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ORIGINAL PAPER

Identification and QTL mapping of whitefly resistance components in *Solanum galapagense*

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Abstract *Solanum galapagense* is closely related to the cultivated tomato and can show a very good resistance towards whitefly. A segregating population resulting from a cross between the cultivated tomato and a whitefly resistant *S. galapagense* was created and used for mapping whitefly resistance and related traits, which made it possible to study the genetic basis of the resistance. Quantitative trait loci (QTL) for adult survival co-localized with type IV trichome characteristics (presence, density, gland longevity and gland size). A major QTL (*Wf-1*) was found for adult survival and trichome characters on Chromosome 2. This QTL explained 54.1 % of the variation in adult survival and 81.5 % of the occurrence of type IV trichomes. A minor QTL (*Wf-2*) for adult survival and trichome characters was identified on Chromosome 9. The major QTL was confirmed in F3 populations. Comprehensive metabolomics, based on GCMS profiling, revealed that 16 metabolites segregating in the F2 mapping population were associated with *Wf-1* and/or *Wf-2*. Analysis of the 10 most resistant and susceptible F2 genotypes by LCMS showed that several acyl sugars were present in significantly higher concentration in the whitefly resistant genotypes, suggesting a role for these components in the resistance as well. Our results show that whitefly resistance in *S. galapagense* seems to inherit relatively simple compared to whitefly resistance from other sources and this offers great prospects for resistance breeding as well as elucidating the underlying molecular mechanism(s) of the resistance.

Introduction

Whiteflies (*Bemisia tabaci* Genn.) can cause serious problems in the cultivation of tomatoes and other vegetable

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crops mainly because they vector a large number of harmful viruses (Morales and Jones 2004). In addition, feeding of whiteflies inhibits plant growth (Schuster et al. 1990) and the honeydew produced by the whiteflies can promote sooty mold growth, which may lead to physiological disorders (McCollum et al. 2004). Natural enemies and/or pesticides are not effective enough in open field cultivation to prevent the unpredictable outbreaks of whiteflies (Hirano et al. 1995). Moreover, pesticides are known to be harmful for growers and can result in pesticide-resistant whiteflies (Erdogan et al. 2008). A plant variety that is naturally resistant to whiteflies may be a good alternative to control *B. tabaci* and the viruses it distributes (Broekgaarden et al. 2011).

Whitefly resistance has been found in wild relatives of tomato such as *S. pennellii*, *S. habrochaites*, *S. chilense*, *S. pimpinellifolium* and *S. galapagense* (Baldin et al. 2005; Fancelli and Vendramim 2002; Firdaus et al. 2012; Muigai et al. 2003; Toscano et al. 2002). The resistance parameters used in these studies were density and/or survival of whitefly adults, eggs and nymphs (Firdaus et al. 2012; Maliepaard et al. 1995; Muigai et al. 2003). The resistance mechanisms were based on antixenosis and/or antibiosis (Baldin et al. 2005; Channarayappa et al. 1992; Toscano et al. 2002). Mortality may be caused by physical barriers preventing the whiteflies to feed on the phloem sap (Toscano et al. 2002) or by toxic compounds (Kehr 2006).

Whitefly resistance in wild relatives of cultivated tomato is suggested to be associated with glandular trichomes (Erb et al. 1994; Oriani et al. 2011; Muigai et al. 2003; Rodriguez-Lopez et al. 2011). Of the seven trichome types found in tomato and its wild relatives, four types are glandular (Luckwill 1943). The presence of type IV and VI trichomes is highly correlated with whitefly resistance (Channarayappa et al. 1992; Dimock and Kennedy 1983; Firdaus et al. 2012; Muigai et al. 2003). Glandular trichomes might play a role as physical barrier and/or source of compounds deterrent and/or toxic to whiteflies (Dimock and Kennedy 1983; Toscano et al. 2002). However, not all tomato accessions with type IV trichomes are resistant (Frelichowski and Juvik 2001; Muigai et al. 2003), suggesting that the content of the trichomes also plays an important role. Acyl sugars are the major exudates of type IV trichomes in *S. pennellii* and *S. pimpinellifolium* (Blauth et al. 1998; Fancelli et al. 2005; Mutschler et al. 1996; Rodriguez-Lopez et al. 2011, 2012; Leckie et al. 2012; Schilmiller et al. 2012). Methyl-ketones and derivates of sesquiterpenes carboxylic acids are major exudates of type IV and VI trichomes in *S. habrochaites* (Chatzivasileiadis and Sabelis 1997; Eigenbrode et al. 1994; Farrar and Kennedy 1991; Frelichowski and Juvik 2001; Kennedy et al. 1991).

Genetic factors underlying whitefly resistance were identified by quantitative trait loci (QTLs) mapping studies (Blauth et al. 1998; Maliepaard et al. 1995; Momotaz et al. 2010; Mutschler et al. 1996). In *S. habrochaites* LA1777, the QTLs for oviposition rate on Chromosomes 9, 10 and 11 co-localized with QTLs for type IV trichomes (Momotaz et al. 2010). However, in *S. habrochaites* CGN1.1561, the QTLs for egg deposition and the QTLs for presence of glandular trichomes did not co-localize, which may point at a different mechanism (Maliepaard et al. 1995). In *S. pennellii*, 12 QTLs were detected for presence and density of type IV trichome and production of acyl sugar (Blauth et al. 1998; Mutschler et al. 1996; Leckie et al. 2012). However, backcross plants containing the five QTLs for acyl sugar production did not produce elevated levels of acyl sugars, suggesting that additional QTLs are needed (Lawson et al. 1997). These studies showed that whitefly resistance can be based on several mechanisms involving many genes. For successful introgression breeding, a comprehensive knowledge of the genetic basis of the different whitefly resistance factors is needed and preferably a closely related wild relative to be able to minimize linkage drag as much as possible. In this study, we used a *S. galapagense* accession with a very high level of resistance to study the genetics of the resistance and to identify components involved in the resistance.

Materials and methods

Plant and whitefly materials

An F2 population of 230 individual seeds was obtained after self-pollination and seed collection of an F1 plant originating from the cross between *S. lycopersicum* cv. Moneymaker^{tmvR} PRI91117 and *S. galapagense* PRI95004 (Firdaus et al. 2012). The parents were obtained from Wageningen UR Plant Breeding, Wageningen, The Netherlands. Seeds were sown in peat-moss soil in a sowing box and seedlings transplanted after the third leaf stage into 1.5 L pots containing peat-moss soil and maintained in an insect-proof greenhouse with a 16 h light and an 8 h dark photoperiod, 20 °C/16 °C (day/night) and 70 % relative humidity from October 2009 to March 2010 in Wageningen, The Netherlands. Plants were pruned to maintain a manageable size. Cuttings were made from each genotype for whitefly screenings in Wageningen, The Netherlands and Purwakarta, Indonesia. Non-viruliferous silverleaf whiteflies (*B. tabaci*, group Mediterranean-Middle East-Asia Minor I), from the collection of the Laboratory of Entomology, Wageningen University, The Netherlands or the local haplotype of *B. tabaci* (group Asia I) of Purwakarta-West Java, Indonesia, were used for screening. Sequence analysis of the mtCOI gene was carried out for whitefly identification (Firdaus et al. 2013).

Whitefly resistance tests

No-choice resistance tests were carried out in Wageningen and Purwakarta. In the Netherlands, three cuttings were made from the two parents, four F1 plants and 120 F2 plants, and shipped to Indonesia in October 2009 for phenotyping. In Indonesia, the plantlets were grafted onto 3-week-old eggplant (*Solanum melongena* cv. EG203) rootstocks to prevent nematode problems. Two cuttings per genotype were randomly arranged on tables 1 m above the ground in an insect-proof screen house. In a screen house, the plants are protected from unwanted insects, heavy rainfall and intense sunshine. There were two rows on each table with 35 cm between rows and 20 cm between plants. Amaranthus plants were put in between genotypes as border plants. Branches and flowers of the tomato plants were pruned regularly to get one main stem and to avoid fruit setting.

Clip-on cages were used for the tests according to Maliepaard et al. (1995). In Indonesia, the test was carried out from November 2009 to February 2010, on 5-week-old plants. Synchronized whiteflies (1–2 days old) were anesthetized by putting them at 4 °C for about 10 min and females were selected. Five whitefly females (n) were collected with an aspirator and transferred to a clip-on cage (2 cm in diameter and 1 cm in height); three cages were attached to the underside of a leaflet of the third or fourth leaf from the top. Four days after infestation (d), the clip-on cages were removed from the leaves and the death and living whiteflies (m) were counted. The number of eggs (e) was counted under a stereo microscope. In Wageningen, one cutting was made from each of the 189 F2 individuals and four cuttings from each parent. Lateral branches and flowers were regularly removed. One week before infestation, the temperature was raised gradually until it reached 27 °C/18 °C (day/night) 2 days before infestation. Whitefly infestation was done 6 weeks after the cuttings were made. Synchronized whiteflies (1–2 days old) were anesthetized with CO_2 for female selection. Female selection and infestation were done in a similar way as in Indonesia. The same parameters as in Indonesia were measured and additionally pupal cases (p) were counted 8 days after the first adult appeared, which was around 17–21 days after infestation. Adult survival (AS), oviposition rate (OR) and pre-adult survival (PS) were calculated using the following equations (Maliepaard et al. 1995).

$$\mathrm{AS} = \left(\frac{m}{n}\right)^{1/d} \text{ survival day}^{-1} \quad (1)$$

$$\mathrm{OR} = \frac{2e}{d(m+n)} \text{ eggs female}^{-1}\text{ day}^{-1} \quad (2)$$

$$\mathrm{PS} = {}^{p}/_{e} \text{ whiteflies egg}^{-1} \quad (3)$$

An ArcSin transformation was used to normalize adult survival and pre-adult survival values, and a square-root transformation was used for oviposition rate.

