenzano and Rosa Ayala for taking care of the plants, Anne Kirkwood and Sabrina Spurgeon for helping with the whitefly bioassays, and Dr. Abul Rabbany for assistance with the QTL analysis.

¹Present address: Sr. Project Scientist, Frito Lay Agricultural Research and Development, Rhinelander, WI-54501.

²Corresponding author. E-mail: jwsc@ufl.edu.

compounds, including germacrene D, dedecatriene, and α -farnesene (Fridman et al., 2005), and have demonstrated high levels of repellent and fumigant activity against *B. tabaci* adults (Muigai et al., 2002). The resistant phytochemicals have been reported to be controlled by polygenes (Frelichowski and Juvik, 2005; Maliepaard et al., 1995; Mutschler et al., 1996; Rahimi and Carter, 1993).

In an earlier study to locate SPWF resistance genes, 94 of the 98 recombinant inbred lines (RILs) of tomato (Monforte and Tanksley, 2000) were tested for *B. tabaci* egg deposition and for the presence of type IV and type VI (four-lobed) glandular trichomes (Momotaz et al., 2005). None of the RILs showed any resistance to SPWF nor had type IV trichomes. These results likely indicate that resistance is controlled polygenically, but resistance could also be controlled by a gene or genes in the 15% of the genome not covered by the RILs tested (Momotaz et al., 2005). Because no RILs were resistant to SPWF, we developed an F_2 population from tomato and *S. habrochaites* accession LA1777 to locate SPWF resistance genes that would be combined in some of the plants.

The objective of this work was to identify quantitative trait loci (QTLs) associated with SPWF resistance derived from LA1777 using selective genotyping, QTL, and multiple regression analyses.

Materials and Methods

PLANT MATERIALS. In Spring 2002, determinate fresh market tomato inbreds Fla. 7771, Fla. 7171, and Fla. 7324 were used as seed parents and separately crossed with *S. habrochaites* accession LA1777 to obtain F_1 seeds. In Fall 2003, bulked pollen from plants of all three F_1 crosses was used to sib-pollinate all the F_1 plants to produce a single F_2 population as this allowed for better seed production than doing separate sib pollinations for each cross.

WHITEFLY AND TRICHOME BIOASSAY. In Spring 2004, 171 F_2 plants, three control plants each of susceptible parent cultivar E6203, resistant parent LA1777, and their F_1 , were grown in an insect-proof greenhouse. Supplemental lighting was provided using 40-W fluorescent and 300-W incandescent light bulbs to obtain a 16/8-h (light/dark) photoperiod. The lights were suspended above the plants and were raised as the plants grew.

Plants were maintained using the cultural practices of Momotaz et al. (2005). After ≈ 3 to 4 weeks, the plants were moved to plant growth rooms with the temperature at about 27 °C and supplemental fluorescent lighting set at a 16/8-h (light/dark) photoperiod. Ten adult nonviruliferous whitefly females were confined in clip cages (2 cm diameter, 1 cm high) on the abaxial surface of a lateral leaflet of the leaf at the fifth node from the top of each plant. After 24 h, the number of living and dead adults and the number of eggs were counted. The bioassays were repeated two more times at about 1-week intervals to confirm the results of the first bioassay. The whitefly data were averaged over the three bioassays. The clip cages were constructed in the vegetable entomology laboratory at Wimauma, FL, with 0.49-mm size mesh, which was whitefly- and predator-proof.

At the first bioassay, the lateral leaflet opposite the one used for the insect assay was used to assess densities of type IV trichomes (Luckwill, 1943). Counts were made on two binocular-dissecting microscope fields at 50 $\times$ magnification from the interior middle section of the abaxial leaf surface and a mean

score was calculated (Momotaz et al., 2005). Eleven plants from the population were selected as resistant (R) based on low numbers of eggs deposited (0–17 eggs), high adult mortality, and high number of type IV trichomes, while 10 plants were selected as susceptible (S) based on their low adult mortality, high numbers of eggs deposited, and 0 to very few type IV trichomes (Table 1).

DNA EXTRACTION AND MARKER SELECTION. Total genomic DNA was isolated from the tissues of fully expanded leaves using a simple DNA isolation procedure (Fulton et al., 1995). Over 400 polymerase chain reaction (PCR)-based molecular markers, polymorphic for the R and S parents, at about 10-cM intervals on each chromosome, were used to find regions associated with resistance. Markers were selected from the integrated Tomato-EXPEN 2000 map of Fulton et al. (2002) to screen the whole genome. These included cleaved amplified polymorphic markers (CAPs), sequence characterized amplified region (SCAR) markers, conserved orthologous sequence (COS), conserved orthologous sequence II (COSII) that were designed from public sequences available at Sol Genomics Network (SGN, 2008) or markers from the literature (Bai et al., 2003, 2004a, 2004b; Balvora et al., 2001; Doganlar et al., 1998; Fulton et al., 2002; Hemming et al., 2004; Yaghoobi et al., 2005; Williamson et al., 1994; Wu et al., 2006). Selected polymorphic markers that were associated with egg deposition or type IV trichome density through selective genotyping analysis were used to screen 138 of 171 F_2 plants for QTL analysis. Codominant PCR-based markers were used because they can distinguish heterozygotes from homozygotes.

