

Resistance to *Bemisia tabaci* biotype B of *Solanum pimpinellifolium* is associated with higher densities of type IV glandular trichomes and acylsugar accumulation

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Abstract

Advances in tomato breeding for pest resistance have been achieved via gene introgression from wild *Solanum* (section *Lycopersicon*) species (Solanaceae). Ninety-nine F₃ families derived from an interspecific cross using as parental lines *Solanum lycopersicum* L. ‘LAM-148’ (susceptible standard) and *Solanum pimpinellifolium* L. ‘TO-937-15’ (multiple pest resistance accession with type IV glandular trichomes and acylsugar accumulation) were evaluated for their resistance against the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotype B in free-choice and no-choice tests for oviposition and adult colonization. The parental lines and eight F₃ families with contrasting levels of resistance against the whitefly were selected and investigated in additional assays, which included the estimation of trichome densities and foliar acylsugar levels. The F₃ families BTR-302 and BTR-331 exhibited low amounts of eggs of whitefly and transgressive segregation for type IV glandular trichome density with values greater than that of TO-937-15 plants. However, the tested families did not surpass the total foliar acylsugar content found in TO-937-15. BTR-331 exhibited low colonization in the free-choice test and it was the least preferred F₃ family in the no-choice test. The higher resistance levels of BTR-331 were associated with a positive combination of higher type IV trichome density and higher acylsugar levels. Some F₃ families displayed reduced fruit set due to the presence of flowers with style exertion of the antheridial-cone. Fruit weight at harvest stage of the selected families (from 4.9 to 14.5 g) was lower than that of LAM-148 (139.5 g) but higher than that of TO-937-15 plants (1.3 g). Therefore, although difficult to reach due to the simultaneous segregation of many polygenic traits, the combination of high *B. tabaci* resistance levels with superior horticultural traits is feasible. These results confirm TO-937-15 as a source of biotype B resistance. From the breeding standpoint, the genetic similarity between *S. lycopersicum* and *S. pimpinellifolium* would allow a more efficient resistance introgression by facilitating recombination and minimizing the potentially undesirable linkage drag associated with this trait.

Introduction

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most important tomato [*Solanum lycopersicum* L. (Solanaceae)] pests throughout tropical and subtropical regions (Hanssen et al., 2010). *Bemisia*

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tabaci is currently recognized as a complex of related cryptic species and biotypes with a wide host range, which includes vegetable, ornamental, and field crops as well as non-cultivated and weed species (De Barro et al., 2011). Whitefly control is difficult as this insect can reach high population densities in a short period of time due to its high fecundity and short life cycle, especially when colonizing susceptible host plants under favorable environmental conditions (De Barro et al., 2011).

Nymphs and adults of *B. tabaci* might cause either direct or indirect damage to tomato plants. Whiteflies can induce physiological disorders such as irregular fruit ripening and imbalance in the vegetative and reproductive development. Indirectly, whitefly colonization is associated with honeydew excretion on the host leaf surface that provides a substrate to black mold mycelium development. However, the worst damage by *B. tabaci* is done in its role as a vector of several viral species of the genus *Begomovirus* (Geminiviridae), which can induce very destructive diseases (Hanssen et al., 2010). Yield losses can reach up to 60% in early begomovirus-infected susceptible tomato cultivars (Giordano et al., 2005a).