Trichome type identification and counting

Different types of trichomes were identified based on Luckwill (1943). Trichomes were counted on the abaxial side of the lateral leaflets which were used for the clip-on cage test 1 or 2 days after the infestation started. The leaflets were cut from the plant and three circles were made using a perforator (each circle was approx. 1 mm^2) on the right and/or left sides of the main vein at the beginning of the leaflet. The trichome types were identified and counted using a stereo dissecting microscope (40× to 100×). R45, which is the ratio type IV/(type IV + type V) trichome density, was calculated. Gland longevity and size of the type IV trichomes were measured in the test done in the Netherlands. Gland longevity is given by the fraction of type IV trichomes still present 3 weeks after infestation (old leaves) compared to the number at the start of the infestation (6 weeks). The scores were: (0) value between 0 and 0.49, (1) value between 0.5 and 0.99, and (2) value of 1 (no type V trichomes, only type IV). For the gland size, the scores were made based on the proportion, small (15–25 μm) and big (45–55 μm) type IV trichomes. The score for gland size was (0) when the minority of the glands were big and (1) when the majority of the glands were big.

Correlation between resistance parameters and trichomes

Correlations between resistance parameters and density of type IV, V and VI trichomes were calculated with the Pearson's correlation method, whereas the correlation between presence of type I and III trichomes and scores for ratio, longevity and size of type IV trichomes were calculated with the Spearman's correlation method (Steel and Torrie 1980). The effect of the presence and absence of type IV or/and V on AS and OR was calculated using Mann–Whitney and Kruskal–Wallis tests (Steel and Torrie 1980). The resistance parameters and traits of the tests in Indonesia and the Netherlands were compared with paired samples t test analyses. The analyses were done using SPSS 19.0.0.1 package (SPSS® Inc. an IBM® Company). Heritability of the resistance parameters and traits were calculated based on Burton (1952): $h^2 = (VF_2 - (VP_1 + VP_2 + VF_1)/3)/VF_2$, where h^2 = broad sense heritability, VF_2 = variance of F_2, VP_1 = variance of parent 1, VP_2 variance of parent 2 and VF_1 = variance of F_1.

Genomic DNA extraction and genotyping

Approximately 2 cm^2 fresh young leaves were collected and ground using the Retsch Mixer Mill MM301® according to the manufacturer's manual. Afterwards, the

genomic DNA was extracted according with the maxiprep method as described in the KingFisher® 96 manual (Thermo Labsystems). The DNA quantity and quality was determined using the NanoDrop 1000 V.3.7 (Thermo Fisher Scientific Inc) and agarose gel electrophoresis. 50 ng/μl DNA solution were prepared for genotyping. For genotyping a single nucleotide polymorphism (SNP), Infinium array (made for other purposes at Wageningen UR Plant Breeding) was used. This array contained 5528 SNPs, of which 1,654 were polymorphic between our *S. lycopersicum* and *S. galapagense* parents. Genotyping was carried out by Service XS, Leiden, The Netherlands.

Genetic linkage analysis and QTL mapping

A genetic linkage map of SNP marker data was calculated using JoinMap® 4.0 (Van Ooijen 2011). SNP markers that were difficult to score were removed. Markers that showed an identical segregating pattern were considered as one marker. Finally, of the 1,654 SNP markers polymorphic between the parents, 589 markers were used to construct a genetic map. The genetic map was constructed based on recombination frequency with a minimum LOD-score 2.0 and maximum likelihood was used as mapping algorithm. In the regression mapping, linkages with recombination frequency less than 0.4 and LOD score greater than 1.0 were used. MapQTL® 6 (Van Ooijen 2009) was used to determine significant associations between markers and phenotypic traits. The genetic linkage and QTL maps were drawn using MapChart 2.2 (Voorrips 2002).

Gas chromatography–mass spectroscopy (GC–MS)

Two cuttings per F2 genotype were grown in a greenhouse at Wageningen University and Research Centre and used to analyze the chemical content by GC–MS. After 6 weeks, the third and fourth leaf from the top (the same leaf stages as used for clip-on cages test) were harvested and immediately frozen in liquid nitrogen and kept at −80 °C until they were prepared for metabolite analyses, which were performed as described by Maharijaya et al. (2012) with minor modifications. Frozen leaves were ground to a fine powder and 400 mg of the powder was dissolved in 3 ml anhydrous dichloromethane (>99.8 %, Sigma-Aldrich) containing 0.75 μg ml^{-1} Heptadecanoic acid methyl ester (methylheptadecanoate) as an internal standard. The solution was homogenized by vortexing and centrifuged at 1500 rpm for 10 min. Chromatography was performed on an ZB-5MS column (Phenomenex, 30 meter, 0.25 mm inner diameter, 0.25 μm film thickness) with a 5 meter retention gap. The temperature of the injector was set to 250 °C for GC and 260 °C for MS. The temperature of the column was programmed at 45 °C for 1 min, and raised gradually by 10 °C min^{-1} up to 300 °C and kept at 300 °C for 7 min. Helium was used as carrier gas and the column flow was 1 ml min^{-1}. The column effluent was ionized by electron impact at 70 eV. Mass spectra were obtained from 35 to 400 m/z. An untargeted approach was applied to process the raw GC–MS data and to identify metabolites as described by Maharijaya et al. (2012).

To identify resistance related metabolites, the mass abundances of metabolites obtained from GC–MS were subjected to QTL mapping. Metabolites which had QTLs at the same position as QTLs for AS were considered as potentially related to whitefly resistance. The effect of parent alleles of the QTLs on the abundance of related metabolites in the F2 population was also compared by using univariate analysis followed by least significant difference (LSD) test of the IBM® SPSS® 19 package (Steel and Torrie 1980).

Liquid chromatography-mass spectroscopy (LC–MS)

The ten most resistant and ten most susceptible genotypes were selected for LC–MS analysis. Extraction and analysis by Liquid Chromatography-Quadrupole Time of Flight-Mass Spectrometry (LC-QTOF-MS, in short LC–MS) was performed as described by De Vos et al. (2007) with slight modifications of the LC gradient to enable a good separation of acyl sugars. In short, five hundred mg of frozen-leaves powder were extracted with 1.5 ml Methanol containing 0.1 % of Formic acid (FA). The extracts were homogenized by shaking for a few seconds and sonicated for 10 min, and then centrifuged at 2,500 rpm for 10 min. The supernatant was filtered using a RC4® minisart 0.45 μm filter. Afterwards, 5 μl per sample was injected in the LC–MS system (Waters QTOF Ultima) and separated on a Phenomenex Luna C18 (2) column (2.0 × 150 mm, 3 mm particle size) using a 5–95 % acetonitrile gradient in H_2O with 0.1 % FA for acidification. Mass signals of *m/z* 80–1,500 were detected with negative electrospray ionization. Leucine encephalin was used as lock mass for local accurate mass corrections. For the annotation of LCMS peaks corresponding to acyl sugars, the mono-isotopic exact masses of negatively charged ions were calculated for a series of possible acyl chain-sugar combinations, from 7 up to 30 carbons acylated to either glucose (G) or sucrose (S) as the sugar backbone, i.e. starting from m/z 333.0827 for G4:7 up to *m/z* 803.5162 for S3:50), as well as their formic acid adducts (additional mass of 46.0055 for CH_2O_2). Under the LCMS conditions applied the acyl sugars were mainly detectable as their formic acid adducts. Metalign software (http://www.metalign.nl) was used for peak picking and alignment and extracted LCMS signals corresponding to the major acyl sugars were annotated based on their unique mono-isotopic accurate mass, using a

threshold of 5 ppm deviation of detected masses from calculated masses.

The abundance of each acyl sugar (signal intensities based on peak heights) obtained from LC–MS was compared between resistant and susceptible bulks, using the student *t* test.

Confirmation of the QTLs in F3 populations

Nine F2 plants were selected that were heterozygous for one or both QTL regions in order to have segregation after selfing. Forty F3 seeds of each selected F2 plant were sown in peat-moss soil in sowing boxes. Genomic DNA was extracted and a number of markers (Table 1) were determined using the KASPar assay (KBiosciences, UK). Markers were chosen in the identified QTL regions. Based on the marker data, 96 F3 plants were selected and phenotyped for whitefly resistance level in a no-choice experiment. The effects of the QTLs on Chromosomes 2 and 9 were calculated.