PCR AMPLIFICATION, ELECTROPHORESIS, AND VISUALIZATION/ MOLECULAR MARKERS. PCR amplification was carried out with a Gene Amp® thermocycler (Applied Biosystems, Foster City, CA) following the procedures of Momotaz et al. (2004) in 10- μ L reaction volumes at various annealing temperatures, mostly 55 °C. The PCR products were then separated on 2% (w/v) agarose gels (molecular biology grade; Fisher, Pittsburgh) containing ethidium bromide (1.0 μ g·mL⁻¹) in 1 $\times$ TBE buffer, visualized under ultraviolet transillumination, and photographed using AlphaImager® Imaging System (Alpha Innotech, San Leandro, CA). For CAPs markers, 4 μ L of PCR product was used for restriction digestion following the manufacturer's recommendations (New England Biolabs, Ipswich, MA) (Momotaz et al., 2007). Restriction fragments were separated on 2% (w/v) agarose gel as documented above.

SELECTIVE GENOTYPING. Plants at the tail ends of the F_2 were selected (Darvasi and Soller, 1992) for R and S based on egg deposition, adult mortality, and type IV trichome numbers. The 11 R and 10 S plants from the F_2 population were each tested separately for the initial marker analysis. The DNA from R and S plants were amplified with selected codominant polymorphic markers covering the whole genome to identify the markers linked to SPWF resistance genes. Use of codominant markers allowed identification of the plants into three groups at each locus: plants homozygous (hh) for the *S. habrochaites* allele, heterozygous plants (he), and plants homozygous (ee) for the tomato allele. The association between markers and resistance to SPWF was assessed by chi-square and with one-way analysis of variance (ANOVA) based on the number of SPWF eggs laid on leaves after 24 h in clip cages (SAS, version 9.1; SAS Institute, Cary, NC). Chi-square analysis was performed to estimate expected frequencies of alleles in the R and S plants

Table 1. Sweet potato whitefly (SPWF) egg deposition, SPWF adult mortality, type IV trichomes, and genotypes at putative SPWF resistance loci for controls and resistant and susceptible F₂ tomato plants derived from LA1777 (*Solanum habrochaites*) crossed with three susceptible tomato lines.

Lines	Eggs (no.)	Adult mortality (%)	Type IV trichomes (no.) ^z	Region 1 (chromosome 10)	Region 2 (chromosome 9)	Region 3 (chromosome 11a)	Region 4 (chromosome 11b)	Total h alleles (no.) ^y
<i>S. habrochaites</i> accession LA1777	0	100	216	hh	hh	hh	hh	
<i>S. lycopersicum</i> cultivar E6203	55	3.6	0	ee	ee	ee	ee	
F ₁	25.6	37.5	9.5					
Resistant F ₂ plants								
17-6-16	0	100	157	hh	hh	he	hh	7
1-1-1	0	100	112	hh	hh	he	he	6
18-6-2	0	100	88	he	hh	he	he	5
17-7-4	0	97.2	133	he	hh	he	hh	6
17-6-19	0	78.6	98	hh	hh	hh	he	7
17-1-8	0	92.9	78	hh	he	hh	he	6
17-6-17	0.5	81.8	60	hh	hh	hh	he	7
18-1-2	5	77.4	111	hh	he	hh	hh	7
17-4-21	9	73.4	98	hh	he	hh	hh	7
18-2-4	10	93.0	136	hh	hh	he	he	6
17-4-10	17	88.2	153	hh	he	hh	he	6
Susceptible F ₂ plants								
18-6-9	51	7.8	0	ee	ee	hh	hh	4
18-6-7	57	12.5	8	ee	ee	he	he	2
18-4-5	58	33.3	4	ee	he	ee	hh	3
18-4-9	63	0	3	hh	he	ee	ee	3
18-3-5	78	0	4	ee	he	ee	ee	1
17-6-6	83	0	5	he	he	ee	he	3
1-2-10	86	0	4	ee	ee	he	he	2
17-6-2	95	45.7	8	ee	he	ee	ee	1
1-2-2	95	0	4	ee	ee	he	he	2
1-2-8	123	0	1	ee	ee	ee	ee	0
χ^2 (1:2:1) ^x	—	—	—	14.02**	11.52**	9.57**	5.19 ^{ns}	

^zNumber per 50× microscope field.

^yhh = homozygous for *S. habrochaites* allele, ee = homozygous for *S. lycopersicum* allele, he = heterozygous.

^xContingency chi-square goodness of fit test of marker frequency for resistant versus susceptible F₂ plants; ** indicates significant at $P \leq 0.01$, note that the ns for Region 4 was barely not significant ($P = 0.07$).

based on observed frequencies. We defined indicator variables for a LA1777 R allele as 1, heterozygote as 0.5, and the S allele as 0 for each marker.

QTL AND STEPWISE MULTIPLE REGRESSION ANALYSES. Analysis was performed on 138 of 171 F₂ plants comparing the genotypic marker data with the phenotypic egg count data and the type IV trichome counts using Map-Maker/QTL 1.1 (Lincoln et al., 1993). Acceptable DNA was not available from the other 33 plants. Interval mapping, which searches for the effects of QTL using sets of linked markers (Lander et al., 1987), was used. A LOD score of 3.0 was considered the threshold for detecting significant QTL locations. The additive effect (a), the dominance deviation (d), and the degree of dominance (d/a) were calculated for each QTL (Lincoln et al., 1993). To further understand the QTL single and cumulative effects on the phenotypic variance, stepwise multiple regression was done using the REG procedure in SAS (version 9.1; SAS Institute, Cary, NC) with all possible marker loci combinations. The phenotypic variance explained by a single QTL or cumulative QTL effect were estimated by the square of the correlation determination (R^2). The variables retained in the

final model were determined by a stepwise selection at a significant level of 5%.

