

## Resistance to *Bemisia tabaci* in tomato wild relatives

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**Abstract** *Bemisia tabaci* is one of the most threatening pests in agriculture, particularly in Solanaceous crops such as tomato and pepper that are cultivated in the open field. Pesticide application is often not effective and hazardous to humans and environment. The exploitation of plant natural defenses that are present in wild relatives of tomato, may offer a solution. To evaluate resistance parameters and to identify plant material with high levels of resistance, we screened a number of accessions of tomato wild relatives using three methods; a free-choice test in a screenhouse in

Indonesia, a no-choice test with clip-on cages in a greenhouse and a leaf disc test in a climate-room in the Netherlands. Antibiosis resulting in low adult survival was the major component for resistance in tomato. However, other resistance component(s) may play a role as well. In some accessions there was a change in the resistance level over time. Several resistance parameters used in the different tests were well correlated. The best resistance source was an accession of *Solanum galapagense*, which had not been identified as being resistant in the past. This is of particular interest as this species is closely related to the cultivated tomato, which may facilitate introgression of the resistance component(s). Whitefly non-preference and resistance were associated with the presence of type IV trichomes. Other mechanisms might be involved since some accessions without type IV trichomes showed low nymphal density. The leaf disc test is a good in vitro alternative for the clip-on cage whitefly resistance screening, as shown by the high correlation between the results obtained with this test and the clip-on cage test. This offers breeders the possibility to carry out tests more efficiently.

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### Introduction

*Bemisia tabaci* (Gennadius) is one of the most important pests in agricultural crops worldwide. This

whitefly is responsible for large reductions in crop yield and quality. Consequently, high costs are made for controlling it (Morales 2007). *Bemisia tabaci* causes direct damage by feeding on the phloem sap and it produces honeydew on which sooty molds can grow (Byrne and Miller 1990). This may result in physiological disorders of the plant, such as leaf wilting and irregular ripening of the fruit (Schuster et al. 1990; McCollum et al. 2004). However, the main problem caused by *B. tabaci* is the damage done by the viruses they transmit (Morales and Jones 2004).

Tomato cultivation and production, particularly in tropical countries is highly dependent on pesticides. However, pesticides are hazardous for the environment, growers and consumers. The exploitation of whitefly resistance originating from wild relatives of cultivated tomato is anticipated to be a more sustainable way of controlling whiteflies (Broekgaarden et al. 2011). Different levels of whitefly resistance have been reported for wild relatives of tomato including *S. pennellii*, *S. habrochaites*, *S. habrochaites* f. *glabratum*, *S. pimpinellifolium*, *S. chilense* (Maliepaard et al. 1995; Fancelli and Vendramim 2002; Muigai et al. 2002; Toscano et al. 2002; Muigai et al. 2003; Maruthi et al. 2003; Baldin et al. 2005).

Whiteflies prefer hairy leaves (Toscano et al. 2002), but the presence and density of type IV and VI trichomes has a negative effect on whitefly adult survival and oviposition rate (Channarayappa et al. 1992; Snyder et al. 1998). Exudates of these trichomes play a major role in whitefly resistance (Fancelli et al. 2005). Compounds implicated in whitefly resistance are acyl-sugars (Liedl et al. 1995; Mutschler et al. 1996), methyl-ketones and derivatives of sesquiterpene carboxylic acid (Frelichowski and Juvik 2005), which might act as repellent and/or natural pesticides.

Mapping studies have identified Quantitative Trait Loci (QTLs) for reduced oviposition of *Trialeurodes vaporariorum* on chromosomes 1 and 12 in *S. habrochaites* f. *glabratum* (Maliepaard et al. 1995). Five QTLs for acyl sugars that confer whitefly resistance in *S. pennellii* LA176 were identified on chromosomes 2, 3, 4 and 11 (Mutschler et al. 1996). *Solanum habrochaites* LA1777 was also the source for QTLs showing a reduced egg deposition; these were located on the chromosomes 9, 10 and 12 (Momotaz et al. 2010). However, the combined effects of these QTLs explained only part of the variation present for whitefly resistance (Maliepaard et al. 1995; Lawson

et al. 1997; Momotaz et al. 2010). From these results it was concluded that many genes might be involved in the whitefly resistance. When many genes/regions are involved, the introduction of the resistance trait from the wild relatives into commercial cultivars is often difficult. Therefore, the identification of genes with a major effect on resistance is of utmost importance. Preferably these resistance components should be present in close relatives of the cultivated tomato as introgression of the resistance is easier in these cases (Hogenboom 1972).

Two types of assessments are used to evaluate whitefly resistance: free-choice test and no-choice test (Romanow et al. 1991; Erb et al. 1994). In a free-choice test, whiteflies are given the choice between two or more different hosts of which it is able to choose the most preferred host(s). In a no-choice test, only one host is accessible for the whitefly and whiteflies that cannot feed on it will be hampered in their growth or die. Therefore, both antibiosis and antixenosis, which may result from repellence or attraction of whiteflies, is assessed in free-choice tests, whereas no-choice tests much more assess antibiosis (Baldin and Beneduzzi 2010). Reliable parameters for whitefly resistance assessments are very important. Parameters used to describe resistances are density and/or survival of a particular developmental stage of whitefly including adults, eggs or nymphs (Maliepaard et al. 1995; Fancelli and Vendramim 2002; Maruthi et al. 2003). Those parameters might measure similar or different resistance factors. Furthermore, relationships between resistance parameters and other supposedly related parameters like honeydew production, sooty-mold growth and plant damages have not been evaluated yet. Also, the development of whitefly resistance during the growth of the tomato plant has not been analyzed. Therefore, the objectives of the present study were to evaluate methods and resistance parameters used for whitefly resistance screening and to identify plant material that has high levels of resistance, preferably based on different mechanisms.

## Materials and methods

### Plant and whitefly material

Forty-six accessions of tomato and related wild species were obtained from the Centre for Genetic

Resources (CGN) and the collection of Plant Research International (PRI)—The Netherlands, the Asian Vegetable Research and Development Center (AV-RDC)—Taiwan and PT East West Seed Indonesia (EWSI)—Indonesia. In case clear differences were seen between individuals of one accession, they were considered as different accessions and they were given an individual number. This made the total number of evaluated accessions 52. Twenty-six accessions (Table 1) were screened in 2008 under free-choice condition in a screen house at EWSI, Purwakarta, West Java, Indonesia. Nine accessions (both resistant and susceptible) together with 26 until then unscreened accessions (Table 2) were evaluated under no-choice conditions using clip-on cages at Wageningen UR Plant Breeding, the Netherlands in 2009.