The resistance to *B. tabaci* reported in some wild *Solanum* accessions is associated with the presence of specific glandular trichome types and with secondary compounds that are produced and stored into the glandular trichome vesicles. The trichomes of wild and domesticated *Solanum* species (section *Lycopersicon*) are categorized into glandular (I, IV, VI, and VII) and non-glandular (II, III, V, and VIII) types (Luckwill, 1943; Channarayappa et al., 1992; Peralta et al., 2008). Some accessions of the wild species *Solanum habrochaites* Knapp & Spooner exhibit resistance to *B. tabaci* and to the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), due to the presence of type VI glandular trichomes and the accumulation of methyl-ketones (e.g., 2-tridecanone and 2-undecanone) (Channarayappa et al., 1992; Heinz & Zalom, 1995; Maliepaard et al., 1995; Fancelli et al., 2008). Some *Solanum pennellii* Correll accessions have type IV glandular trichomes and secrete acylsugars, which are related to the resistance against a wide range of herbivores, including *B. tabaci* biotype B (Gentile et al., 1968; Goffreda et al., 1989; Lawson et al., 1997; Freitas et al., 2002; Maluf et al., 2010). Likewise, selected lines derived from *Solanum pimpinellifolium* L. accession 'TO-937' have shown resistance against a wide spectrum of pests due to the presence of type IV glandular trichomes and acylsugar accumulation as a result of repellence, mortality, and adverse developmental effects (Fernández-Muñoz et al., 2000; Alba et al., 2009; Escobar et al., 2010), including the Mediterranean *B. tabaci* biotype Q (Rodríguez-López et al., 2011).

Although resistance genes against begomovirus infection have already been introgressed from wild species into cultivated tomatoes (Giordano et al., 2005b; Boiteux et al., 2007; García-Cano et al., 2008), the whitefly resistance identified in many wild species has not yet successfully been incorporated into elite tomato lines. The resistance detected in *S. habrochaites* accessions is associated with undesirable sensorial attributes of the fruits (Fancelli et al., 2008). On the other hand, the introgression of the acylsugar-mediated resistance detected in *S. pennellii* accessions has been a difficult process due to linkage with some undesirable agronomic traits such as delayed and reduced fruit set, small fruits, and poor seed germination (Lawson et al., 1997). These repulsion phase linkages are more difficult to be broken because recombination frequencies vary over the distinct genome regions and are dependent upon the phylogenetic distances between the parental accessions. Genetic recombination is often greatly reduced in taxonomically distant tomato species (Bonnema et al., 1997). In this scenario, introgression breeding programs of the type IV trichome/acylsugar-mediated resistance derived from *S. pimpinellifolium* (Fernández-Muñoz et al., 2000; Alba et al., 2009; Rodríguez-López et al., 2011) appears to be more advantageous than other wild *Solanum* sources as this species is closely related to the cultivated tomato (Tomato Genome Consortium, 2012).

The main objective of the present work was to characterize the oviposition preference, attraction of adults, and developmental impacts on *B. tabaci* (Middle East Asia Minor 1 species, biotype B) in tomato progenies derived from the interspecific cross between the multiple pest resistant accession of *S. pimpinellifolium* TO-937-15 and one highly whitefly susceptible *S. lycopersicum* line (LAM-148) from the variety group 'Santa Cruz' or 'Chonto'. Additionally, densities of glandular trichomes and the levels of acylsugars were quantified and used to correlate these traits with the biological performance of the host genotypes regarding their interaction with *B. tabaci*. The development of elite Santa Cruz/Chonto lines with resistance against *B. tabaci* will provide a significant agronomic and environmental contribution for integrated pest management programs in many tomato-producing regions in South America, where this variety group is widely cultivated (Melo et al., 2009).

Materials and methods

Bemisia tabaci biotype B colony

Begomovirus-free *B. tabaci* biotype B colonies were reared on cabbage [*Brassica oleracea* L. var. *capitata* (Brassicaceae)] and cucumber [*Cucumis sativus* L. cv. Curumin

(Cucurbitaceae)] plants. These host plants were cultivated in 2-l plastic pots filled with commercial solid substrate, which was composed of pine bark, vermiculite, rice husk, coconut fiber, and nutrients (Bioplant Prata HT[®]; Bioplant Agrícola, Nova Ponte, Brazil). These plants were kept in a greenhouse (5 m long × 4 m high × 4.5 m wide) at 26.8 ± 1 °C and $59.8 \pm 5\%$ relative air humidity. A set of distinct bioassays was carried out under greenhouse, protected house, and growth chamber conditions at the National Center for Vegetable Crops Research (CNPq)/Embrapa Hortaliças, Brasília, DF, Brazil.