Results

PHENOTYPIC EVALUATION OF THE F₂ POPULATION. The number of eggs on leaves after 24 h of inoculation in clip cages for plants in the F₂ population ranged from 0 to 123, and Type IV trichome counts ranged from 0 to 157 (Figs. 1 and 2). Both traits were continuously distributed, indicating control by polygenes. Type IV trichome production appears to be recessively controlled because the F₁ was skewed strongly toward the susceptible parent (Fig. 2). No F₂ plants had as many trichomes as LA1777. Thus, more than three genes may be required to obtain the type IV trichome density of LA1777 (Fig. 2) because two to three LA1777-like plants would be expected with control by three recessive genes. However, acceptable resistance for a tomato line may not require the level of resistance of LA1777. For instance, there were five plants in the F₂ population that had no eggs deposited on their leaves but had type IV trichome numbers of 78 to 157. The 11 resistant plants had 0 to 17 eggs,

Fig. 1. Frequency distribution of sweetpotato whitefly egg numbers in a 171 plant F_2 population of *Solanum lycopersicum* cultivar E6203 (P_1) $\times$ *Solanum habrochaites* accession LA1777 (P_2). Arrows indicate mean of respective parents and their F_1 .

Fig. 2. Frequency distribution of type IV trichome numbers in a 171 plant F_2 population of *Solanum lycopersicum* cultivar E6203 (P_1) $\times$ *Solanum habrochaites* accession LA1777 (P_2). Arrows indicate mean of respective parents and their F_1 .

73% to 100% SPWF mortality, and 60 to 157 type IV trichomes compared with the 10 susceptible plants, which had 51 to 123 eggs, 0% to 46% SPWF mortality, and 0 to 8 type IV trichomes.

SELECTIVE GENOTYPING ANALYSIS. Markers in four regions (R1, R2, R3, and R4) on three different chromosomes (also designated R1/10, R2/9, R3/11a, and R4/11b, where the number after the slash indicates the chromosome number) cosegregated with R and S plants (Table 1). The resistant lines carried 5 to 7 *S. habrochaites* alleles and were homozygous or heterozygous at each region, while the susceptible lines carried 0 to 4 *S. habrochaites* alleles in three or less regions. Contingency chi-square analysis comparing 1:2:1 ratios of h and e alleles in R versus S groups indicated that the h markers in three regions (R1, R2, and R3) were significantly associated with resistance, while the h markers in the R4 region were weakly associated ($P = 0.07$). In other words, the four regions had unacceptable fits to 1:2:1 ratios because of an excess of h alleles in the R group and an excess of e alleles in the S group (Table 1). To verify further the association of these regions to SPWF resistance, the individual marker genotypes were tested for significant association with the mean number of eggs deposited using single-factor ANOVA analysis. Markers at R1, R2, and R3 regions showed highly significant associations with SPWF, while the markers at the R4 region showed a weak association (Table 2). Because R4 had the weak association, we included two more markers on that region in attempts to identify a more closely linked marker. The F-values for markers in that region ranged from 2.29 to 2.77 (Table 2). An additional region (R5/8) was associated with type IV trichome production, but not with egg deposition or adult mortality.

QTL ANALYSIS. Based on selective genotyping, the five target regions mentioned above were analyzed for numbers of eggs and type IV trichome QTL, while other regions of the chromosome were analyzed only for the numbers of eggs QTL. We were not confident in the type IV trichome analysis for the whole genome. Whereas the LOD scores in these regions were

Table 2. Analysis of variance of sweetpotato whitefly (SPWF) egg deposition for marker alleles at putative SPWF resistance loci in an F_2 population of *Solanum habrochaites* accession LA1777 crossed with three susceptible tomato lines.

Region/chromosome no.	Marker	Genotype ^z	Plants (no.)	Mean eggs (no.)	Range of eggs (no.)	F value
R1/10	TG313	hh	56	37.3	0–115	13.72**y
		he	59	38.9	0–83	
		ee	23	67.1	20–113	
R2/9	C2_At2g41680	hh	34	31.8	0–115	6.50**
		he	88	43.9	0–132	
		ee	16	61.0	24–123	
R3/11	TG523	hh	41	32.4	0–86	5.86*
		he	70	43.4	0–132	
		ee	17	56.5	15.5–123	
R4/11	cLEG-G17–1	hh	50	35.3	0–123	2.77 ^{ns}
		he	61	45.3	0–132	
		ee	19	50.3	2.5–75	
R4/11	TG400	hh	39	39.8	0–107	2.29 ^{ns}
		he	69	39.4	15.5–123	
		ee	27	52.4	0–132	
R4/11	cTOE-14-L16	hh	40	38.8	0–107	2.59 ^{ns}
		he	73	41.4	0–132	
		ee	25	57.2	15.5–123	

^zhh = homozygous for *S. habrochaites* allele, ee = homozygous for *S. lycopersicum* allele, he = heterozygous.

^y*,** indicate significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; ^{ns} = not significant.