Non-viruliferous silverleaf whiteflies (*B. tabaci* B-biotype), from the collection of the Laboratory of Entomology, Wageningen University—the Netherlands or the Plant-Pathology Department of Bogor Agricultural University—Indonesia, were used for screening.

#### Free-choice test

Twenty-six tomato accessions were evaluated using a free-choice test in an insect proof screenhouse. The experiment was conducted from September until December 2008 at EWSI. The screenhouse protected the plants from out-side insects, heavy rainfall as well as strong sunlight. Seeds were sown in insect-free boxes and moved to clean cages after 1 week. One month later, three plants which had four or more shoots were selected from each accession. Four shoots of each selected plants were grafted onto 2 week old eggplants to avoid root diseases such as *Fusarium* wilt and nematodes. Two weeks after grafting, the plants were transplanted into a four liter-black bucket containing a rice husk and peat moss soil mixture. Four grafted plants originating from one original plant of each accession were placed in a square together on a table one meter above ground level. There were two lines on each table with 35 cm between lines, 20 cm between plants within the line and 100 cm between tables. The plants were supported by a bamboo stick. Branches and flowers were pruned in order to get one main stem. Two amaranth plants were placed in between every accessions (Fig. 1). There were three

replications (derived from three individual plants) for each accession in the screenhouse. Five weeks after grafting, virus-free *B. tabaci* were introduced by placing heavily infested eggplants in the middle of the plants of each accession. During 1 week, the eggplants were shaken twice a day and left without watering. In this way, the whitefly adults were forced to look for other plants because the eggplants desiccated and died after 6 days (Muigai et al. 2003). The whitefly population development was studied by counting the number of adult whiteflies, eggs and nymphs, and also registering the whitefly related parameters including honeydew production, sooty-mold growth and plant damages at three different time points. The first evaluation was carried out on day 8 and 9 after infestation, the second evaluation was on day 22 and 23 and the third evaluation was on day 36 and 37. The number of adult whiteflies was determined by counting directly on the abaxial leaf surface of lateral leaflets on the 3rd or 4th and 7th or 8th leaf from the apex; this direct counting is more reliable (less variance) than beating of the plant and counting in a tray (Gusmao et al. 2005). Egg and nymph numbers were determined on the same leaflets as where the adults were counted. The leaflets were cut from the plant to facilitate egg and nymph counting under a stereo microscope (10×). Also the leaf area was measured. Honeydew production, sooty-mold growth and plant damages were visually scored on a scale of 0–4. Scores used for honeydew production were (0) no honeydew, (1) one to five honeydew droplets on one leaf, (2) honeydew present on two or more leaves, (3) severe honeydew (more than five honeydew droplets per leaflet) present on one or two leaves, (4) severe honeydew present on three or more leaves. Scores for sooty-mold growth were (0) no sooty mold, (1) some sooty mold present on one leaf, (2) sooty mold present on two or more leaves, (3) heavy sooty mold (thick and covering >10 % of leaflet area) present on one or two leaves and the others show no or a bit sooty mold, (4) heavy sooty mold present on three or more leaves. Scores for plant damages were (0) no necrosis or wilting, (1) light necrosis or wilting present of one leaf, (2) light necrosis or wilting present of two or more leaves, (3) heavy necrosis or wilting (>30 % of leaf area) of two leaves but the plant is still growing, (4) heavy necrosis or wilting of three or more leaves and plant growth is inhibited or the plant is dead.

**Table 1** Means of adult whitefly density (whitefly cm<sup>-2</sup>) and egg density (egg cm<sup>-2</sup>) in the resistance screening of tomato accessions under free-choice condition