Parental tomato lines and development of the F₃ families

The F₃ families under evaluation were derived from the following interspecific cross: *S. lycopersicum* LAM-148 (= female parent; domesticated Santa Cruz/Chonto tomato type; standard for *B. tabaci* susceptibility) × *S. pimpinellifolium* (= male parent with wild tomato species characteristics, standard for *B. tabaci* resistance). The whitefly resistant line (TO-937-15) was previously obtained in Spain after selection for production of high levels of acylsugars within one multiple pest resistant *S. pimpinellifolium* TO-937 accession (Fernández-Muñoz et al., 2003; Escobar et al., 2010; Rodríguez-López et al., 2011). Ninety-nine F₃ families were obtained by selfing individual plants of the F₂ generation. These families were codified with the acronym BTR (*Bemisia tabaci* resistance). The two parental lines and 99 F₃ families (n = 101 genotypes) were initially used in the experiments. The parental lines LAM-148 and TO-937-15 were employed in the assays as standards for whitefly susceptibility and resistance, respectively. The seedlings were produced in polystyrene trays (68 × 34 cm; 128 cells, each 3.6 cm wide × 6.0 cm deep; Tecnocell[®], Joinville, SC, Brazil) filled with commercial solid substrate (Bioplant Prata HT[®]). The trays were kept in a greenhouse free of whitefly infestation with daily irrigation until transplanting and without pesticide applications.

Oviposition preference of *Bemisia tabaci* biotype B adults in free-choice tests with plants of different age

The free-choice tests were carried out from January to July 2011 with the tomato genotypes being exposed to the whiteflies at two plant ages (23 and 50 days) in two independent assays. The first free-choice oviposition test was carried out with the two parental lines plus the 99 F₃ families (with 16 plants each), comprising a total of 1 616 plants under evaluation. The 23-day-old plants were kept in 128-cell polystyrene trays (Tecnocell). The polystyrene trays were randomly organized (spaced 0.7 m) by placing them in the center of eight 2-m-wide benches in a greenhouse (12 × 4.5 × 4 m). The tomato

plants were then exposed to *B. tabaci* adults using infested cabbage plants as the primary insect source. The cabbage plants (192 pots with on average 105 whitefly adults per plant) were uniformly placed around the trays during 7 days. The trays with tomato plants had their position changed daily to minimize the potential variability caused by their location within the greenhouse. The assay was set in a randomized block design with each polystyrene tray representing a block containing one plant of each tomato genotype. On day 7 after start of exposure to the whiteflies, the third fully expanded true leaf from each tomato plant top was carefully removed, placed into a Petri dish, and stored at 4 °C (Lourenção & Yuki, 1982; Simmons, 1994; Toscano et al., 2002a). Egg counts were carried out within 12 h after collection. Ten leaf disks (two per leaflet) were sampled from each leaf with the aid of a metal cutter (0.8 cm diameter), avoiding the main leaflet vein. Egg counts were conducted on the abaxial leaf surface of each leaf disk with the aid of a stereomicroscope (20× magnification) and expressed as a standardized unit (number of eggs cm⁻²). Conditions in the greenhouse were 26.5 ± 1.1 °C and $82 \pm 1\%$ r.h., and natural photoperiod.

The second free-choice oviposition trial was also conducted under greenhouse conditions with a separate batch of 35-day-old tomato plants. However, in this second trial, families (with 16 plants of each tomato genotype) were transplanted into soil using two adjacent greenhouses (67 × 8 m × 4.2 m) with arched ceiling and covered with agricultural plastic film. The plants were cultivated using eight rows (60 m long) per greenhouse with between-row spacing of 1 m and within-row spacing of 0.55 m. The experimental design consisted of eight rows/blocks per greenhouse and one plant of each genotype was randomly assigned to each row. Plants were individually identified with plastic labels placed on the soil surface near each stem. The plants were watered using a drip irrigation system with one pipeline per bed. Fifteen days after transplanting (i.e., 50-day-old plants), whitefly-infested cabbage plants (single plants kept in 2-l plastic pots) were uniformly distributed in each greenhouse (total of 264 pots). The cabbage plants exhibited, at this time, a whitefly infestation averaging 113.6 adults per plant. This estimation was done by counting adults on 10 randomly selected plants (ca. 30 000 adults). The whitefly-infested cabbage plants were placed among the beds (2 m apart), and they had their position changed every 2 days. The cabbage plants were watered once a day. Seven days after releasing the whiteflies, the third fully expanded leaf (counting from the top) of all tomato plants (now 57 days old) was harvested and the eggs laid were counted, following the same procedure

as described above. Environmental conditions were 26.1 ± 1.3 °C, $80 \pm 1\%$ r.h., and natural photoperiod.