Fig. 3. QTL associated with low sweetpotato whitefly egg deposition from an F_2 population derived from susceptible tomato lines crossed with *S. habrochaites* accession LA1777. Four regions with LOD scores >3 were considered significant and are indicated with a solid line. A fifth region on chromosome 8 was associated with type IV trichomes, but not egg deposition in the selective genotyping analysis, and is indicated by the hatched line. The distances between markers are from Fulton et al., (2000).

all less than 3, the analysis gave error messages that likely related to the low number of plants (138) in the analysis. A total of 124 polymorphic markers was used to associate markers and phenotypes in the F_2 population (Fig. 3). Regions R1 to R4 previously identified through selective genotyping were identified here as QTLs for low numbers of eggs deposited. The LOD values for R1, R2, R3, and R4 for the numbers of eggs were 4.87, 4.70, 5.96, and 3.50, respectively. Figure 3 shows the map position of the four major QTLs marked with bold lines. The chromosomal locations of marker intervals, effect of each QTL, and coefficients of determination are presented in Table 3. On the basis of the d/a ratio, dominance effects were detected for R1 and R3, additive effects for R2, and overdominance for R4. Regions 1 to 4 were also significantly associated with type IV trichomes (Table 3). We did not find any other chromosomal region to be associated with resistance variables through QTL analysis, which is consistent with the selective genotyping analysis (Fig. 3). Source, fragment size, and map position of molecular markers linked to low numbers of eggs deposited, high adult SPWF mortality, and/or high type IV trichome density are presented in Table 4.

STEPWISE MULTIPLE REGRESSION ANALYSIS. Through the single-marker analysis, significant QTLs were detected for numbers of eggs deposited for all marker loci in regions R1 to R4. Only R2 was significant for adult mortality. Regions R1, R2, and R5 were significant for type IV trichomes. For the multiple QTL models, if statistical significance was found for numbers of eggs, it was also found for adult mortality, although often the variation explained was higher for the former. The QTL combinations that were significant for type IV trichomes were often not significant for the numbers of eggs deposited or adult mortality. Regions R1, R2, and R3 were significant for

egg deposition/mortality in all three models where these regions were tested, with R2 being of key importance (Table 5). In some models, significant egg deposition/mortality effects were found with R1 + R2 or R2 + R3. Regions R4 and R5 were not significant for egg deposition or adult mortality. For type IV trichomes, R1 + R4 + R2 were significant for both models where this combination was tested. Some combinations of R1 + R2 or R2 + R3 were significant for type IV trichomes, but R5 was not a significant factor in the multiple QTL models. For the numbers of eggs deposited, single regions had R^2 values of 0.1 or less; with R1 + R2, the R^2 increased to 0.13; with R2 + R3, the R^2 increased to 0.20 to 0.21 and when R1 was added to R2 and R3, the R^2 ranged from 0.23 to 0.25. These data suggest R2 and R3 combined account for much of the variation identified. For adult mortality, R2 accounted for essentially all the variation. For type IV trichomes, R1, R2, and R5 taken singly had R^2 values of 0.8 to 0.11. When R1 + R2 or R2 + R4 were combined, the R^2 increased to 0.16 or 0.17. When R1 + R2 + R4 were combined, the R^2 increased to 0.22. Thus, it seems that loci in these three regions primarily control type IV trichome production.

Discussion

The genome-wide scan for QTLs significantly affecting SPWF resistance revealed four genomic regions on three different chromosomes using chi-square tests, single-marker ANOVA analysis, QTL analysis, and multiple regression analysis. SPWF resistance (number of eggs) has been found to be correlated with the density of type IV trichomes (Muigai et al., 2003). In our study, four QTLs for numbers of eggs deposited and type IV trichome numbers were mapped to the same chromosomes. Maliepaard et al. (1995) found two QTL

Table 3. Logarithm of odds (LOD), degree of dominance, and the percentage of explained phenotypic variance (R^2) for QTL associated with sweetpotato whitefly egg deposition and type IV trichomes from an F_2 population of *Solanum habrochaites* accession LA1777 crossed with three susceptible tomato lines.

Regions/chromosome no.	Flanked markers	LOD	Degree of dominance (d/a)	R^2 (%)
Oviposition (no. of eggs)				
R1/10	TG313 - C2_At3g21610	4.87	0.89	15.0
R2/9	C2_At2g41680 - C2_At3g09920	4.70	0.36	55.2
R3/11a	T0408 - TG523	5.96	0.97	52.9
R4/11b	cLEG-37-G17 - TG400	3.50	1.55	43.3
Type IV trichome				
R1/10	TG313	3.0	—	—
R1/10	C2_At5g06430 - C2_At3g01440	5.6	0.69	22.5
R2/9	C2_At2g41680 - C2-09920	12.6	0.78	69.7
R3/11a	C2_At5g09880 - TG523	9.2	0.99	69.0
R4/11b	cTOL16 - TG400	8.0	—	—

Table 4. Source, fragment size, and map position of molecular markers in chromosomal regions associated with sweetpotato whitefly egg deposition or type IV trichome density from an F_2 population of *Solanum habrochaites* accession LA1777 crossed with three susceptible tomato lines.

Regions/chromosome no.	Associated marker	Source ^z	PCR fragment size (bp)	Map position ^y (cM)
R1/10	TG313	Own	580	0.0
R2/9	C2_At2g41680	SGN, 2008	600	15.5
R3/11	TG523/T0408	Bai et al., 2004/own	342/340	26–29
R4/11	TG400/cLEG-37-G17	SGN/own	404/320	53–57
R5/8	T0718	Own	340	20

^zOwn = developed in our laboratory by A. Momotaz, SGN = Sol Genomics Network.