No.	Accession name	Observation time <sup>a</sup>					
		Adult whitefly density			Egg density		
		1	2	3	1	2	3
1	<i>S. galapagense</i> PRI95004/ PY-8027	0.0 (a) [a]	0.1 (a) [a]	0.0 (a) [a]	0.0 (a) [a]	0.0 (a) [a]	0.5 (a) [a]
2	<i>S. galapagense</i> PRI95004/ PY-8030	0.5 (e) [b]	0.5 (cde) [b]	0.3 (def) [a]	4.4 (d) [a]	8.1 (d) [ab]	8.4 (cd) [b]
3	<i>S. cheesmaniae</i> CGN15916	2.1 (jk) [a]	2.4 (k) [a]	1.4 (o) [a]	69.3 (m) [b]	52.1 (k) [a]	65.2 (i) [ab]
4	<i>S. cheesmaniae</i> CGN24039	0.8 (g) [a]	1.2 (ij) [a]	0.9 (mn) [a]	37.8 (j) [a]	79.7 (l) [b]	55.7 (i) [ab]
5	<i>S. cheesmaniae</i> CGN17086	2.2 (k) [b]	1.2 (j) [a]	0.9 (mn) [a]	60.4 (lm) [c]	14.5 (fg) [b]	9.1 (d) [a]
6	<i>S. arcanum</i> CGN14355	1.8 (j) [b]	0.3 (b) [a]	0.2 (cd) [a]	45.5 (jk) [a]	62.1 (k) [a]	37.7 (gh) [a]
7	<i>S. arcanum</i> CGN15877	0.9 (g) [b]	0.6 (ef) [b]	0.5 (ghi) [a]	9.5 (ef) [a]	12.8 (ef) [ab]	13.1 (e) [b]
8	<i>S. glandulosum</i> CGN15803	1.3 (hi) [c]	0.9 (gh) [b]	0.7 (jkl) [a]	26.3 (i) [a]	23.8 (i) [a]	21.6 (f) [a]
9	<i>S. glandulosum</i> CGN14357	0.5 (e) [a]	0.1 (a) [b]	0.1 (b) [b]	10.2 (ef) [a]	20.3 (hi) [b]	22.1 (f) [b]
10	<i>S. glandulosum</i> CGN14358	1.0 (g) [b]	0.5 (cde) [a]	0.5 (hij) [a]	5.4 (d) [a]	10.9 (e) [a]	9.0 (d) [a]
11	<i>S. habrochaites</i> f. <i>glabratum</i> CGN24035	0.4 (cd) [a]	0.9 (ghi) [b]	0.8 (lm) [b]	5.1 (d) [a]	6.7 (d) [ab]	10.4 (de) [b]
12	<i>S. habrochaites</i> f. <i>glabratum</i> PRI921237	0.2 (b) [a]	0.2 (a) [a]	0.2 (c) [a]	1.3 (b) [a]	2.9 (b) [a]	30.4 (g) [b]
13	<i>S. habrochaites</i> CGN15391	1.4 (i) [a]	3.5 (l) [c]	2.3 (p) [b]	18.8 (h) [a]	62.2 (k) [b]	62.2 (i) [b]
14	<i>S. habrochaites</i> LA1777	0.4 (de) [a]	0.4 (bcd) [a]	0.4 (fgh) [a]	3.0 (c) [a]	34.8 (j) [b]	41.6 (h) [c]
15	<i>S. habrochaites</i> LA1033	0.1 (b) [a]	0.8 (fg) [b]	0.8 (klm) [b]	2.3 (c) [a]	58.5 (k) [b]	54.0 (i) [b]
16	<i>S. lycopersicoides</i> CGN23973	0.3 (c) [a]	1.2 (j) [b]	1.1 (no) [b]	28.1 (i) [a]	35.8 (j) [a]	37.7 (gh) [a]
17	<i>S. lycopersicum</i> PRI91117(control)	1.5 (i) [b]	0.9 (ghij) [a]	0.7 (jkl) [a]	11.3 (f) [a]	39.6 (j) [b]	42.4 (h) [b]
18	<i>S. lycopersicum</i> EWSI24294	2.1 (jk) [b]	0.6 (ef) [a]	0.6 (ijk) [a]	2.6 (c) [a]	40.2 (j) [b]	36.7 (gh) [b]
19	<i>S. lycopersicum</i> EWSI49444	0.5 (de) [a]	0.8 (gh) [b]	0.8 (lm) [b]	4.4 (d) [a]	4.7 (c) [a]	6.6 (bc) [a]
20	<i>S. neorickii</i> CGN15816	0.3 (c) [a]	0.5 (cde) [c]	0.4 (efg) [b]	10.2 (ef) [b]	4.6 (c) [a]	5.4 (b) [a]
21	<i>S. neorickii</i> CGN15815	0.7 (f) [b]	0.4 (bc) [a]	0.3 (de) [a]	14.6 (g) [b]	4.9 (c) [a]	6.2 (bc) [a]
22	<i>S. pennellii</i> CGN23952	3.3 (l)					
23	<i>S. peruvianum</i> CGN17052	1.2 (h) [a]	0.6 (de) [a]	0.5 (ghij) [a]	4.3 (d) [a]	16.8 (gh) [b]	19.1 (f) [b]
24	<i>S. peruvianum</i> CGN17047	2.0 (jk) [b]	0.9 (ghi) [a]	0.8 (klm) [a]	55.0 (kl) [b]	33.0 (j) [a]	42.2 (h) [ab]
25	<i>S. pimpinellifolium</i> CGN14401	0.5 (e) [a]	1.2 (j) [b]	1.2 (o) [b]	8.1 (e) [a]	37.8 (j) [b]	35.8 (gh) [b]
26	<i>S. pimpinellifolium</i> PRI91059	0.7 (f) [a]	1.1 (hij) [b]	0.8 (lm) [ab]	10.9 (f) [a]	12.4 (ef) [a]	13.6 (e) [a]

The mean followed by different letters in the parenthesis within columns are different according to Duncan's multiple range test and different letters in the brackets within lines are different according to Fisher's student test in 0.05 *p*-significance

<sup>a</sup> Observation time: (1) 8 and 9 days after infestation; (2) 22 and 23 days after infestation; and (3) 36 and 37 days after infestation

Data on adults, eggs, nymphs and leaf area in the free-choice test were used to determine adult-whitefly density (number of adult whiteflies/cm<sup>2</sup> of leaf), egg density (number of eggs/cm<sup>2</sup>), and

nymphal density (number of nymphs/cm<sup>2</sup>). Log transformation was used to normalize adult-whitefly density data and ln transformation for egg and nymphal density.

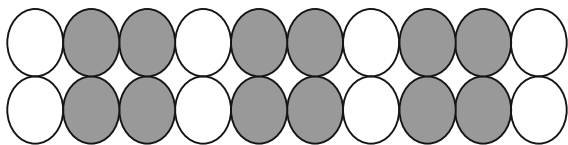
**Table 2** Means of whitefly resistance parameters and type-trichome density in clip-on cage test