Results

Whitefly resistance and trichome properties

The average values for adult survival (AS), oviposition rate (OR), pre-adult survival (PS) and trichomes of the parents, F1 and F2 populations are shown in Table 2. *Solanum lycopersicum*, the susceptible parent, did not have type IV trichomes, whereas, *S. galapagense*, the resistant parent, did not have type V trichomes. The presence of type IV trichomes was dominant in the Netherlands, but under Indonesian conditions both trichome types were present on the F1 leaves (Table 2). Also in the F2 population there were differences in trichome distribution between the Netherlands and Indonesia. Of the F2 population 100 genotypes were analyzed in both countries. In the Netherlands both types of trichomes were simultaneously present on 15 % of the population and in Indonesia this was on 49 %. In the Netherlands more genotypes (63 %) had only type IV trichomes compared to Indonesia (32 %). The proportion of genotypes without type IV trichomes did not differ much between the two countries (22 % in the Netherlands and 19 % in Indonesia). The average AS and OR of the F2 population in Indonesia were significantly lower than in the Netherlands and the trichome type IV and VI densities in the Netherlands were significantly higher than in Indonesia. The AS screening in the Netherlands showed 75 resistant and 92 susceptible genotypes. Furthermore, all 75 resistant genotypes had type IV and no type V trichomes (R45 = 1), 30 other genotypes that also had type IV trichomes and no type V trichomes were susceptible. All together 124 genotypes had type IV trichome and 43 genotypes did not have it.

In the Netherlands there was a high correlation between AS and OR and a lower correlation between AS and PS (Table 3). The AS of the tests in Indonesia and the Netherlands were significantly correlated. Presence of type I trichomes and properties of type IV trichomes such as density, ratio, gland longevity and size, were negatively correlated with AS and OR and to a lesser extent also with PS (Table 3). Type IV and type V trichomes can both be present but there was a high positive correlation of 0.85 between the presence of type IV and the absence of type V trichomes. High correlations were also found among trichome type I, III, IV and V; no correlation was found between type VI and the other trichome types (Table 4). The presence of trichome type IV and/or type V had a significant effect on AS and OR. The presence of type IV resulted in low AS and OR, whereas the presence of type V alone or the combined presence of type IV and V resulted in high AS and OR (Table 5).

Linkage map of the SNP markers

Fourteen genetic linkage groups were constructed based on 589 segregating markers (loci) using a population of 182

Table 1 SNP markers used in confirmation of QTLs in the F3 population

No.	Marker position[a]	Chromosome	Sequence	Allele	
				S. galapagense	*S. lycopersicum*
1	47987080	02	ATCATTTTTTAGGAC[**G/A**]GATTTATATTCTTGT	G	A
2	48838393	02	AAACTTGCAGGTACT[**G/A**]CGACCTCCTATGATC	G	A
3	49271930	02	GATTCTTCCACGCCT[**A/C**]GCTCTTCTTCTGCAG	A	C
4	49456289	02	GGAAAATAGTTTGTG[**T/C**]ATTAAAAGAGCAGAA	T	C
5	49486944	02	GTTGCCTAGTTCAAC[**G/A**]TTTGTTTACGCAACA	A	G
6	14931856	09	TCAGATGGTGATTCC[**T/C**]CACCTTACAGAAAAT	T	C
7	23385770	09	GCAGCCGTTGCAGTC[**T/C**]CAATTTCGCCCACAA	T	C

[a] Marker position was according to version 2.30 of the tomato sequence

Table 2 Whitefly resistance related characteristics of the plant material evaluated in the Netherlands and Indonesia

Country	Adult survival	Oviposition rate	Pre-adult survival	Trichome presence		Trichome density			Gland characters of type IV trichomes	
				Type I	Type III	Type IV	Type V	Type VI	Longevity	Size
The Netherlands										
P1 (*S. lycopersicum*)	1 ± 0	4.63 ± 0.12	0.30 ± 0.06	0	1	0 ± 0	283.8 ± 71.3	7.5 ± 2.4	nd	nd
P2 (*S. galapagense*)	0 ± 0	0 ± 0	0 ± 0	1	0	326.3 ± 21.9	0 ± 0	15.0 ± 1.0	2	1
F1	0.45 ± 0.20	2.15 ± 0.60	0.60 ± 0.1	1 ± 0	0.1 ± 0.1	281.8 ± 28.6	0 ± 0	7.7 ± 1.4	1	1
F2	0.52 ± 0.03	2.86 ± 0.21	0.35 ± 0.02	0.79 ± 0.03	0.25 ± 0.03	173.8 ± 9.4	92.9 ± 10.6	9.2 ± 0.4	1.5 ± 0.1	0.46 ± 0.04
Heritability	0.98	0.72	0.72	1	0.78	0.83	0.74	0.61	1	1
Indonesia										
P1 (*S. lycopersicum*)	1 ± 0	1.55 ± 0.15	nd	0 ± 0	1 ± 0	0 ± 0	279.0 ± 3.6	14.1 ± 0.5	nd	nd
P2 (*S. galapagense*)	0 ± 0	0 ± 0	nd	1 ± 0	0 ± 0	208.7 ± 9.6	0 ± 0	3.1 ± 0.2	nd	nd
F1	nd	nd	nd	1 ± 0	0 ± 0	146.4 ± 14.5	62.0 ± 31.3	11.5 ± 1.3	nd	nd
F2	0.28 ± 0.04	0.80 ± 0.13	nd	0.77 ± 0.04	0.35 ± 0.04	100.3 ± 7.6	87.6 ± 8.7	6.9 ± 0.5	nd	nd
Heritability	0.87	0.99	nd	1.00	1.00	0.89	0.72	0.98	nd	nd
p value	<0.001	<0.001	nd	0.657	0.01	<0.001	0.97	<0.001	nd	nd

Mean (followed by standard error of mean) and heritability of resistance parameters and trichome properties of parents, F1 and F2 population in the resistance tests in Indonesia and the Netherlands and significance of the difference (*p* value) between both countries. Presence of type I and III was scored: 0 for absence and 1 for presence. Trichome density is expressed as the number of trichomes per square mm. Gland longevity was scored 0–2 based on division of type IV density in old leaflet (value 2 or 3 days after infestation compared to value 3 weeks after infestation); score 0 for 0.0–0.49, score 1 for 0.5–0.99 and score 2 for 1 or the absent of type V trichomes in the old leaflet. Gland sizes were scored as 0 if most glands were small (15–25 µm) and 1 if most glands were large (45–55 µm)

nd not determined

Table 3 Correlation between whitefly resistance parameters and trichome properties of experiments carried out in the Netherlands and Indonesia. Adult survival in the Netherlands (ASNL) was used as a reference point; number of plants in the analysis is in parenthesis

Test location	Parameters	ASNL	Presence of trichome type		Density of trichome type			Type IV trichome properties		
			I	III	IV	V	VI	R45[a]	Longevity	Size
The Netherlands	Adult survival		−0.55[b] (169)	0.56[b] (168)	−.63[b] (168)	0.62[b] (169)	−0.15 (169)	−0.74[b] (168)	−0.61[b] (125)	−0.58[b] (132)
	Oviposition rate	0.80[b] (169)	−0.45[b] (168)	0.49[b] (167)	−0.57[b] (167)	0.54[b] (168)	−0.14 (168)	−0.68[b] (167)	−0.64[b] (124)	−0.52[b] (131)
	Pre-adult survival	0.39[b] (111)	−0.41[b] (110)	0.42[b] (109)	−0.35[b] (109)	0.30[b] (110)	−0.09 (110)	−0.44[b] (109)	−0.17 (67)	−0.09 (73)
Indonesia	Adult survival	0.57[b] (61)	−0.26[c] (74)	0.36[b] (73)	−0.69[b] (74)	0.54[b] (74)	0.04 (74)	−0.65[b] (74)	nd	nd
	Oviposition rate	0.45[b] (61)	−0.18 (74)	0.31[b] (73)	−0.49[b] (74)	0.59[b] (74)	−0.01(74)	−0.59[b] (74)	nd	nd

nd not determined

[a] Ratio between type IV and V densities

[b] Correlation is significant at the 0.01 level (2-tailed)

[c] Correlation is significant at the 0.05 level (2-tailed)

Table 4 Correlation between trichome types based on test in the Netherlands

Trichome type	Type III	Type IV	Type V	Type VI
Type I	−0.86[a] (175)	1[a] (176)	−0.71[a] (176)	0.11 (176)
Type III		−0.86[a] (175)	0.75[a] (176)	−0.07 (175)
Type IV			−0.85[a] (176)	0.11 (176)
Type V				−0.02 (176)

Number of plants is in parenthesis

[a] Correlation is significant at the 0.01 level (2-tailed)

Table 5 Mean of adult survival (AS) and oviposition rate (OR) of F2 population grouped based on the presence of type IV and/or V trichomes

Trichomes	AS	OR	Number of genotypes
Type IV			
Present	0.39a	2.05a	125
Absent	0.92b	5.19b	43
Type V			
Present	0.90b	5.21b	64
Absent	0.30a	1.40a	104
Type IV and Type V			
Type IV present–type V absent	0.30a	1.40a	104
Type IV absent–type V present	0.92b	5.19b	43
Both types present	0.85b	5.24b	21

Different letters after the mean between presence and absence of type IV or type V trichomes or among combination of type IV and type V trichomes for AS or OR show significant differences based on the Mann–Whitney tests for type IV or type V and Kruskal–Wallis for the combined presence of type IV and V trichomes at the 0.05 significance level

F2 plants (Fig. 1). Two chromosomes (Chromosomes 1 and 3) were represented by two linkage groups probably due to recombination hot spots (Segal et al. 1992). In total, the genetic map spans 1,259 cM and the order of the SNP markers in genetic linkage map was in accordance with what was expected based on the known tomato sequence (The tomato Genome Consortium 2012) (results not shown). Figure 1 also shows the distribution of the markers on the physical map.