^yMap position adapted from Tomato-EXPEN 2000 map (Fulton et al., 2000).

Table 5. Stepwise multiple regression analysis of putative QTL linked to sweetpotato whitefly resistance in an F₂ population of *Solanum habrochaites* accession LA1777 crossed with three susceptible tomato lines. All significant associations are shown.

Model	Eggs	Adult mortality (%) Model R ²	Type IV trichomes Model R ²
Single marker analysis – Locus R ² (single QTL)			
Marker 1 (R1/10)	0.10 ^z	0.06 ^{ns}	0.10*
Marker 2 (R2/9)	0.08*	0.13**	0.11**
Marker 3 (R3/11)	0.10*	0.01 ^{ns}	0.01 ^{ns}
Marker 4 (R4/11)	0.08*	0.02 ^{ns}	0.07 ^{ns}
Marker 5 (R5/8)	0.04 ^{ns}	0.02 ^{ns}	0.08*
Significant marker model – Model R ² (QTL × QTL effects)			
Model 1 + 2			
1 + 2	0.13 *	0.13* (2) ^y	0.16 * (2 + 1)
Model 1 + 2 + 3			
1 + 2			0.17** (1 + 2)
3 + 2 + 1	0.25**	0.13* (2)	
Model 1 + 2 + 3 + 4			
2 + 3 + 1	0.23*	0.12* (2)	—
1 + 4 + 2	—		0.22**
Model 1 + 2 + 3 + 4 + 5			
2 + 3 + 1	0.23*	0.12* (2)	—
1 + 4 + 2	—		0.22**
Model 2 + 3			
3 + 2	0.21*	0.13* (2)	0.11* (2)
Model 2 + 3 + 4 + 5			
2 + 3	0.20*	0.12* (2)	
2 + 4			0.16*

^z*,** indicate significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; ^{ns} = not significant.

^yNo marker met the $P \leq 0.05$ significance level for entry into the model except marker 2.

for numbers of eggs deposited; one on chromosome 1 (TG142) and one on chromosome 12 (TG296) and two type IV trichome QTL; one on chromosome 5 (TG379) and one on chromosome 9 (TG223) for greenhouse whitefly in *S. habrochaites* f. *glabratum* accession CGN.1561. Our study covered regions TG142 (chromosome 1), TG296 (chromosome 12), and TG379 (chromosome 5) using QTL and selective genotyping analysis, and for TG223 (chromosome 9) using selective genotyping analysis only. We did not find any association of these markers or any nearby marker for numbers of eggs deposited or type IV trichomes. We found one QTL for number of eggs and type IV trichomes on chromosome 9 at a different position near the marker C2_At2g41680, which is about 17 cM away from the reported type IV trichome QTL marker TG223 from accession CGN.1561. Differences in the studies could be due to the use of different accessions and/or differences in whitefly species. No effect was detected for the six QTLs reported by Blauth et al. (1999) for acylsugar accumulation in *S. pennellii*. Although we did not study the chemical composition of the type IV trichome glands, our results could support the findings of Frelichowski and Juvik (2005) that found segregation for high levels of sesquiterpene carboxylic acids (SCA) in the populations of *S. habrochaites* LA1033 and *S. habrochaites* LA1777 and have suggested that inheritance of SCA is polygenic. Zamir et al. (1984) reported that the inheritance of 2-tridecanone produced by type IV trichomes in *S. habrochaites* f. *glabratum* also is polygenic. The present study also suggests polygenic inheritance for SPWF resistance, as measured by reduced egg deposition and increased adult mortality. Zamir et al. (1984), using isozyme markers with an F₂ BC₁ population of tomato

and *S. habrochaites* f. *glabratum*, detected significant associations with five of the isozyme markers on four different chromosomes (3, 4, 6, and 7). We found no association of type IV trichome density with any of these chromosomal regions using selective genotyping analysis or on chromosomes 3 and 4 using selective genotyping and QTL analyses. However, we only studied trichome density and not the chemicals contained in trichome glands. One R plant in the present study had 60 type IV trichomes but had only 0.5 eggs deposited per leaflet, while another plant had 153 type IV trichomes but had 17 eggs deposited (Table 1). Thus, it is not necessarily the high number of type IV trichomes resulting in a high level of resistance (low egg deposition).

FUTURE RESEARCH. Four QTLs on three different chromosomes were identified through selective genotyping, QTL analyses, and stepwise multiple regression analysis for reduced egg deposition and/or type IV trichome production. This is the first step toward our overall goal of developing SPWF-resistant breeding

lines without linkage drag from the wild species. The amount of variation explained by any single QTL was not high, and combining QTL did not explain much more variation. One reason for this could be that different combinations of resistance genes can provide resistance, and thus any particular gene combination can only account for a part of the resistance variation. It is apparent that accumulating all four regions did not increase the resistance variation accounted for over lesser numbers of regions. Perhaps there was enough resistance with fewer genes, thus the additional ones were superfluous. Presently, we have made crosses and are using the molecular markers to test all combinations of the four regions for resistance. Once this is done, we will have a better concept as to what regions are necessary to attain resistance. We will then proceed to use markers and test crosses to fine map the actual resistance genes. The results presented here will enable high-resolution genetic mapping using large populations and increasing marker density within regions, which will be needed to more closely identify the regions on the chromosomes associated with resistance.