No.	Accession name	Whitefly resistance parameters				Trichome density (trichomes/mm <sup>2</sup> )	
		Adult survival	Oviposition rate	Pre-adult survival	Development period	Type IV	Type V
1	<i>S. galapagense</i> PRI95004/PY-8027	0.0 a	0.1 a	0.0 a	No data	30	0
2	<i>S. galapagense</i> PRI95004/PY-8028	0.98 efg	6.9 hij	0.9 hi	22.7 abc	0 <sup>(a)</sup>	40
3	<i>S. galapagense</i> PRI95004/PY-8029	0.97 efg	9.7 klm	0.9 ghi	23.5 hijk	0 <sup>(a)</sup>	19
4	<i>S. galapagense</i> PRI95004/PY-8030	0.96 ef	5.7 gh	0.8 fghi	23.1 def	0 <sup>(b)</sup>	20
5	<i>S. galapagense</i> PRI95004/PY-8031	0.97 ef	5.6 gh	0.8 fghi	23.7 jkl	0 <sup>(a)</sup>	27
6	<i>S. cheesmaniae</i> LA1448	1.0 g	10.7 lm	0.9 hi	23.6 ijkl	0	17
7	<i>S. arcanum</i> CGN15531	0.98 efg	9.5 klm	0.9 ghi	23.6 ijkl	0	35
8	<i>S. arcanum</i> CGN14356	0.99 fg	9.4 klm	0.9 ghi	22.8 bcd	0	34
9	<i>S. arcanum</i> CGN15801	0.97 efg	3.8 ef	0.8 ghi	23.7 jkl	0	2
10	<i>S. arcanum</i> CGN15392	0.99 fg	5.2 fgh	0.3 b	24.5 m	0	45
11	<i>S. arcanum</i> CGN15799	0.97 efg	4.0 fg	0.9 ghi	23.9 l	0	25
12	<i>S. glandulosum</i> CGN14357	0.99 fg	10.1 klm	0.9 ghi	24.0 l	0	56
13	<i>S. habrochaites</i> f. <i>glabratum</i> CGN15792	0.79 bc	0.8 abc	0.5 cde	23.2 efgh	7	7
14	<i>S. habrochaites</i> f. <i>glabratum</i> CGN15879	0.72 b	1.8 cd	0.7 cde	23.3 efghi	3	13
15	<i>S. habrochaites</i> f. <i>glabratum</i> PI134417	0.72 b	0.3 ab	0.4 cd	25.9 n	29	0
16	<i>S. habrochaites</i> f. <i>glabratum</i> PI134418	0.0 a	0.2 a	0.0 a	No data	36	0
17	<i>S. habrochaites</i> f. <i>glabratum</i> PRI921237	0.82 c	1.9 cd	0.6 cd	23.3 fghij	21	10
18	<i>S. habrochaites</i> LA1718	0.78 bc	0.3 ab	0.0 a	No data	5	11
19	<i>S. habrochaites</i> LA4137	0.98 efg	4.3 ef	0.6 cdef	22.6 abc	8	0
20	<i>S. habrochaites</i> LA1777	0.89 d	1.2 bc	0.4 bc	22.6 ab	14	0
21	<i>S. lycopersicum</i> MoneyMaker	1.0 g	6.3 hij	0.9 fghi	23.8 kl	0	30
22	<i>S. lycopersicum</i> PRI91117	1.0 g	5.9 ghi	0.6 cde	23.6 ijkl	0	24
23	<i>S. neorickii</i> CGN15816	0.97 efg	5.1 fgh	0.9 hi	23.1 def	0	43
24	<i>S. neorickii</i> LA2072	0.98 efg	5.2 fgh	0.7 defg	22.5 a	0	31
25	<i>S. neorickii</i> LA2133	0.83 c	2.4 de	0.7 cdef	23.0 cde	25	0
26	<i>S. peruvianum</i> CGN17052	0.94 e	3.6 ef	0.9 ghi	23.6 ijkl	0	16
27	<i>S. peruvianum</i> CGN17046	1.0 g	10.7 lm	1.0 i	23.4 fghij	0	17
28	<i>S. peruvianum</i> PI126928/PY-8037	0.99 fg	8.1 jk	0.9 ghi	23.1 defg	0	44
29	<i>S. peruvianum</i> PI126928/PY-8038	0.99 fg	11.4 m	0.9 hi	23.5 ghijk	0	51
30	<i>S. pimpinellifolium</i> PRI91059	0.98 efg	10.9 lm	0.9 hi	22.9 bcde	0	28
31	<i>S. pimpinellifolium</i> LA1261	0.98 efg	8.6 jkl	0.9 ghi	22.8 abcd	0	32
32	<i>S. pimpinellifolium</i> LA1584/PY-8040	0.71 b	0.6 ab	0.7 efgh	23.3 efgh	21	2
33	<i>S. pimpinellifolium</i> LA1584/PY-8039	0.97 efg	6.7 hij	0.9 ghi	22.8 bcd	0 <sup>(b)</sup>	26
34	<i>S. pimpinellifolium</i> CGN15912	0.97 efg	7.6 hij	0.6 cd	22.8 bcd	0	21
35	<i>S. pimpinellifolium</i> CGN15808	0.99 fg	7.8 ijk	0.9 hi	23.6 hijk	0	20

Mean followed by different letters within columns are different by Duncan's multiple range test in 0.05 *p*-significance

<sup>(a)</sup> Type IV trichomes were not found on the leaf lamina, but a few were present on the stem and leaf petioles

<sup>(b)</sup> A few type IV trichomes were found on the leaf lamina



**Fig. 1** Setup in the screenhouse, four grafts of one individual plant of an accession are grown together (*grey*) and are separated from four plants of the next accession by two amaranth plants (*white*). Whitefly infested leaves were put in between the four grafts

### No-choice tests

No-choice tests were carried out in April and May 2009 by using clip-on cages (Maliepaard et al. 1995) and leaf discs in Wageningen, The Netherlands. Seeds of each tomato accession were sown in peat-moss soil in a sowing box and after the third leaf stage, the plants were transplanted into 1.5 L pots containing peat-moss soil and placed on stainless steel tables in two randomized blocks (compartments) with one plant per accession per block. The temperature in the compartments was set to 20/15 °C (day/night), a 16L:8D photoperiod was used and relative humidity was kept at 70 %. One week before infestation, the temperature was raised gradually until it reached 27/18 °C (day/night) two days before infestation. Two plants per accession were ready to be tested with clip-on cages and leaf discs.

### Clip-on cage test

Plants were infested with whiteflies 6 weeks after sowing. Five synchronized whitefly females (one to two days old) ( $n$ ) were anesthetized with CO<sub>2</sub> and put into a clip-on cage (2 cm in diameter and 1 cm in height) and placed immediately on the abaxial surface of a leaflet of the 3rd or 4th leaf from the apex. Five clip-on cages were attached per plant. 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 (10× magnification). The clip-on cages were reassembled at their original positions on each leaflet before new adult whiteflies started to emerge from the eggs. The emerging adults ( $a_i$ ) were counted and removed from the cages every day ( $t_i$ ) during a week. Pupal cases ( $p$ ) were counted 7 days after the first-emerging adult whiteflies. Adult survival

(AS), oviposition rate (OR), pre-adult survival (PS) and development periods (DP) were calculated by using the equations (Maliepaard et al. 1995) as shown below.

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

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

$$PS = P/e \quad (\text{whiteflies/egg}) \quad (3)$$

$$DP = \frac{(\sum t_i \cdot a_i)}{\sum a_i} \quad (\text{days}) \quad (4)$$

An *Arcsin* transformation was used to normalize data of adult survival and pre-adult survival, and a square-root transformation for oviposition rate and development period.