QTL mapping

QTLs were identified for resistance parameters that were measured in the no-choice evaluations carried out in the Netherlands and Indonesia (Table 6). QTLs for AS in the Netherlands were identified on Chromosomes 2 and 9 (Fig. 2). A major QTL (LOD = 28.1) was found on Chromosome 2 and named *Wf-1*. *Wf-1* explained 54.1 % of genetic variation (Table 6). Another QTL (LOD = 5.8) was located on Chromosome 9 and named *Wf-2*. The *Wf-2* QTL explained 14.8 % of the genetic variation. The presence of the *Wf-1* allele of the resistant parent (*Wf-1gal*) in homozygous state reduces adult survival in the F2 lines to that of the resistant parent (Table 7). In the F2 population

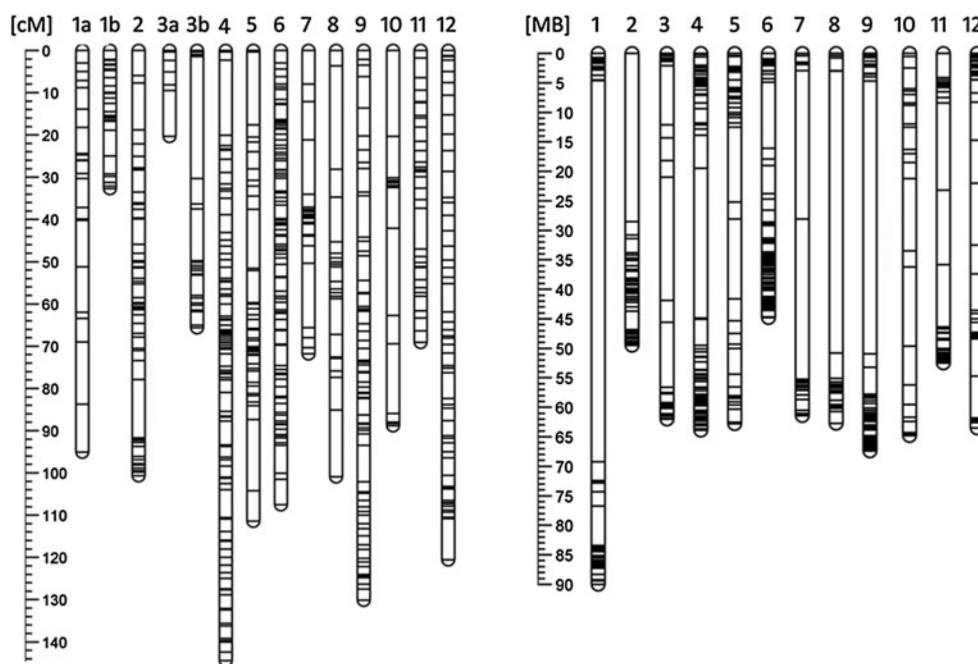

Fig. 1 Genetic linkage map based on a F2 population of a *S. lycopersicum* × *S. galapagense* cross and calculated by JoinMap 4.1 package (van Ooijen, 2011) (*left*). In total, 589 SNP markers were used, the marker positions are also given on the physical map of *S. lycopersicum* version 2.30 (*right*)

Wf-2 does not have an effect on AS and ratio of type IV/V trichome (R45) when *Wf-1gal* is present in homozygous state (Table 7). With the data obtained in Indonesia, only the major QTL on Chromosome 2 could be detected. The QTLs for trichome type I, III, IV and V co-localized with the QTLs for AS and OR (Table 6). The QTLs for type IV trichome density and R45 are also shown in Fig. 2. The major QTL on Chromosome 2 was also associated with gland longevity and size of type IV trichomes. Additional QTLs for gland longevity and for gland size were found on Chromosome 5 and Chromosome 7 (Table 6). Trichome QTLs were also detected with the data collected in Indonesia, but with lower LOD scores.

GC–MS and LC–MS analysis

In the GC–MS analysis a total of 96 metabolites were detected in the F2 population. The segregation of the abundance of these 96 metabolites was analyzed. A total of 16 out of the 96 metabolites were associated with *Wf-1* and/or *Wf-2* (Table 8). Eleven out of the 16 could be putatively identified. Nine metabolites had a QTL at *Wf-1* or *Wf-2*, three metabolites had QTLs at *Wf-1* and *Wf-2* of which one had a third QTL at Chromosome 3. The remaining four metabolites had a QTL at *Wf-1* or *Wf-2* in combination with another one on Chromosome 6 or 7. Table 8 also shows the effect of the alleles of *S. galapagense* or *S. lycopersicum* on the relative abundance of the metabolites. Seven metabolites were more abundant when the *S. galapagense* allele was homozygously present, and seven metabolites were more abundant when the *S. lycopersicum* allele was homozygously present; two metabolites had the highest abundance in the heterozygous state. Metabolites that were more abundant in *S. galapagense* were associated with *Wf-1*, while those being more abundant in *S. lycopersicum* were not associated with *Wf-1* (Table 8).

Using LC–MS profiling of aqueous-methanol extracts, we subsequently compared the 10 most resistant with the 10 most susceptible genotypes for the relative abundance of acyl sugars, as these compounds are known to be related to whitefly resistance (Fancelli et al. 2005; Leckie et al. 2012; Rodriguez-Lopez et al. 2011, 2012). A total of 28 acyl sugars (all present as acyl sucroses) could be annotated, based on the matching of their exact masses with calculated masses and in-source fragmentation revealing their sucrose unit. Nine out of these 28 acyl sugars were present at higher relative abundance ($p < 0.001$) in the 10 most resistant F2-plants than in the 10 most susceptible F2-plants (Fig. 3).

Confirmation and reduction of QTLs in F3 population

The confirmation experiment in the F3 populations gave somewhat different results. The effect of *Wf-1* on AS and R45 was confirmed (Table 7). As in the F2 population, *Wf-1gal* had a strong effect on adult survival and R45. The *Wf-2* allele in this analysis did not affect adult survival (Table 7).

Table 6 Results of QTL mapping for whitefly resistance and presence of different trichome types in the mapping population derived from a cross between *Solanum lycopersicum* × *S. galapagense*

No.	Traits	QTL location	The Netherlands					Indonesia				
			Position (cM)	LOD score	% Expl.	Additive	Dominance	Position (cM)	LOD score	% Expl.	Additive	Dominance
1	Adult survival (survival day^{-1})	Ch# 2 (*Wf-1*)	99.5	28.1	54.1	−0.41	0	98.1	15.6	65.7	−0.34	−0.27
		Ch# 9 (*Wf-2*)	57.6	5.8	14.8	−0.21	−0.07					
2	Oviposition rate (eggs female^{-1} day^{-1})	Ch# 2 (*Wf-1*)	98.9	19.5	41.7	−2.39	−0.13	77.9	5.96	33.6	−0.83	−0.75
		Ch# 9 (*Wf-2*)	76	4.3	11.1	−1.26	−0.28					
3	Pre-adult survival (whiteflies egg^{-1})	Ch# 2 (*Wf-1*)	96.1	3.4	13.3	−0.14	0.01	nd				
4	Type I presence	Ch# 2 (*Wf-1*)	98.9	31.2	56.4	0.34	0.32	93.7	6.1	21.2	0.22	0.19
		Ch# 9 (*Wf-2*)	70.5	4.9	12.2	0.19	0.11	76	3.5	12.7	0.18	0.20
5	Type III presence	Ch# 2 (*Wf-1*)	97.8	33.5	59	−1.02	−1.09	92.8	5.8	21.1	−0.31	−0.05
		Ch# 9 (*Wf-2*)	44.2	3.8	9.7	−0.39	−0.53	57.6	3.25	12.4	−0.25	0.01
6	Type IV presence	Ch# 2 (*Wf-1*)	98.9	31.2	56.4	0.34	0.32	98.9	10.6	35.1	0.29	0.21
		Ch# 9 (*Wf-2*)	70.5	4.9	12.2	0.19	0.11					
7	Type IV density	Ch# 2 (*Wf-1*)	98.9	40.9	66.3	120.01	89.11	98.9	16.8	49	80.52	14.07
		Ch# 9 (*Wf-2*)	76	3.4	8.7	51.49	3.66					
8	Type IV gland longevity	Ch# 2 (*Wf-1*)	96.1	14.5	38.9	0.77	0.25	nd				
		Ch# 5	62.6	3.1	10.1	−0.203	0.394					
9	Type IV gland size	Ch# 2 (*Wf-1*)	91.7	6.2	19.1	0.36	0.09	nd				
		Ch# 7	37.9	3.5	11.2	0.012	0.33					
10	Type V density	Ch# 2 (*Wf-1*)	99.5	57.5	78.4	−140.85	−123.96	98.9	13.3	41.2	−80.30	−38.02
		Ch# 9 (*Wf-2*)						57.6	3.8	14.4	−0.25	0.04
11	R45	Ch# 2 (*Wf-1*)	99.5	63.1	81.5	46.15	38.82	99.5	16.8	49.3	39.08	12.81
		Ch# 9 (*Wf-2*)	64.7	4	10.1	16.17	16.89					
12	Type VI density	No QTL										