Preference of whitefly adults for the selected sub-group of tomato genotypes

Additional free-choice assays were conducted using six F_3 families identified with the lowest egg densities and two F_3 families with the highest whitefly egg densities. These families were selected after analysis of the former two free-choice oviposition trials. The two parental lines (LAM-148 and TO-937-15) were also included in these studies as standard for susceptibility and resistance, respectively. Eight 40-day-old plants of each of eight selected F_3 families and the parental lines were transplanted into a greenhouse ($8 \times 4 \times 4.5$ m). The plants were cultivated in 5-l plastic pots. The test was setup as a randomized block design, considering a greenhouse bench as a block and eight benches in total (eight replicates). Each bench held 10 tomato plants (one plant of each selected F_3 family plus one of both parental lines). Infestation of these plants was obtained using 208 whitefly-infested cabbage plants cultivated in 2-l plastic pots filled with sterile soil. The estimated whitefly number at the beginning of the experiment was around 20 000 adult insects. Twenty-six pots with whitefly-infested cabbage plants were placed on the borders of each bench. Evaluations were done at 24, 48, and 72 h after exposing the tomato genotypes to the whitefly-infested plants, by counting the whitefly adults resting on the abaxial leaf surface of the third and fifth fully expanded leaf from the plant top. Insects were counted with the aid of a mirror (Baldin et al., 2005). Conditions during the study were 27.1 ± 1.5 °C, $82 \pm 1\%$ r.h., and natural photoperiod.

***Bemisia tabaci* biotype B oviposition in no-choice test with a group of selected tomato genotypes**

Sixteen plants of each of the 10 selected genotypes were cultivated in plastic pots (one plant per pot) and kept initially free from whitefly infestation. Plants were then transferred to a greenhouse and organized on 16 benches with 10 plants each (= one plant per genotype per bench) in a completely randomized design. Cylindrical cages (14.2×15 cm) made with transparent plastic (covered with organdie fabric) and with two 5-cm-diameter openings on their sides were used to cage whitefly adults on individual 40-day-old tomato plants. Fifty whitefly adults (both sexes) were collected from the stock colony with the aid of an aspirator and released into each cage. Seventy-two h after the whitefly release into the cages, the third fully expanded leaves were collected and the eggs laid were counted. Conditions were 27.3 ± 1 °C, $81 \pm 1\%$ r.h., and natural photoperiod.

Development and survival of *Bemisia tabaci* biotype B reared on a group of selected tomato genotypes

Eight plants of each of the 10 selected genotypes were cultivated in climatic growth chambers adjusted to 26 ± 1 °C, $73 \pm 1\%$ r.h., and L12:D12 photoperiod. Five whitefly pairs were collected from the stock colony and caged on the middle (central) portion of the third fully expanded leaf from the top of each plant. Cages consisted of organdie bags (7 cm wide, 10 cm long). The adults were discarded after 48 h, cages were removed, and the eggs laid per leaflet were counted under a stereomicroscope ($40\times$ magnification). The stereomicroscope was set up using a support adjusted to the height of the leaflet under analysis. Egg hatching, nymph molting, and adult eclosion were determined daily (always at the same time of the day) with the aid of a stereomicroscope ($40\times$). To monitor adult eclosion, the leaflets were caged again when the molt to the fourth instar was observed and cages were kept until the last adult in a given cage died. Thus, egg and nymphal viability and developmental times from adult caging to adult eclosion were determined for *B. tabaci* reared on each tomato genotype.