### Leaf disc test

Nine accessions, selected on the basis of adult survival and oviposition rate in the clip-one cage test were used for the leaf disc test. One week after whitefly infestation in the clip-on cages test, four young leaflets at the 3rd, 4th or 5th node from the top were cut from each accession and put on a petri dish containing 1 % agar and covered with paper which had four symmetrical holes (Fig. 2). Each hole was two cm in diameter and therefore of the same size as the area under the clip-on cage. Twenty synchronized whitefly females (1–2 days old) were anesthetized with CO<sub>2</sub> and placed on the paper, the disc was closed with a cage (eight cm in diameter and six cm in height) containing nylon mesh (for air circulation). The cages were reversed and placed in the climate room at 27 °C, a relative humidity of 70 % and a photoperiod of 16L:8D. Alive and dead whiteflies as well as egg number were counted 4 days after infestation. The test was done in three replications. Adult survival and oviposition rate were calculated and normalized in the same way as in the clip-on cage test.

### Observation of trichomes

Classification and identification of trichome types were made according to Luckwill (1943) based on the morphology and presence of glands. The leaflet opposite of the leaflet with the clip-on cage was taken

from the plant. The trichomes were identified and counted in an area of  $11.11 \text{ mm}^2$  ( $1/3 \times 1/3 \text{ cm}$ ) on the right and left side of main vein at the leaflet base using a stereo dissecting microscope (40–100 times magnification).

### Statistical analysis

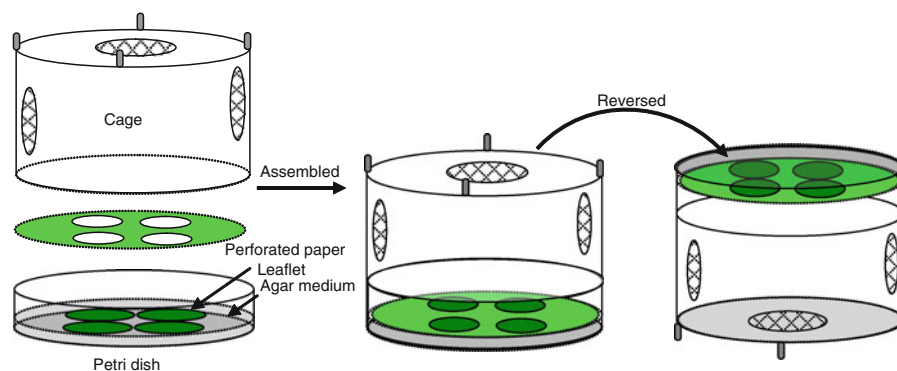
Pearson's  $r$  correlation coefficients were calculated between whitefly parameters in both no-choice and free-choice tests and between whitefly resistance parameters and trichome density in no-choice test. Spearman's  $\rho$  correlation was used to calculate the correlation between honeydew production, sooty-mold growth and plant damages in free-choice test. Data were also subjected to two-way repeated measurement analysis of variance for free-choice test and analysis of variances for no-choice tests. Afterward, mean differentiation by Duncan's Multiple Range Test (DMRT) for accession and Least Significant Difference (LSD) for observation time of each accession. Statistical analysis was conducted with the software package SPSS 17.0 for Windows (SPSS Inc., Chicago, IL).

## Results

### Whitefly resistance in free-choice test

In the free-choice test, several parameters were measured including adult-whitefly, egg and nymphal density at three successive time points as well as honeydew production, sooty-mold growth and plant damage. Table 3 shows the Pearson's  $r$  correlations

between the different parameters. The data underlying these can be found in the Supplementary Tables 1, 2, 3, 4. Significant correlations were named slight if  $r = 0.20\text{--}0.40$  moderate from 0.41 to 0.60, high from 0.61 to 0.80 and very high from 0.81–1.00. Adult-whitefly density was moderately correlated with egg and nymphal density, whereas, egg and nymphal density had a high correlation. Honeydew production was strong correlated with nymphal density, but less to adult-whitefly and egg density (Table 3). Table 1 shows for all accessions the development of adult and egg density over time in the free-choice test. The results of nymphal density, honeydew production, sooty-mold growth and plant damages are presented in the Supplementary Tables 1, 2, 3, 4. Adult-whitefly and egg density on most tomato accessions changed over time. For instance, the number of adults decreased sharply over time for *S. arcanum* CGN14355 and *S. lycopersicum* EWSI24294, whereas, the number of adults increased on most *S. habrochaites* and *S. pimpinellifolium* accessions. Furthermore, egg density also decreased on *S. cheesmaniae* CGN17086, *S. neorickii* CGN15816 and *S. neorickii* CGN15815, whereas egg density for all *S. habrochaites* accessions developed the other way around. The changes mostly occurred between the first and second observation time and less between the second and third. The change can also be seen from the correlation between the three time points. The correlation of adult-whitefly density between first and second observation time was 0.454 ( $N = 75$ ), between first and third observation time 0.413 ( $N = 75$ ) and the correlation between second and third observation time was 0.931 ( $N = 75$ ). The correlations between observations for the other parameters are presented in Supplementary Table 5.



**Fig. 2** Experimental setup for the leaf disc test



**Table 3** Pearson's  $r$  correlation between parameters used in the whitefly resistance screening of tomato accessions in the free-choice experiment

Parameters	WD	ED	ND	HD	SM	DM
Whitefly density (WD)		0.48** (228)	0.43** (225)	0.38** (225)	0.35** (228)	0.24** (228)
Egg density (ED)			0.86* (225)	0.49** (225)	0.20** (228)	0.35** (228)
Nymphal density (ND)				0.62** (225)	0.26** (225)	0.47** (225)
Honeydew (HD)					0.30** (234)	0.50** (234)
Sooty mold (SM)						0.61** (234)
Plant damages (DM)						

\*\* Correlation is significant at the 0.01 level (two-tailed)

\* Correlation is significant at the 0.05 level (two-tailed)

*Solanum galapagense* PRI95004/PY-8027 showed the lowest adult density at all time points. *S. habrochaites* f. *glabratum* PRI921237 behaved similarly, whereas other accessions such as *S. habrochaites* LA1033 showed only a low adult density at the first observation point but not at the other two. For *S. glandulosum* CGN14357 this was the opposite.