% Expl. percent of explained phenotypic variation, *nd* not determined

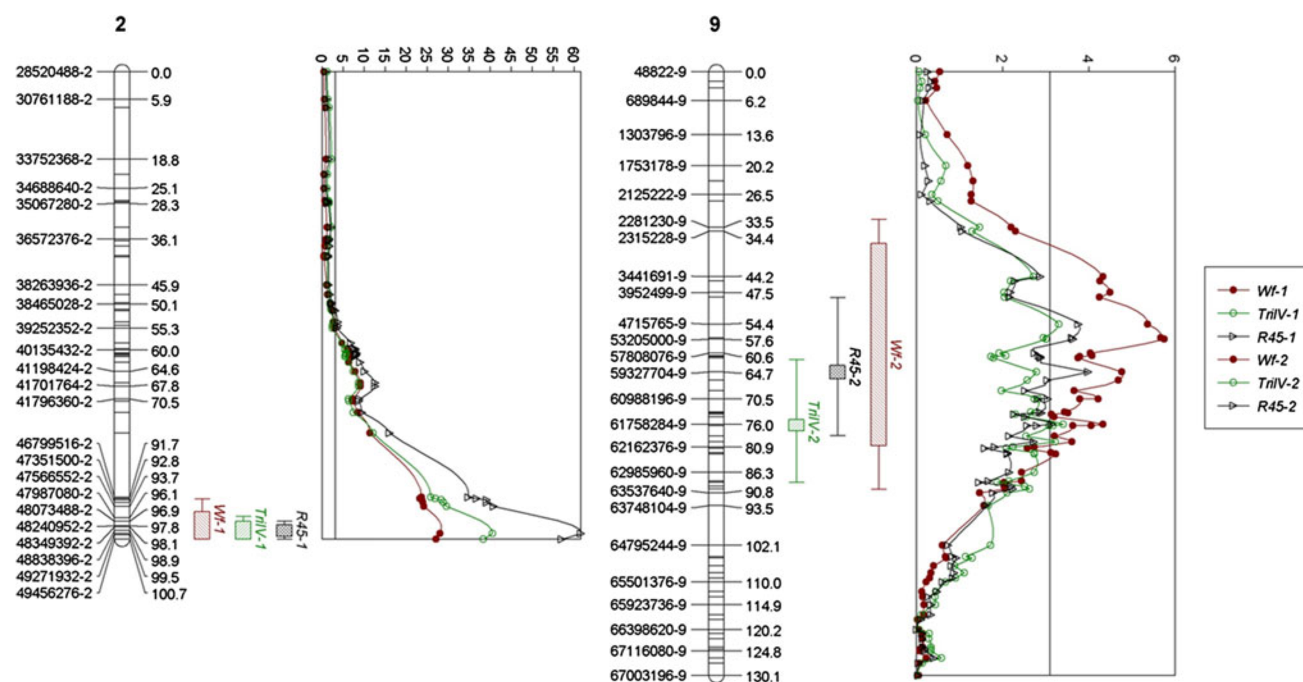

Fig. 2 The whitefly resistance loci on Chromosomes 2 and 9. Adult survival (*Wf-1, Wf-2*), type IV trichome density (*TriIV*) and type IV divided by the sum of types IV and V (*R45*) are associated with both loci. The *numbers in left* of the schematic chromosomes show the physical position of the SNP markers (ITAG version 2.30) and the *right numbers* show the genetic distance in centiMorgan (cM). The LOD values are at the *top of the graph*

Discussion

A major QTL confers high levels of whitefly resistance

Solanum galapagense PRI95004 is very resistant to whiteflies (Firdaus et al. 2012). The QTL mapping revealed two QTLs for adult survival, one major QTL on Chromosome 2 (*Wf-1*) and one minor QTL on Chromosome 9 (*Wf-2*). The F2 plants with *Wf-1* allele of *S. galapagense* (*Wf-1gal*) in homozygous state had an equally low adult survival as the resistant *S. galapagense* parent. During the screening in Indonesia, only the *Wf-1* locus was detected. The different climatical conditions in the Netherlands and Indonesia may have caused this, as also in the number and ratio of the different trichomes, differences were seen between both locations. Alternatively, it might be due to the different whitefly populations that were used in the Netherlands and Indonesia. In the F2 population, the locus *Wf-2* played only a role in plants heterozygous for *Wf*-1, and plants with the *Wf-2gal* allele homozygous were clearly more resistant. In the F3 populations, *Wf-1* was confirmed but, although it is not significantly different, it seems that *Wf-2* is also needed to get complete resistance (Table 7). Maybe this was due to the small sample of the F3 populations but this will be further investigated. The results show that a homozygous introgression with *Wf-1gal* will lead to resistant plants.

So far, there was no QTL detected for whitefly adult survival in tomato. However, QTLs for oviposition rate were found in *S. habrochaites* CGN1.1561 (Maliepaard et al. 1995) and *S. habrochaites* LA1777 (Momotaz et al. 2010). In *S. habrochaites* CGN1.1561, two QTLs for oviposition rate of the greenhouse whitefly (*Trialeurodes vaporariorum*) were detected on Chromosomes 1 (*tv-1*) and 12 (*tv-2*). Besides the different locations of the QTLs, the effect of the QTLs was also less obvious and probably more loci are required to get low levels of oviposition of the greenhouse whitefly in *S. habrochaites* CGN1.1561. In *S. habrochaites* LA1777, QTLs for oviposition rate were detected on Chromosomes 9, 10 and 11 (Momotaz et al. 2010). The QTL on Chromosome 9 was not located in the same region as our *Wf-2*.

Whitefly resistance and trichomes

The QTL for the absence/presence of type I, III, IV and V trichomes and type IV properties such as gland longevity and size co-localized with the resistance QTLs, suggesting an important role of the trichomes in whitefly resistance. This is in agreement with our previous study where whitefly resistance was shown to be dependent on the presence of type I and IV glandular trichomes in several tomato wild relatives (Firdaus et al. 2012). QTLs for trichomes were identified in *S. habrochaites* (Maliepaard et al.

Table 7 Mean of adult survival of parents and F2 and F3 populations grouped based on alleles of *Wf-1* (in Chromosome 2) and *Wf-2* (Chromosome 9)

Adult survival	F2 population			F3 population		
	Chromosome 9			Chromosome 9		
	Wf-2lyc\Wf-2lyc	*Wf-2lyc\Wf-2gal*	*Wf-2lyc2gal\Wf-2gal*	*Wf-2lyc\Wf-2lyc*	*Wf-2lyc \Wf-2gal*	*Wf-2gal\Wf-2gal*
Chromosome 2						
Wf-1lyc\Wf-1lyc	0.91e (22)	0.87de (16)	0.89e (13)	0.99c (5)	0.97c (5)	0.98c (18)
Wf-1lyc\Wf-1gal	0.66cd (16)	0.55c (40)	0.30b (21)	0.59b (15)	0.68b (10)	0.61b (9)
Wf-1gal\ Wf-1gal	0.0a (3)	0.07ab (19)	0.08ab (16)	0.17a (15)	0.49b (8)	0.02a (9)
S. lycopersicum	0.99e (3)			0.99c (6)		
S. galapagense			0.0a (3)			0.0a (6)
Ratio of type IV trichomes (R45)	Chromosome 9			Chromosome 9		
	Wf-2lyc\Wf-2lyc	*Wf-2lyc\Wf-2gal*	*Wf-2gal\Wf-2gal*	*Wf-2lyc\Wf-2lyc*	*Wf-2lyc\Wf-2gal*	*Wf-2gal\Wf-2gal*
Chromosome 2						
Wf-1lyc\Wf-1lyc	0.04a (22)	0.06 a (17)	0.14a (13)	0.0a (5)	0.0a (5)	0.0a (18)
Wf-1lyc\Wf-1gal	0.86b (16)	0.92 b (43)	1.0b (22)	0.69c (15)	0.36b (10)	0.42b (9)
Wf-1gal\Wf-1gal	1.0b (3)	1.0 b (19)	1.0b (18)	1.0d (15)	0.99d (8)	1.0d (9)
S. lycopersicum	0.0a (3)			0.0a (6)		
S. galapagense			1.0b (3)			1.0d (6)

Wf-1lyc and *Wf-2lyc* are alleles of *S. lycopersicum*; *Wf-1gal* and *Wf-2gal* are alleles of *S. galapagense*. Different letters after the mean within the F2 or F3 population show significant differences based on Duncan's multiple range test in level significance of 0.05. Number of plants is indicated in parentheses

1995; Momotaz et al. 2010) and *S. pennellii* (Blauth et al. 1998). In the Maliepaard study, the QTLs for presence of type IV and VI trichomes were not on the same chromosomes as the QTLs for OR, showing that the lower OR of the greenhouse whitefly in *S. habrochaites* was not related to the presence of these trichomes. In the Momotaz et al. (2010) study, the QTLs for oviposition rate of *B. tabaci* and the type IV trichome density did co-localize. Although the resistance was correlated with the density of type IV trichomes, the differences in number, position and effect of the QTLs described in Momotaz et al. (2010) show that the resistance of *S. habrochaites* is quite different from that found in *S. galapagense*.