Quantification of trichomes and acylsugars in a group of selected tomato genotypes

The densities (no. trichomes mm^{-2}) of type IV glandular trichomes of the parental lines and the eight F_3 families were determined by sampling two leaflets from eight plants. The leaflets from the central part of the third fully expanded leaf from the plant top were harvested and placed inside Petri dishes avoiding damage to the trichomes and maintained at 5 °C during the 3 days of counting. The leaf trichome types were identified following the method described by Alba et al. (2009), and they were counted on an area of 0.6 mm^2 of the middle part of both sides of the leaflets under a stereomicroscope ($40\times$).

The level of acylsugars in the leaves of eight plants of each of the 10 selected genotypes (eight F_3 families plus the two parental lines) was determined following the procedure described in Lin & Wagner (1994). Briefly, the harvested leaves were dipped in a vial containing 100% acetonitrile for 20 s to extract the trichome exudates. The leaves were removed and the acetonitrile was evaporated using Q344B rotatory evaporator (Quimis Aparelhos Científicos, Diadema, SP, Brazil). Then, 0.5% rhodamine B in aqueous solution was added to the vials and let to rest for 10 min to bind the acylsugars and precipitate, followed by five careful washes with distilled water to eliminate the excess of rhodamine B. In the last step, 2 ml of 50% acetonitrile was added to dissolve the stained acylsugars. Absorbance was read at 550 nm on an ELx808 microtiter reader (Bio-Tek Instruments, Winooski, VT, USA). Simulta-

neously, a standard curve was measured using known concentrations of sucrose to estimate the amount of acylsugar produced. The foliar acylsugar concentration values were expressed in nmol cm^{-2} .

Horticultural traits of the parental lines and of the group of selected tomato genotypes

Flower structure of each plant within the selected families and parental lines was observed and assigned for the presence/absence of style exertion out of the antheridial-cone, a trait derived from the resistant parent (*S. pimpinellifolium* TO-937-15), which is associated with low fruit set (Levy et al., 1978). Fruit mass of the selected genotypes was also evaluated during the growing cycle using 10 fruits per plant and 16 replicates (plants) per genotype.

Statistical analysis

Due to lack of independence of treatments in free-choice tests, the 101 genotypes in the free-choice test for oviposition and the 10 selected genotypes in the free-choice test for adult preference were ranked within block/replication, from one (= least preferred) to the highest preferred, depending on the number of genotypes tested (Menezes et al., 2005). The sum of the ranks obtained for each genotype in relation to possible maximal ranking (101 or 10) was calculated. The Friedman test for block design was performed to determine whether there were differences among tomato genotypes based on the rank sums (Conover, 1999). Multiple comparisons based on rank sum differences were conducted between pairs of genotypes, using a sequential Holm adjustment for significance level (Holm, 1979). To check consistency in genotype preference ranking obtained between the two greenhouses in the free-choice test using the 50-day-old plants for whitefly oviposition, the Kendall's rank correlation coefficient was used (Conover, 1999). The outcome of the analysis showed a high degree of concordance between the two sets of ranks to whitefly preference for oviposition ($\tau = 0.96072$, $n = 808$, $P < 0.0001$). Then, the data from both greenhouses were combined and a new round of analyses was carried out considering a total of 16 replications.

Data from additional trials comparing the 10 selected genotypes for oviposition in the no-choice test, whitefly developmental parameters, density of type IV glandular trichomes, and acylsugar level were subjected to analysis of variance (ANOVA). To meet the assumptions of this analysis, $\log(x + 1)$ transformation was applied for densities of type IV glandular trichomes, acylsugar concentration, and average fruit mass values, whereas $\sqrt{(x + 0.5)}$ transformation was applied to percentage of egg and nymphal viability. Tukey's honestly significant difference test

was performed for mean separation, considering the Bonferroni and Šidák correction for the significance level ($\alpha = 0.05/n$, where n equals the number of means; Abdi, 2007). Pearson's correlation analysis was used to assess the relationship among the number of eggs laid and the number of adults with trichome density and acylsugar level. All analyses were done using the statistical packages available in SAS (SAS Institute, 2001).