The lowest egg density at all time-points was also found on *S. galapagense* (PRI95004/PY-8027). Some accessions were less preferred at a particular developmental stage. For instance, on all *S. habrochaites* f. *glabratum* accessions there were fewer eggs at the first and second observation point compared to the third. On *S. habrochaites* LA1777, LA1033 and *S. lycopersicum* EWSI24294 the number of eggs was low at the first observation point, compared to observations 2 and 3. For *S. lycopersicum* EWSI49444, *S. neorickii* CGN15816 and CGN15815 it was the opposite; they had fewer eggs during the second and third observation.

#### Whitefly resistance in no-choice tests

Two types of no-choice tests were used; clip-on cage and leaf disc tests. The results of the clip-on cage test are shown in Table 2. Pearson's correlation coefficient between adult survival (AS) and oviposition rate (OR) was 0.726 ( $N = 315$ ), between AS and pre-adult survival (PS) was 0.591 ( $N = 315$ ) and between OR and PS was 0.623 ( $N = 288$ ). The development period (DP) did not correlate with AS, OR or PS ( $-0.12$  to  $-0.07$ ;  $N = 288$ ). Adult survival, OR, PS and DP were not significantly different between the two blocks used and among replications. However, PS was different between the two blocks ( $p$  value  $<0.05$ ). There were

significant differences among tomato accessions for all parameters ( $p$  values  $<0.01$ ) for instance the adult survival ranged from 0.0 to 1.0 (Table 2). *Solanum pimpinellifolium* LA1584/PY-8040 and four *S. habrochaites* accessions were slightly less resistant than the two most resistant accessions (*S. galapagense* PRI95004/PY-8027 and *S. habrochaites* f. *glabratum* PI134418). Oviposition rate ranged from 0.1 to 11.4 eggs female<sup>-1</sup> day<sup>-1</sup>. Six accessions (*S. galapagense* PRI95004/PY-8027, *S. habrochaites* f. *glabratum* PI134418, PI134417, CGN15792, *S. habrochaites* LA1718 and *S. pimpinellifolium* LA1584/PY-8040), with a low AS, were also having the lowest OR (Table 2). Three accessions (*S. galapagense* PRI95004/PY-8027, *S. habrochaites* f. *glabratum* PI134418 and *S. habrochaites* LA1718) which were low in AS and OR, were also low in PS. The development period ranged from 22.5 to 25.9 days. The shortest DP was found on *S. neorickii* LA2072, *S. pimpinellifolium* LA1261, *S. galapagense* PRI95004/PY-8028, *S. habrochaites* LA4137 and LA1777.

In the leaf disc test we compared 9 accessions to the clip-on cage test. The resistance levels observed for the resistant (*S. galapagense* PRI95004/PY-8027, *S. habrochaites* f. *glabratum* PI134417 and PI134418, and *S. pimpinellifolium* LA1584/PY-8040), moderately resistant (*S. habrochaites* f. *glabratum* CGN15879 and *S. habrochaites* LA1718) and susceptible accessions (*S. galapagense* PRI95004/PY-8028, *S. peruvianum* PI126928/PY-8038 and *S. lycopersicum* MM) were similar in the leaf disc and clip-on cage tests (additional Table 6). This was also clear from the high correlation between the leaf disc and clip-on cage tests ( $R = 0.88$  for AS and  $R = 0.93$  for OR).



**Table 4** Pearson correlation between parameters in free-choice and no-choice tests ( $N = 9$ )

Parameters	Observation time	Clip-on cage test			
		AS	OR	PS	DP
Adult whitefly	1	0.79*	0.55	0.54	0.66
	2	0.59	0.45	0.47	0.44
	3	0.70*	0.46	0.52	0.63
Egg density	1	0.87**	0.87**	0.79*	0.66
	2	0.74*	0.42	0.33	0.65
	3	0.64	0.22	0.18	0.74*
Nymphal density	1	0.76*	0.81**	0.80**	0.59
	2	0.81*	0.54	0.48	0.68*
	3	0.86**	0.48	0.46	0.84**

\*\* Correlation is significant at the 0.01 level (two-tailed)

\* Correlation is significant at the 0.05 level (two-tailed)

### Correlation between no-choice and free-choice tests

Nine accessions were tested in both free-choice and clip-on cage tests. Pearson correlation between parameters in free-choice and no-choice tests can be seen in Table 4. Adult survival correlated with adult-whitefly, egg and nymphal density at all time-points. On the other hand, OR and PS highly correlated with egg and nymphal density at the first observation time only.

### Trichome diversity and its relationship to whitefly resistance parameters

Of the seven types of trichomes (I–VII) type IV and/or V trichomes were predominantly present on the abaxial leaf surface. Type I, III and VII were mostly absent. Type VI was present on the abaxial side of the leaves of all accessions, but in low numbers. It was frequently found on stem and leaf petioles. Trichome type I and/or III were found on the stem of the plant, leaf petiole and on the veins and only rarely on the adaxial leaf surface. The number of type IV and V trichomes was different among the tomato accessions (Table 2). The occurrence of type IV trichomes ranged from 0.0 to 36.2 trichomes/cm<sup>2</sup>. All accessions which were resistant as shown by low AS, OR and PS had type IV trichomes causing a high correlation between type IV trichomes and whitefly resistance parameters (Table 5). Most susceptible accessions had many type V trichomes and no type IV trichomes, which also shows from the correlation between susceptibility and resistance with presence of trichomes V and IV respectively (Table 5). However, three accessions, *S.*

**Table 5** Pearson's  $r$  correlation between parameters in non-choice test with different types of trichomes ( $N = 140$  for adult survival, oviposition rate and pre-adult survival; and  $N = 128$  for developmental period)

Parameters	Trichome density		
	Type IV	Type V	Type VI
Adult survival	-0.82**	0.67**	-0.42**
Oviposition rate	-0.79**	0.75**	-0.30**
Pre-adult survival	-0.64**	0.60**	-0.24**
Developmental periods	0.07	0.03	0.06

\*\*Correlation is significant at the 0.01 level (two-tailed)

\*Correlation is significant at the 0.05 level (two-tailed)

*arcanum* CGN 14355 and *S. glandulosum* CGN14358, which were evaluated in free-choice test, and *S. arcanum* CGN15392 which was only evaluated in the clip-on cage test, did combine the absence of type IV trichomes with whitefly resistance.