The presence of glands distinguishes type I/III and type IV/V. The relationship among these trichome types has also been reported by others based on their inheritance and metabolomic profiles (Blauth et al. 1998; McDowell et al. 2011).

In earlier studies (Maliepaard et al. 1995; Momotaz et al. 2010), QTLs were described for type IV density on Chromosome 9, but these QTL were positioned 56.5–59.0 Mb apart from the *Wf-2* QTL. This shows that we found a previously not identified QTL.

The high negative correlation between the presence of type I or IV trichomes and AS shows that these trichomes play an important role in adult survival probably by the production of sticky and/or toxic exudates. Trapped whiteflies were often seen on this kind of trichomes, which was also observed by Toscano et al. (2002). This supports the hypothesis that this resistance mechanism acts before feeding, which may also prevent virus transmission (Rodriguez-Lopez et al. 2012). Negative correlations between type IV trichomes and insect survival were also reported in *S. pennellii*, *S. habrochaites* and *S. pimpinellifolium* (Channarayappa et al. 1992; Dimock and Kennedy 1983; Muigai et al. 2003; Firdaus et al. 2012; Rodriguez-Lopez et al. 2012; Leckie et al. 2012).

No resistant genotype was found without type IV trichomes and the resistance was highly correlated with the presence of type IV trichome density and R45 (Table 3). However, also a number of susceptible genotypes had type IV trichomes and no type V trichomes, showing that the resistance was not only determined by merely the presence of type IV trichomes but also by additional characters such as size, longevity and specific exudates. Similar results were also reported for accessions of *S. habrochaites* (Frelichowski and Juvik 2001; Momotaz et al. 2010). The very low correlation between type VI trichomes and resistance showed that type VI trichomes do not play a role in the resistance mechanism in our mapping population. This was different in *S. habrochaites* where the resistance was associated with type VI trichomes (Chatzivasileiadis and Sabelis 1997; Lin et al. 1987).

The gene involved in formation of type IV trichomes was dominant in the Netherlands and intermediate in Indonesia. An interdependent presence of type IV and V trichomes was also found in mapping populations of *S. lycopersicum* × *S. habrochaites* (Maliepaard et al. 1995; Momotaz et al. 2010; Snyder and Carter 1984) and an intraspecific cross of *S. pennellii* (Blauth et al. 1998). The

Table 8 Metabolites detected by GC–MS analysis and co-localizing with QTLs for adult survival on Chromosome 2 and/or 9

No.	Putative metabolites Identity	QTL description						Alleles		
		Chromosome	Position	LOD	% Explained	Additive	Dominance	*ll*	*gl*	*gg*
1	Unknown (Met 1202)	2	99.5	7.18	18.5	0.4	−0.3	2.1a	2.2a	2.9b
2	Unknown (Met1376)	2	99.5	7.98	20.3	3.0	−2.7	15.1a	15.4a	21.0b
3	Phytol (Met2279)	2	96.1	4.61	12.3	0.3	−0.3	1.9a	1.9a	2.6b
4	Isovaleric anhydride (only spectrum, no RI, Met1319)	2	99.5	17.39	39	8.8	−6.3	13.1a	15.7a	30.8b
		9	54.4	3.46	9.4	4.4	−0.5	14.1a	18.0b	22.0c
5	Unknown (Met2620)	2	92.8	4.47	11.9	−0.1	0.1	1.30b	1.32b	1.18a
		9	45.1	3.12	8.5	0.0	0.1	1.29ab	1.33b	1.22a
6	Heptacosane (Met3988)	2	96.9	6.79	17.5	27.5	−19.1	109.47a	115.46a	157.04b
		9	73.8	4.65	12.4	23.6	−7.9	109.78a	115.17a	144.20b
		3	0.3	5.08	13.4	29.4	−10.1			
7	Dodecanoic acid (Met1160)	2	99.5	11.87	28.6	15.7	−11.7	11.5a	15.5a	43.0b
		6	49.1	3.79	10.2	7.1	−10.9			
8	Dodecanoic acid chloride (Met1332)	2	99.5	17.54	39.3	4.3	−3.2	5.2a	6.2a	13.7b
		6	49.1	3.8	10.2	1.6	−2.6			
9	Tetramethyl-2-hexadecene isomer (Met1411)	9	73.5	3.36	9.1	−2.1	−0.3	16.4b	14.2a	13.2a
10	Neophytadiene isomer I (Met1487)[a]	9	73.5	4.02	10.8	−1967.9	−231.7	17051.4b	15043.9a	13756.2a
11	Tetramethyl-2-hexadecene isomer (Met1593)	9	73.5	4.16	11.2	−26.6	−6.1	202.6b	173.4a	160.5a
12	Neophytadiene isomer II (Met1637)[a]	9	73.5	3.89	10.5	−102.0	−14.6	882.1b	774.6a	711.4a
13	Neophytadiene isomer III (Met1825)[a]	9	73.5	4.19	11.2	−74.4	−7.1	633.5b	555.2a	507.5a
14	Alkane (Met4051)	9	50.6	3.19	8.7	1.5	10.1	26.8a	36.2b	28.9a
15	Unknown (Met1741)	9	73.5	4.28	11.5	−28.5	−12.5	199.3b	167.5a	156.9a
		7	21.2	3.2	8.7	10.2	−33.8			
16	Unknown (Met1953)	9	73.5	3.27	8.9	−10.9	−7.3	93.24b	81.04a	77.38a
		7	16.1	3.33	9.0	4.7	−17.6			

The average of metabolite abundance is followed by letters of least significance difference (LSD) test between group based on alelles of resistant parent (*g*) and susceptible parent (*l*). Different letters indicated significantly different of mean at *p* value less than 0.05

[a] Likely chlorophyll breakdown product

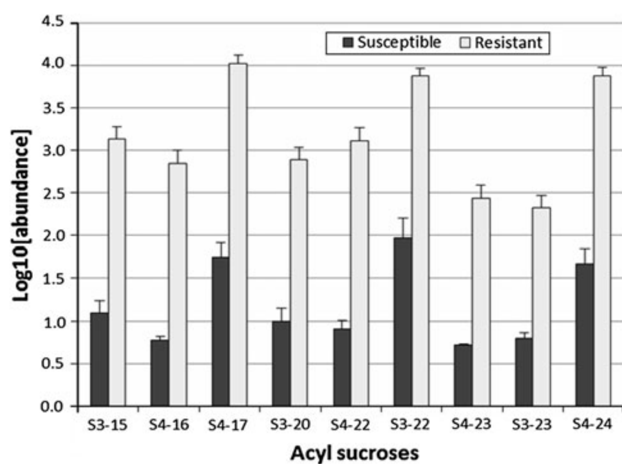

Fig. 3 Abundance of acyl sugars that were significantly different ($p < 0.01$) between resistant and susceptible bulks. The *S* shows sucrose, *number after S* shows number of acyl groups and *number after hyphen* shows total carbon atoms of all the acyl moieties

presence and density of type IV trichomes are known to be influenced by light intensity and leaf age (Wilkens et al. 1996), but other factors like humidity and/or temperature may also play a role in the formation of the different trichomes. Our results show that the presence of type IV trichomes was controlled dominantly by one gene, but there might be interaction of genes for the ratio of type IV/V trichomes (R45). Lenke and Mutschler (1984) found that the inheritance of type IV trichomes in an interspecific cross of *S. lycopersicum* × *S. pennellii* was not complex and segregation patterns indicated that only two unlinked genes were involved. A similar trichome inheritance was also observed in a study with *S. habrochaites* (Freitas et al. 2002).

Metabolites involved in whitefly resistance

Both mono- and sesquiterpenes, methyl ketones and acyl sugars are secondary metabolites that have been associated

with whitefly resistance in tomato (Chatzivasileiadis and Sabelis 1997; Eigenbrode et al. 1994; Fancelli et al. 2005; Farrar and Kennedy 1991; Frelichowski and Juvik 2001; Lin et al. 1987; Mutschler et al. 1996; Oriani and Vendramim 2010; Rodriguez-Lopez et al. 2011). Monoterpenes such as *p*-cymene are abundantly present in *S. pennellii* LA716 and play a role as repellent of *B. tabaci* (Bleeker et al. 2009) and western flower thrips (*Frankliniella occidentalis*) (Janmaat et al. 2002). In our study, we did not find QTLs for monoterpenes on Chromosome 2 and/or Chromosome 9. All eight metabolites with a QTL on Chromosome 2 in our study were present at a higher concentration when the *S. galapagense* allele was present. Phytol, a diterpenoid compound, is one of the putative whitefly resistance-related metabolites. Its role may be related to the fact that it is a precursor of tocopherols (Valentin et al. 2006) that were shown to play a role as anti-herbivory agent (Neupane and Norris 1991). QTLs for the neophytadiene isomers I, II and III, and tetramethyl-2-hexadecene isomers were found on Chromosome 9. These compounds are metabolites resulting from the degradation of phytol (Didyk et al. 1978). Heptacosane is a metabolite that can be produced at high levels in tomato (Srinivasan et al. 2006) and is the main constituent of the leaf cuticle (Reina-Pinto and Yephremov 2009). So far, this compound was reported in studies on the oviposition rate in *Helicoverpa armigera* (Srinivasan et al. 2006) and as attractant of parasitoids (Paul et al. 2008). The neophytadiene isomers I, II and III, and tetramethyl-2-hexadecene isomers had lower relative concentrations in the presence of the *S. galapagense* allele and seem to enhance whitefly susceptibility in the cultivated tomato, but further investigations are needed to elucidate their exact role.