Literature Cited

- Alba, J.M., J. Cuartero, and R. Fernandez-Muñoz. 2005. Resistance to *Bemisia tabaci* in *L. pimpinellifolium* accession TO-937 and advance-backcross line. XVth EUCARPIA Tomato Working Group. p. 19 (Abstr).
- Bai, Y., C.C. Huang, R. Van der Hulst, F. Meijer-Dekens, G. Bonnema, and P. Lindhout. 2003. QTLs for tomato powdery mildew resistance (*Oidium neolyopersici*) in *Lycopersicon parviflorum* G1.1601 co-localize with two quantitative powdery mildew resistance genes. Mol. Plant-Microbe Interact. 16:169–176.

- Bai, Y., R. Van der Hulst, C.C. Huang, L. Wei, P. Stam, and P. Lindhout. 2004a. Mapping *Ol-4*, a gene conferring resistance to *Oidium neolycopersici* and originating from *Lycopersicon peruvianum* LA2172, requires multi-allelic, single-locus markers. *Theor. Appl. Genet.* 109:1215–1223.
- Bai, Y., X. Feng, R. van der Hulst, and P. Lindhout. 2004b. A set of molecular markers converted from sequence specific RFLP markers on tomato chromosomes 9 to 12. *Mol. Breed.* 13:281–287.
- Balvora, A., S. Schornack, B.J. Baker, M. Ganai, U. Bonas, and T. Lahye. 2001. Chromosome landing at the tomato *Bs4* locus. *Mol. Genet. Genomics* 266:639–645.
- Berlinger, M.J., R. Dahan, and E. Shevach-Urkin. 1983. Breeding for resistance in tomato to the tobacco whitefly (*Bemisia tabaci*). *Phytoparasitica* 11:132. (Abstr.).
- Blauth, S.L., G.A. Churchill, and M.A. Mutschler. 1998. Identification of quantitative trait loci associated with acylsugar accumulation using interspecific populations of the wild tomato, *Lycopersicon pennellii*. *Theor. Appl. Genet.* 96:458–467.
- Blauth, S.L., J.C. Steffens, G.A. Churchill, and M.A. Mutschler. 1999. Identification of QTLs controlling acylsugar fatty acid composition in an interspecific population of *Lycopersicon pennellii* (Corr.) D'Arcy. *Theor. Appl. Genet.* 99:373–381.
- Byrne, F.J., S. Castle, N. Prabhaker, and N. Toscano. 2003. Biochemical study of resistance to imidacloprid in B biotype *Bemisia tabaci* from Guatemala. *Pest Manag. Sci.* 59:347–352.
- Dahan, R. 1985. *Lycopersicon pennellii* as a source for resistance to the tobacco whitefly *Bemisia tabaci* in tomato. M.S. thesis, Ben-Gurion Univ. Negev, Be'er Sheva, Israel.
- Darvasi, A. and M. Soller. 1992. Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. *Theor. Appl. Genet.* 85:353–359.
- Denholm, I., M. Cahill, F.J. Byrne, and A.L. Devonshire. 1996. Progress with documenting and combating insecticide resistance in *Bemisia*, p. 577–603. In: D. Gerling and R.T. Mayer (eds.). *Bemisia*: 1995 Taxonomy, biology, damage, control and management. Intercept, Andover, UK.
- Doganlar, S., J. Dodson, B. Gabor, T. Beck-Bunn, C. Crossman, and S.D. Tanksley. 1998. Molecular mapping of the *py-1* gene for resistance to corky root rot (*Pyrenochaeta lycopersici*) in tomato. *Theor. Appl. Genet.* 97:784–788.
- Elbert, A. and R. Nauen. 2000. Resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticide in southern Spain with special reference to neonicotinoids. *Pest Manag. Sci.* 56:60–64.
- Frelichowski J.E., Jr. and J.A. Juvik. 2005. Inheritance of sesquiterpene carboxylic acid synthesis in crosses of *Lycopersicon hirsutum* with insect-susceptible tomatoes. *Plant Breed.* 124:277–281.
- Fridman, E., J. Wang, Y. Lijima, J.E. Froehlich, and D.R. Gang. 2005. Metabolic, genomic and biochemical analyses of glandular trichomes from the wild tomato species *Lycopersicon hirsutum* identify a key enzyme in the biosynthesis of methylketones. *Plant Cell* 17:1252–1267.
- Fulton, T., R. van der Hoeven, N. Eannetta, and S.D. Tanksley. 2002. Identification, analysis, and utilization of a conserved ortholog set (COS) markers for comparative genomics in higher plants. *Plant Cell* 14:1457–1467.
- Fulton, T.M., J. Chunwongse, and S.D. Tanksley. 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Mol. Biol. Rpt.* 13:207–209.
- Fulton, T.M., S. Grandillo, T. Beck-Bunn, E. Fridman, A. Frampton, J. Lopez, V. Petiard, J. Uhlig, D. Zamir, and S.D. Tanksley. 2000. Advanced backcross QTL analysis of a *Lycopersicon esculentum* × *Lycopersicon parviflorum* cross. *Theor. Appl. Genet.* 100:1025–1042.