## Discussion

### Parameters for whitefly resistance assessments

#### *Whitefly developmental stages as parameter for resistance*

In the initial stage of a whitefly infestation the adults have to choose a host plant for feeding and/or oviposition. Selection of the host plant may depend on several factors such as leaf architecture and color (Sippell et al. 1987), leaf pubescence and trichome type and density (McAuslane 1996; Snyder et al. 1998;

Toscano et al. 2002), cuticle thickness (Channarayappa et al. 1992) and compounds that play a role in repelling or attracting whiteflies (Chermenskaya et al. 2009). Subsequent stages of the whitefly development depend on the initial selection or survival of the adults. Therefore the different stages in the whitefly development may be correlated in a free-choice test and this is also what we observe (Table 3). However, some of these resistance parameters are much stronger correlated than others. Although very significant, we see a correlation of only 0.48 between whitefly density and egg density on a host plants whereas egg density and nymph density are highly correlated (0.86). Lower correlation between adult density and egg or nymphal densities shows that resistance factors in adult density and egg/nymphal density may be different.

The high correlation between egg and nymphal densities suggest that egg hatching is not influenced. All nymphal stages including instar 1 to instar 4 were observed, so oviposition (egg density) was apparently affected by an antibiosis and/or preference factor(s) which were recognized by the adult female. This hypothesis was already proposed by van Lenteren and Noldus (1990) and Nomikou et al. (2003) who suggest that oviposition preference and host plant selection by the female whitefly has a profound effect on the fitness of its offspring.

Adult and egg density in free-choice test, especially at the beginning of the infestation, may be influenced by preference factors. However, the high correlation between whitefly density in the free-choice test and adult survival in no-choice test (Table 4), which much more assesses antibiosis than antixenosis, points at antibiotic factors as the main cause for the differences. Only the first observation showed a high correlation of egg and nymphal densities in the free-choice test and oviposition rate and pre-adult survival in the no-choice test (Table 4). However, the correlation is only there for egg and nymphal density. This observation time was relatively similar with that for oviposition rate and pre-adult survival in clip-on cage test; plants were about 6 weeks old. The poorer correlations for the other time points may therefore be caused by the different development changes of the host plant, which may affect resistance. The level of resistance of some accessions increased whereas for others the level decreased (Table 1).

Adult survival in the no-choice test highly correlated with other parameters determined in the same

test and also with all parameters in the choice test (Table 4). This strongly suggests that factor(s) affecting adult survival are the major factor in tomato defense. Antibiotic agents, such as acyl-sugar and methyl ketones, have been identified affecting insect growth in some tomato wild relatives (Lin et al. 1987; Liedl et al. 1995).

The development period was not correlated to the other parameters. This indicates that it is regulated by other mechanisms and in our studies it is not playing a major role in whitefly resistance. Also others showed the developmental period was not simply linked to adult survival, oviposition rate and pre-adult survival (Romanow et al. 1991; Bas et al. 1992; van Giessen et al. 1995).

#### *Correlation between honeydew production, sooty mold growth, plant damage and whitefly resistance*

Whiteflies produce honeydew (Blua and Toscano 1994) which contains several sugars and amino acids (Byrne and Miller 1990), that are good substrates for sooty-mold growth (McCollum et al. 2004). Whitefly infestation and sooty-mold growth were found to result in physiological disorder and plant damages (Morales 2007). Our results show that only nymphal density correlates with honeydew production (Table 3), although both adult whiteflies and nymphs produce honeydew (Blua and Toscano 1994). The high correlation is most likely due to the fact that honeydew production of nymphs is much more regular than that of adults. Plant damage did slightly to moderately correlate with resistance parameters as well as honeydew production, and it highly correlated with sooty-mold growth (Table 3). Sooty mold growth contributes in several ways to the plant damage as it inhibits light transmission into leaf tissue which result in reducing photosynthesis and physiological disorders (Filho and Paiva 2006; Morales 2007).

#### *Leaf disc test for whitefly resistance assessment*

Adult survival and oviposition rate in the leaf disc test is highly correlated with adult survival and oviposition rate in the clip-on cage test. Therefore, leaf disc test may be a good alternative for whitefly resistance assessment using the clip-on cages. As an in vitro test, the leaf disc test has some advantages. It allows conducting the test in a more controlled environment,

less space is needed and it is safer especially when there are viruses involved in the experiment. It is also possible to carry out the test in a free-choice situation. However, some improvements such as the addition of appropriate nutrition and antifungal agents are needed when one would like to assess the whole whitefly life cycle. The fact that we find a high correlation between the clip-on cage (in vivo test) and the leaf disc test (in vitro test) suggests that detaching or wounding tomato leaves does not or slightly effect on the resistance. Similar results were reported for an in vitro test used to screen for thrips resistance in pepper (Maharajaya et al. 2011).

Whitefly resistance and preference in accessions of tomato wild relatives

*Level of whitefly resistance and preference in tomato accessions*

The results show accession dependent responses to the whiteflies in the no-choice and choice test. Some accessions were fully resistant, whereas others were completely susceptible (Tables 2, 3). One of the most striking examples was accession PRI95004. This *S. galapagense* (syn: *Lycopersicum cheesmanii* f. *minor*) accession is derived from the a genetically heterogeneous *S. galapagense* accession. Both morphological characters and trichome types varied between individuals of this accession. In total five different homogenous groups were found, of which PY-8027 and PY-8030 are shown (Tables 1, 2). *Solanum galapagense* PY-8027, was highly resistant with high density of type IV trichomes. The four others were susceptible and were lacking the high density of type IV trichomes, like PY-8030. The resistant selection PY-8027 gave no adult survival and almost no oviposition in the no-choice test and was not preferred in the free-choice tests. The accession has never before been reported to be resistant to *B. tabaci*. *Solanum galapagense* is genetically close to commercial tomato (Perralta et al. 2008) which may make it easier to use in commercial breeding programs. After testing with *Keiferia lycopersicella* (Walsingham) less damage and lower numbers of larvae were found on another *S. galapagense* accession (Schuster 1977).