One of the other identified GC–MS metabolites was dodecanoic acid, a free fatty acid that is one of the intermediate metabolites in methyl ketone synthesis. In *S. habrochaites*, a fatty acid can be hydrolysed and decarboxylated into a methyl ketone (Fridman et al. 2005). However, in our population, we did not find any difference in the concentration of methyl ketones between susceptible and resistant genotypes. Therefore, it is not likely that methyl ketones play a role in the whitefly resistance coming from *S. galapagenese*.

Fatty acid compounds are very abundant in glandular trichomes of *S. pennellii* LA716 and are the main constituent in acyl sugar biosynthesis (Blauth et al. 1999; Burke et al. 1987; Mutschler et al. 1996; Schilmiller et al. 2012; Walters and Steffens 1990).

Whitefly resistance was shown to be associated with acyl sugar production in *S. pennellii* (Leckie et al. 2012) and *S. pimpinellifolium* (Rodriguez-Lopez et al. 2011, 2012). Isovaleric acid and dodecanoic acid both have a QTL on Chromosome 2 and might be involved in acyl sugar biosynthesis (Blauth et al. 1999; Walters and Steffens 1990). In an interspecific cross of *S. pennellii* and *S. lycopersicum*, five QTLs related to acyl sugars have been mapped on Chromosomes 2, 3, 4 and 11 (Mutschler et al. 1996) and on Chromosomes 4, 5, 6, 10 and 11 (Leckie et al. 2012). Two of them were located on Chromosome 2 but 5 and 67 cM away from *Wf-1*. Although the position of one of the QTLs was close to *Wf-1*, the effect was different; *Wf-1* has a strong effect on whitefly resistance, whereas the acyl sugar QTL had only a small additive effect on acyl sugar accumulation in *S. pennellii* (Lawson et al. 1997; Mutschler et al. 1996; Leckie et al. 2012). These studies showed that acyl sugar production is controlled by many genes in *S. pennellii*. Unfortunately, we did not map loci for acyl sugar accumulation in our population, but we showed that some acyl sugars were present at much higher abundance in resistant F2 plants than in the susceptible plants (Fig. 3) indicating that the production of acyl sugars may be an important resistance component in *S. galapagense*. It may be expected that also the acyl sugars that are higher expressed in the resistant plants will map to the *Wf-1* and/or *Wf-2* locus.

Prospects for resistance breeding in cultivated tomato based on the *Wf-1* locus

The resistant parent (*S. galapagense*) and susceptible cultivated tomato (*S. lycopersicum*) are close relatives that are grouped within the same clade of the phylogenetic tree (Rodriguez et al. 2009; Peralta et al. 2008). The use of a more closely related species in introgression breeding makes breeding more straight forward because the differences between the parents are smaller, resulting in more easy crosses (Hogenboom 1972). The difference between almost complete resistant plants and almost complete susceptible plants could be traced back to two loci: *Wf-1* and *Wf-2*. Since whitefly resistance, trichome properties and the abundance of specific metabolites all map within these loci, it is possible that a single gene that affects all traits is involved.

The *Wf-1* locus is located at the bottom of Chromosome 2. This region is on the genetic linkage map 7.8 cM and 2.5 Mb on the physical map. In tomato, the gene density in euchromatin region is about 6.7 kb/gene (Wang et al. 2006). Therefore, this region would cover approximately 370 genes and this was confirmed with the known annotated tomato sequence where approximately 360 genes are predicted in this region (http://solgenomics.net). In future studies, we will carry out recombinant screens to further delineate the chromosomal fragment harboring *Wf-1gal*. Better characterisation of the resistance will make a very focussed breeding possible, which may result in new varieties with higher levels of whitefly resistance based on the *S. galapagense* source.

As we have shown in this paper, the resistance mechanism identified in *S. galapagense* is active against whiteflies and likely based on chemicals that are toxic to the insect or prevent it from normal feeding. It is very conceivable that such a mechanism is also functional against other insects, such as aphids, pinworm and thrips. This would make the genetic source even more valuable.

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References

Baldin ELL, Vendramim JD, Lourencao AL (2005) Resistance of tomato genotypes to the whitefly *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae). Neotrop Entomol 34(3): 435–441

Blauth SL, Churchill GA, Mutschler MA (1998) Identification of quantitative trait loci associated with acylsugar accumulation using intraspecific populations of the wild tomato, *Lycopersicon pennellii*. Theor Appl Genetics 96(3–4):458–467

Blauth SL, Steffens JC, Churchill GA, Mutschler MA (1999) Identification of QTLs controlling acylsugar fatty acid composition in an intraspecific population of *Lycopersicon pennellii* (Corr.) D'Arcy. Theor Appl Genetics 99(1–2):373–381

Bleeker PM, Diergaarde PJ, Ament K, Guerra J, Weidner M, Schutz S, de Both MT, Haring MA, Schuurink RC (2009) The role of specific tomato volatiles in tomato–whitefly interaction. Plant Physiol 151(2):925–935

Broekgaarden C, Snoeren TAL, Dicke M, Vosman B (2011) Exploiting natural variation to identify insect-resistance genes. Plant Biotechnol J 9:819–882

Burke BA, Goldsby G, Mudd JB (1987) Polar epicuticular lipids of *Lycopersicon pennellii*. Phytochemistry 26(9):2567–2571

Burton GW (1952) Quantitative inheritance in grasses. Proc Int Grassland Congr 1:277–283

Channarayappa SG, Muniyappa V, Frist RH (1992) Resistance of *Lycopersicon* species to *Bemisia tabaci*, a tomato leaf curl virus vector. Can J Bot (Revue Canadienne De Botanique) 70(11): 2184–2192

Chatzivasileiadis EA, Sabelis MW (1997) Toxicity of methyl ketones from tomato trichomes to *Tetranychus urticae* Koch. Exp Appl Acarol 21(6–7):473–484

De Vos RC, Moco S, Lommen A, Keurentjes JJ, Bino RJ, Hall RD (2007) Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. Nat Protoc 2(4): 778–791

Didyk BM, Simoneit BRT, Brassell SC, Eglinton G (1978) Organic geochemical indicators of paleoenvironmental conditions of sedimentation. Nature 272:216–222

Dimock MB, Kennedy GG (1983) The role of glandular trichomes in the resistance of *Lycopersicon hirsutum* f. *glabratum* to *Heliothis zea*. Entomol Exp Appl 33(3):263–268

Eigenbrode SD, Trumble JT, Millar JG, White KK (1994) Topical toxicity of tomato sesquiterpenes to the beet armyworm and the role of these compounds in resistance derived from an accession of *Lycopersicon hirsutum* f. *typicum*. J Agric Food Chem 42(3): 807–810

Erb WA, Lindquist RK, Flickinger NJ, Casey ML (1994) Resistance of selected interspecific *Lycopersicon* hybrids to greenhouse whitefly (Homoptera: Aleurodidae). Fla Entomologist 77(1): 104–116

Erdogan C, Moores GD, Gurkan MO, Gorman KJ, Denholm I (2008) Insecticide resistance and biotype status of populations of the tobacco whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Turkey. Crop Prot 27(3–5):600–605

Fancelli M, Vendramim JD (2002) Development of *Bemisia tabaci* (Gennadius,1889) biotype B on *Lycopersicon* spp. genotypes. Scientia Agricola 59:665–669

Fancelli M, Vendramim JD, Frighetto RTS, Lourencao AL (2005) Glandular exudate of tomato genotypes and development of *Bemisia tabaci* (Genn.) (Sternorryncha: Aleyrodidae) biotype B. Neotrop Entomol 34(4):659–665

Farrar RR, Kennedy G (1991) Inhibition of *Telenomus sphingis* an egg parasitoid of *Manduca* spp. by trichome 2-tridecanone-based host plant-resistance in tomato. Entomol Exp Appl 60(2): 157–166

Firdaus S, van Heusden AW, Hidayati N, Supena EDJ, Visser RGF, Vosman B (2012) Resistance to *Bemisia tabaci* in tomato wild relatives. Euphytica 187:31–45

Firdaus S, Vosman B, Hidayati N, Supena EDJ, Visser RGF, van Heusden AW (2013) The *Bemisia tabaci* species complex: additions from different parts of the world. Insect Sci 00:1–11. doi:10.1111/1744-7917.12001

Freitas JA, Maluf WR, Cardoso MD, Gomes LAA, Bearzotti E (2002) Inheritance of foliar zingiberene contents and their relationship to trichome densities and whitefly resistance in tomatoes. Euphytica 127(2):275–287

Frelichowski JE, Juvik JA (2001) Sesquiterpene carboxylic acids from a wild tomato species affect larval feeding behavior and survival of *Helicoverpa zea* and *Spodoptera exigua* (Lepidoptera: Noctuidae). J Econ Entomol 94(5):1249–1259