- Heinz, K.M. and F.G. Zalom. 1995. Variation in trichome-based resistance to *Bemisia argentifolii* (Homoptera: Aleyrodidae) oviposition on tomato. *J. Econ. Entomol.* 88:1494–1502.
- Hemming, M.N., S. Basuki, D.J. McGrath, B.J. Carroll, and D.A. Jones. 2004. Fine mapping of the tomato *I-3* gene for fusarium wilt resistance and elimination of a co-segregating resistance gene analogue as a candidate for *I-3*. *Theor. Appl. Genet.* 109:409–418.
- Ji, Y., J.W. Scott, P. Hanson, E. Graham, and D.P. Maxwell. 2007. Sources of resistance, inheritance and location of genetic loci conferring to members of the tomato infecting begomoviruses, p. 343–362. In: H. Czosnek (ed.). *Tomato yellow leaf curl virus disease: Management, molecular biology, and breeding for resistance*. Springer, Dordrecht, The Netherlands.
- Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M. Daley, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181.
- Lapidot, M., M. Friedmann, O. Lachman, A. Yehzekel, S. Nahon, S. Cohen, and M. Pilowsky. 1997. Comparison of resistance level to tomato yellow leaf curl virus among commercial cultivars and breeding lines. *Plant Dis.* 81:1425–1428.
- Lawson, D.M., C.F. Lunde, and M.A. Mutschler. 1997. Marker-assisted transfer of acylsugar-mediated pest resistance from the wild tomato, *Lycopersicon pennellii*, to the cultivated tomato *Lycopersicon esculentum*. *Mol. Breed.* 3:307–317.
- Liedl, B.E., D.M. Lawson, K.K. White, J.A. Shapiro, D.E. Cohen, W.G. Carson, J.T. Trumble, and M.A. Mutschler. 1995. Acylsugars of wild tomato *Lycopersicon pennellii* alters settling and reduces oviposition of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 88:742–748.
- Lincoln, S., M. Daly, and E.S. Lander. 1993. Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1: A tutorial and reference manual. 2nd ed. Whitehead Institute Technical Report, Cambridge, MA.
- Luckwill, L.C. 1943. The genus *Lycopersicon*: a historical, biological and taxonomic survey of the wild and cultivated tomatoes. *Studies* No. 120. Aberdeen University Press, Aberdeen, UK.
- Maliepaard, C., N. Je Bas, S. Van Heusden, J. Kos, G. Pet, R. Verkerk, R. Vrielink, P. Zabel, and P. Lindhout. 1995. Mapping of QTLs for glandular trichome densities and *Trialeurodes vaporariorum* (greenhouse whitefly) resistance in an F₂ from *Lycopersicon esculentum* × *Lycopersicon hirsutum* f. *glabratum*. *Heredity* 75:425–433.
- Momotaz, A., J.W. Forster, and T. Yamada. 2004. Identification of cultivars and accessions of *Lolium*, *Festuca* and *Festulolium* hybrids through the detection of simple sequence repeat polymorphism. *Plant Breed.* 123:370–376.
- Momotaz, A., J.W. Scott, and D.J. Schuster. 2005. Searching for silverleaf whitefly and geminivirus resistance genes from *Lycopersicon hirsutum* accession LA1777. *Acta Hort.* 695:417–422.
- Momotaz, A., J.W. Scott, and D.J. Schuster. 2007. *Solanum habrochaites* accession LA1777 recombinant inbred lines are not resistant to tomato yellow leaf curl virus or tomato mottle virus. *HortScience* 42:1149–1152.
- Monforte, A.J. and S.D. Tanksley. 2000. Development of a set of near isogenic and backcross recombinant inbred lines containing most of the *Lycopersicon hirsutum* genome in a *L. esculentum* genetic background: A tool for gene mapping and gene discovery. *Genome* 43:803–813.
- Muigai, S.G. 1997. Enhancement of wild *Lycopersicon* germplasm for resistance to *Bemisia argentifolii* (Homoptera: Aleyrodidae). Ph.D. Diss. University of Florida, Gainesville.
- Muigai, S.G., D.J. Schuster, J.C. Snyder, J.W. Scott, M.J. Bassett, and H.J. McAuslane. 2002. Mechanisms of resistance in *Lycopersicon* germplasm to *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Phytoparasitica* 30:347–360.
- Muigai, S.G., M.J. Bassett, D.J. Schuster, and J.W. Scott. 2003. Greenhouse and field screening of wild *Lycopersicon* germplasm for resistance to the whitefly *Bemisia argentifolii*. *Phytoparasitica* 31:27–38.
- Mutschler, M.A., R.W. Doerge, S.C. Liu, J.P. Kuai, B.E. Liedl, and J.A. Shapiro. 1996. QTL analysis of pest resistance in the wild