The level of whitefly resistance in the *S. habrochaites* accessions was variable. This species has been exploited as resistance source to several pests (Lin

et al. 1987; Eigenbrode and Trumble 1993; Momotaz et al. 2010). In our no-choice test *S. habrochaites* LA 1718 showed some level of resistance (Table 2), due to a low oviposition rate. In the choice assay (Table 1) LA1777 and LA1033 showed resistance only in the beginning of whitefly infestation and they became more susceptible over time. Previous research showed that LA 1777 was less preferred in a choice test (Muigai et al. 2003) and less virus incidence was detected after infestation by viruliferous whitefly (Maruthi et al. 2003). In our evaluation most accessions of *S. habrochaites* f. *glabratum* were not preferred by whitefly with PI134418 being the most resistant accession. Our results confirm earlier results (Toscano et al. 2002; Fancelli and Vendramim 2002; Muigai et al. 2003; Baldin et al. 2005).

*Solanum pimpinellifolium* LA1584 also showed heterogeneity within the accession. Resistance observed was due to a low adult survival and oviposition rate. This accession was also reported as resistant due to low nymphal survival (Fancelli and Vendramim 2002), but it was preferred in a free-choice test (Baldin et al. 2005). Some other accessions from *S. arcanum*, *S. glandulosum*, *S. lycopersicum* and *S. neorickii* showed partial resistance for adult density, egg density or pre-adult survival. From those accessions, only *S. arcanum* was reported to be partially resistant to whitefly (Channarayappa et al. 1992; Muigai et al. 2003).

*Whitefly resistance changes over time*

The number of whiteflies that can be sustained by an accession depends on the suitability of the host as food resources (Hirano et al. 1995), resistance levels (antibiosis) and microclimatic factors (Horowitz 1986). Resistance of most accessions changed between the first and second observation (Table 1). On the other hand, only minor changes occurred between the second and the third observation time for most accessions. These results show that successful whitefly colonization on a new host is largely dependent on host suitability at the time the first infestation takes place. During that period, interactions between the host plant and phloem-feeding insects occur that may change host plant suitability (Broekgaarden et al. 2010). The interaction can increase the resistance in the host plant (induction) or decrease it (suppression) (Broekgaarden et al. 2007). Bas et al. (1992) also

observed resistance differences between younger and older plants. Effects of tomato age and infestation time were also reported in the resistance of tomato plants against potato moth (*Phthorimata operculella*) (Gurr and McGrath 2001).

#### Influence of trichome types on whitefly preference and resistance

Trichomes have been considered as the most important pest resistance factor. Seven types of trichomes are known in tomato of which type I, IV, VI and VII are glandular trichomes, and type II, III and V are non-glandular trichomes (Gurr and McGrath 2001; Simmons and Gurr 2005).

The presence, density and distribution of the trichome types depends on the tomato genotype, organs/tissue, age and environmental conditions (Wilkins et al. 1996; Gurr and McGrath 2001; Kang et al. 2010). *Solanum galapagense* has no type II and III, few type I, VI and VII, very few type V, but very abundant type IV trichomes (Simmons and Gurr 2005; Simmons et al. 2005). In our results type IV trichomes are present on the most resistant *S. galapagense* accession. Also *S. habrochaites* and *S. habrochaites* f. *glabratum* had high densities of type IV and VI trichomes (Eigenbrode and Trumble 1993; Simmons and Gurr 2005).

From our results it is clear that the most resistant and not preferred tomato accessions had a high density of glandular type IV trichomes. Other researchers also reported that the presence of this trichome type highly correlated with resistance to whiteflies and other pests (Dimock and Kennedy 1983; Channarayappa et al. 1992; Snyder et al. 1998; Muigai et al. 2003). Although glandular trichomes seem to play an important role in whitefly resistance, it is actually the compounds within the trichomes that are decisive. For instance, *S. habrochaites* LA 1777 and LA 1033, have a similar density of type IV trichomes, but they differ in resistance to *Helicoverpa zea* and *Spodoptera exigua* and in the constitution of trichome exudates (Frelchowski and Juvik 2001). Examples of such exudates are methylketones such as 2-tridecanone and 2-undecanone which are present at high concentrations in type IV and VI of trichomes and are believed to have an insecticidal effect on several arthropods (Lin et al. 1987; Kashyap et al. 1991; McDowell et al. 2011). Glandular trichomes can also produce zingiberene and sesquiterpene compounds which play role as

repellence (Maluf et al. 2001; Bleeker et al. 2009; Kang et al. 2010). Different compounds were identified in *S. pennellii* and *S. pimpinellifolium* type IV trichomes. Here, the type IV trichomes contain a high amount of acyl-sugars which make the trichomes sticky (Liedl et al. 1995; Mutschler et al. 1996; Fancelli et al. 2005; Rodriguez-Lopez et al. 2011). Importance of the trichome content is also shown by the fact that the metabolite content of different types of trichomes within an accession/species is more similar than the same type of trichome from different accessions/species of tomato (McDowell et al. 2011).

In contrast to glandular trichomes, non-glandular trichomes, especially type V, are not involved in pest resistance. Whiteflies prefer hairy leaf (Toscano et al. 2002). Non-glandular trichomes provide also a more suitable microclimate for oviposition and protects the eggs and larvae from their enemies (Butter and Vir 1989). A glandular trichome-based resistance mechanism is not the only mechanism in tomato to get whitefly resistance. Whitefly resistance was also found in accessions without glandular trichomes such as in *S. arcanum* CGN14355 and CGN15392, and *S. glandulosum* CGN14358. Other mechanisms such as leaf-surface hardness and cuticle thickness or mesophyll-leaf compounds may play a role in the whitefly resistance mechanism as well. Thick cuticles cannot be pierced by the whitefly's stylet (Janssen et al. 1989).

#### Conclusions

Correlations of parameters within and between free-choice and no-choice tests show that antibiosis is the major factor for whitefly resistance in tomato accessions. Leaf disc tests are an alternative in vitro method that can be used for whitefly resistance screening. Whitefly resistance level of tomato accessions varied and can change over time. *Solanum galapagense* PRI95004/PY-8027, which is closely related to commercial tomato, is highly resistant to whitefly over time. Some other accessions from *S. habrochaites* f. *glabratum*, *S. pimpinellifolium*, *S. arcanum* and *S. glandulosum* showed partial resistance. These accessions are potential sources for resistance factor(s), which may be exploited in breeding programs in tomato aimed at whitefly resistance.

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