Fridman E, Wang J, Iijima Y, Froehlich JE, Gang DR, Ohlrogge J, Pichersky E (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(4):1252–1267

Hirano K, Budiyanto E, Swastika N, Fujii K (1995) Population dynamics of the whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), in Java, Indonesia, with special reference to spatiotemporal changes in the quantity of food resources. Ecol Res 10(1):75–85

Hogenboom NG (1972) Breaking breeding barriers in *Lycopersicon*. 1. Genus *Lycopersicon*, its breeding barriers and importance of breaking these barriers. Euphytica 21(2):221–227

Janmaat AF, de Kogel WJ, Woltering EJ (2002) Enhanced fumigant toxicity of p-cymene against *Frankliniella occidentalis* by simultaneous application of elevated levels of carbon dioxide. Pest Manag Sci 58(2):167–173

Kehr J (2006) Phloem sap proteins: their identities and potential roles in the interaction between plants and phloem-feeding insects. J Exp Bot 57:767–774

Kennedy GG, Farrar RR, Kashyap RK (1991) 2-Tridecanone glandular trichome-mediated insect resistance in tomato: effect on parasitoids and predators of *Heliothis zea*. ACS Symp Ser Am Chem Soc 449:150–165

Lawson DM, Lunde CF, Mutschler MA (1997) Marker-assisted transfer of acylsugar-mediated pest resistance from the wild tomato, *Lycopersicon pennellii*, to the cultivated tomato, *Lycopersicon esculentum*. Mol Breed 3(4):307–317

Leckie BM, De Jong DM, Mutschler MA (2012) Quantitative trait loci increasing acylsugars in tomato breeding lines and their impacts on silverleaf whiteflies. Mol Breed 30(4):1621–1634. doi:10.1007/s11032-012-9746-3

Lenke CA, Mutschler MA (1984) Inheritance of glandular trichomes in crosses between *Lycopersicon esculentum* and *Lycopersicon pennellii*. J Am Soc Hortic Sci 109:592–596

Lin SYH, Trumble JT, Kumamoto J (1987) Activity of volatile compounds in glandular trichomes of *Lycopersicon* species against 2 insect herbivores. J Chem Ecol 13(4):837–850

Luckwill LC (1943) The genus *Lycopersicon*: an historical, biological, and taxonomic survey of wild and cultivated tomatoes. Aberd Univ Study 120:44

Maharijaya A, Vosman B, Verstappen F, Steenhuis-Broers G, Mumm R, Purwito A, Visser RGF, Voorrips RE (2012) Resistance factors in pepper inhibit larval development of thrips (*Frankliniella occidentalis*). Entomol Exp Appl 145(1):62–71

Maliepaard C, Bas N, van Heusden S, Kos J, Pet G, Verkerk R, Vrielink R, Zabel P, Lindhout P (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

McCollum TG, Stoffella PJ, Powell CA, Cantliffe DJ, Hanif-Khan S (2004) Effects of silverleaf whitefly feeding on tomato fruit ripening. Postharvest Biol Technol 31(2):183–190

McDowell ET, Kapteyn J, Schmidt A, Li C, Kang JH, Descour A, Shi F, Larson M, Schilmiller A, An LL, Jones AD, Pichersky E, Soderlund CA, Gang DR (2011) Comparative functional genomic analysis of *Solanum* glandular trichome types. Plant Physiol 155(1):524–539

Momotaz A, Scott JW, Schuster DJ (2010) Identification of quantitative trait loci conferring resistance to *Bemisia tabaci* in an F2 population of *Solanum lycopersicum* × *S. habrochaites* accession LA1777. J Am Soc Hortic Sci 135(2):134–142

Morales FJ, Jones PG (2004) The ecology and epidemiology of whitefly-transmitted viruses in Latin America. Virus Res 100(1): 57–65

Muigai SG, Bassett MJ, Schuster DJ, Scott JW (2003) Greenhouse and field screening of wild *Lycopersicon* germplasm for resistance to the whitefly *Bemisia argentifolii*. Phytoparasitica 31(1):27–38

Mutschler MA, Doerge RW, Liu SC, Kuai JP, Liedl BE, Shapiro JA (1996) QTL analysis of pest resistance in the wild tomato *Lycopersicon pennellii*: QTLs controlling acylsugar level and composition. Theor Appl Genetics 92(6):709–718

Neupane FP, Norris DM (1991) Alpha-tocopherol alteration of soybean antiherbivory to *Trichoplusia ni* larvae. J Chem Ecol 17:1941–1951

Oriani MA, Vendramim JD (2010) Influence of trichomes on attractiveness and ovipositional preference of *Bemisia tabaci* (Genn.) B biotype (Hemiptera: Aleyrodidae) on tomato genotypes. Neotrop Entomol 39(6):1002–1007

Oriani MAD, Vendramim JD, Vasconcelos CJ (2011) No-choice ovipositional nonpreference of *Bemisia tabaci* (Gennadius) B biotype on tomato genotypes. Scientia Agricola 68(2):147–153

Paul AVN, Srivastavaa M, Durejaa P, Singha AK (2008) Semiochemicals produced by tomato varieties and their role in parasitism of *Corcyra cephalonica* (Lepidoptera: Pyralidae) by the egg parasitoid *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). Int J Trop Insect Sci 28:108–116

Peralta IE, Spooner DM, Knapp S (2008) Taxonomy of wild tomatoes and their relatives (*Solanum* sect. *Lycopersicoides*, sect. *Juglandifolium*, sect. *Lycopersicon*; Solanaceae. Syst Bot Monogr 84:1–186

Reina-Pinto JJ, Yephremov A (2009) Surface lipids and plant defenses. Plant Physiol Biochem 47:540–549

Rodriguez F, Wu F, Ane C, Tanksley S, Spooner DM (2009) Do potatoes and tomatoes have a single evolutionary history, and what proportion of the genome supports this history? BMC Evol Biol 9:191

Rodriguez-Lopez MJ, Garzo E, Bonani JP, Fereres A, Fernandez-Munoz R, Moriones E (2011) Whitefly resistance traits derived from the wild tomato *Solanum pimpinellifolium* affect the preference and feeding behavior of *Bemisia tabaci* and reduce the spread of tomato yellow leaf curl virus. Phytopathology 101(10):1191–1201

Rodriguez-Lopez MJ, Garzo E, Bonani JP, Fernandez-Munoz R, Moriones E, Fereres A (2012) Acylsucrose-producing tomato plants forces *Bemisia tabaci* to shift its preferred settling and feeding site. PLoS One 7(3):e33064

Schilmiller AL, Charbonneau AL, Last RL (2012) Identification of a BAHD acetyltransferase that produces protective acyl sugars in tomato trichomes. Proc Nat Acad Sci USA 109(40):16377–16382

Schuster DJ, Everett PH, Price JF, Kring JB (1990) Suppression of the sweet-potato whitefly on commercial fresh-market tomatoes. Proc Fla State Hortic Soc 102:374–379

Segal G, Sarfatti M, Schaffer MA, Ori N, Zamir D, Fluhr R (1992) Correlation of genetic and physical structure in the region surrounding the I2 *Fusarium oxysporum* resistance locus in tomato. Mol Genetic 231(2):179–185

Snyder JC, Carter CD (1984) Leaf trichomes and resistance of *Lycopersicon hirsutum* and *Lycopersicon esculentum* to spider mites. J Am Soc Hortic Sci 109(6):837–843

Srinivasan R, Uthamasamy S, Talekar NS (2006) Characterization of oviposition attractants of *Helicoverpa armigera* in two solanaceous plants, *Solanum viarum* and *Lycopersicon esculentum*. Curr Sci 90:846–850

Steel RGD, Torrie JH (1980) Principles and procedures of statistics: a biometrical approach, 2nd edn. McGraw-Hill, New York

The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641

Toscano LC, Boiça JAL, Maruyama WI (2002) Nonpreference of whitefly for oviposition in tomato genotypes. Scientia Agricola 59:677–681

Valentin HE, Lincoln K, Moshiri F, Jensen PK, Qi QG, Venkatesh TV, Karunanandaa B, Baszis SR, Norris SR, Savidge B, Gruys KJ, Last RL (2006) The *Arabidopsis* vitamin E pathway *gene5-1* mutant reveals a critical role for phytol kinase in seed tocopherol biosynthesis. Plant Cell 18:212–22

Van Ooijen JW (2009) Software for the mapping of quantitative trait loci in experimental populations of diploid species. In: Kyazma BV (ed) Wageningen, The Netherlands

Van Ooijen JW (2011) Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. Genetic Res 93:343–349

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Walters DS, Steffens JC (1990) Branched-chain amino acid metabolism in the biosynthesis of *Lycopersicon pennellii* glucose esters. Plant Physiol 93(4):1544–1551

Wang Y, Tang X, Cheng Z, Mueller L, Giovannoni J, Tanksley SD (2006) Euchromatin and pericentromeric heterochromatin: comparative composition in the tomato genome. Genetics 172(4): 2529–2540

Wilkens RT, Shea GO, Halbreich S, Stamp NE (1996) Resource availability and the trichome defenses of tomato plants. Oecologia 106(2):181–191