- tomato *Lycopersicon pennellii*: QTLs controlling acylsugar level and composition. *Theor. Appl. Genet.* 92:709–718.
- Palumbo, J.E., A.R. Horowitz, and N. Prabhaker. 2001. Insecticidal control and resistance management for *Bemisia tabaci*. *Crop Prot.* 20:739–765.
- Pico, B., M. Ferriol, M.J. Diez, and F. Nuez. 1999. Developing tomato breeding lines resistant to tomato yellow leaf curl virus. *Plant Breed.* 118:537–542.
- Polston, J.E. 2001. Tomato yellow leaf curl virus: Economic impact, p. 89–90 In: K. Hopkins (ed.). *Post-global crop protection compendium*. CAB International, Wallingford, UK.
- Polston, J.E. and P.K. Anderson. 1997. The emergence of whitefly-transmitted geminiviruses in tomato in the western hemisphere. *Plant Dis.* 81:1358–1369.
- Rahimi, F.R. and C.D. Carter. 1993. Inheritance of zingiberene in *Lycopersicon*. *Theor. Appl. Genet.* 92:709–718.
- Saikia, A.K. and V. Muniyappa. 1989. Epidemiology and control of tomato leaf curl virus in southern India. *Trop. Agr. (Trinidad)* 66:350–354.
- Schuster, D.J., P.A. Stansly, and J.E. Polston. 1996. Expressions of plant damage by *Bemisia*, p. 153–165. In: D. Gerling and R.T. Mayer (eds.). *Bemisia: 1995 taxonomy, biology, damage, control, and management*. Intercept, Andover, UK.
- Schuster, D.J., R.S. Mann, M. Toapanta, R. Cordero, S. Thompson, S. Cyman, A. Shurtleff, and R.F. Morris, II. 2010. Monitoring neonicotinoid resistance in biotype B of *Bemisia tabaci* in Florida. *Pest Manag. Sci.* 66:186–195.
- Scott, J.W. 2007. Breeding for resistance to viral pathogens, p. 447–474. In: M.K. Razdan and A.K. Mattoo (eds.). *Genetic improvement of solanaceous crops*. Vol. 2: Tomato. Science Publishers, Enfield, NH.
- Sharaf, N. 1986. Chemical control of *Bemisia tabaci*. *Agr. Ecosyst. Environ.* 17:111–127.
- Shevach-Urkin, E. 1983. Comparison of the tobacco whitefly in the cultivated tomato *Lycopersicon esculentum* and wild species *Lycopersicon hirsutum* f. *glabratum*. M.S. thesis. Univ. Jerusalem, Rehovot, Israel. (in Hebrew with English summary).
- Snyder, J.C., A.M. Simmons, and R.R. Thacker. 1998. Attractancy and ovipositional response of adult *Bemisia argentifolii* (Homoptera: Aleyrodidae) to type IV trichome density on leaves of *Lycopersicon hirsutum* grown in three day-length regimes. *J. Entomol. Sci.* 33:270–281.
- Sol Genomics Network. 2008. Tomato-EXPEN 2000: *S. lycopersicum* LA925 × *S. pennellii* LA716 type F2.2000. 18 Dec. 2008. <http://www.sgn.cornell.edu/cview/map.pl?map_id=9&show_offsets=1&show_ruler=1>.
- U.S. Department of Agriculture. 2008. National statistics, Vegetables, Tomato (fresh). 2 Feb. 2009. <<http://www.nass.usda.gov/QuickStats/index2.jsp>>.
- Vidavsky, F. 2007. Exploitation of resistance genes found in wild tomato species to produce resistant cultivars: Pile up of resistant genes, p. 910–914. In H. Czosnek (ed.). *Tomato yellow leaf curl virus disease: Management, molecular biology, and breeding for resistance*. Springer, Dordrecht, The Netherlands.
- Vidavsky, F. and H. Czosnek. 1998. Tomato breeding lines resistant and tolerant to tomato yellow leaf curl virus (TYLCV) issued from *Lycopersicon hirsutum*. *Phytopathology* 88:910–914.
- Williamson, V.M., J.Y. Ho, F.F. Wu, N. Miller, and I. Kaloshian. 1994. A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato. *Theor. Appl. Genet.* 87:757–763.
- Wu, F., L.A. Mueller, D. Crouzillat, V. Petiard, and S.D. Tanksley. 2006. Combining bioinformatics and phylogenetics to identify large sets of single copy, orthologous genes (COSII) for comparative, evolutionary and systematics studies: A test case in the euasterid plant clade. *Genetics* 174:1407–1420.
- Yaghoobi, J., J.L. Yates, and V.M. Williamson. 2005. Fine mapping of the nematode resistance gene *Mi-3* in *Solanum peruvianum* and construction of a *S. lycopersicum* DNA contig spanning the locus. *Mol. Genet. Genomics* 274:60–69.
- Yorit, M. 1986. The wild species *Lycopersicon hirsutum* Humb. & Bonpl. as a source for resistance to the tobacco whitefly (*Bemisia tabaci* Gennadius) in the cultivated tomato *Lycopersicon esculentum* Mill. M.S. thesis. Hebrew Univ. Jerusalem, Rehovot, Israel. (in Hebrew with English summary).
- Zamir, D., I. Ekstein-Michelson, Y. Zakay, N. Navot, M. Zeidan, M. Sarfatti, Y. Eshed, E. Harel, H. Pleben, H. Van-Oss, N. Kedar, H.D. Rabinowitch, and H. Czosnek. 1994. Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, *Ty-1*. *Theor. Appl. Genet.* 88:141–146.
- Zamir, D., T. Seila Ben-David, J. Rudich, and J.A. Juvik. 1984. Frequency distributions and linkage relationships of 2-tridecanone in interspecific segregating generations of tomato. *Euphytica* 33:481–488.
- Zeidan, M., S.K. Green, D.P. Maxwell, M.K. Nakhla, and H. Czosnek. 1999. Molecular analysis of whitefly-transmitted tomato geminiviruses from southeast and east Asia. *Trop. Agr. Res. Sta.* 1:107–115.