Oviposition data (no-choice and free-choice tests) and adult preference tests were used to group tomato genotypes into categories according to their resistance to *B. tabaci*. Genotypes were allocated to one of three resistance levels based on the 95% confidence intervals (95% CI), considering the number of eggs and whitefly adults per plant relative to the standard susceptible genotype (LAM-148): resistant (R) for values below the lower limit of the 95% CI, susceptible (S) for values within the 95% CI, and highly susceptible (HS) for values greater than the upper limit of the 95% CI.

Results

Oviposition preference of *Bemisia tabaci* biotype B adults in free-choice tests with plants of different age

Whitefly preference for oviposition was significantly different across the 101 genotypes in the evaluations conducted at the two host plant ages, 30 and 57 days old (Friedman tests: $Fr = 643.16$ and 275.75 , both $P < 0.0001$; $n = 16$). The 30-day-old plants were classified into seven groups regarding their resistance to whiteflies (Table 1). Thus, based upon the 95% confidence interval of the standard susceptible genotype (LAM-148), 61 genotypes were classified as highly susceptible, 23 as susceptible (including the standard susceptible parental line LAM-148), and 17 genotypes were classified as resistant (including the standard for resistance parental line TO-937-15) (Table 1).

Based on the whitefly oviposition preference towards the 57-day-old plants, the genotypes were clustered into three groups: 59 genotypes were rated as resistant (including TO-937-15), whereas the 42 remaining genotypes were classified as susceptible (Table 2).

Based on the overall analysis of the two initial bioassays, BTR-063, and BTR-343 were selected as the genotypes with the highest oviposition levels on the 30- and 57-day-old plants, respectively. The following were selected as genotypes with lower oviposition levels: BTR-042, BTR-026, and BTR-331 (using 30-day-old plants), and BTR-302, BTR-228, and BTR-142 (using 57-day-old plants). These eight genotypes plus the two contrasting parental lines (TO-937-15 and LAM-148) were employed in additional studies of adult preference, developmental time, and

Table 1 Mean (\pm SE; $n = 16$) density of eggs (no. cm^{-2}) laid by *Bemisia tabaci* biotype B in free-choice tests on 30-day-old tomato plants of the parental lines and 99 BTR (*B. tabaci*-resistant) F_3 families derived from the interspecific cross *Solanum lycopersicum* LAM-148 \times *S. pimpinellifolium* TO-937-15. Eggs were counted 7 days after exposure to adult whiteflies

Genotype	Egg density ¹ (no. eggs cm^{-2})	Classification ²
BTR-063	27.7 \pm 4.65 (1545)a	HS
BTR-309, BTR-057, BTR-244, BTR-312, BTR-074	20.4 \pm 0.34 (1430–1304)b	HS
BTR-232, BTR-306, BTR-279, BTR-229, BTR-141, BTR-273, BTR-041, BTR-275, BTR-222, BTR-206, BTR-227, BTR-238, BTR-055, BTR-217, BTR-292, BTR-285, BTR-300, BTR-252, BTR-118, BTR-045, BTR-182, BTR-046, BTR-327, BTR-235, BTR-315, BTR-142, BTR-077, BTR-263, BTR-179, BTR-216, BTR-078	11.5 \pm 0.28 (1261.5–963)c	HS
BTR-010, BTR-067, BTR-280, BTR-242, BTR-261, BTR-313, BTR-254, BTR-343, BTR-015, BTR-294, BTR-091, BTR-297, BTR-255, BTR-066, BTR-324, BTR-354, BTR-124, BTR-017, BTR-148, BTR-099, BTR-013, BTR-339, BTR-094, BTR-290	7.8 \pm 0.16 (879.5–804)d	HS
BTR-352, BTR-302, BTR-051, BTR-211, BTR-299, BTR-011, BTR-107, BTR-165, BTR-039, BTR-228, BTR-357, LAM-148 , BTR-369, BTR-006, BTR-156, BTR-289, BTR-001, BTR-022, BTR-323, BTR-188, BTR-301, BTR-111, BTR-104	5.2 \pm 0.14 (669–526)e	S
BTR-363, BTR-346, BTR-034, BTR-237, BTR-152, BTR-248, BTR-268	3.9 \pm 0.13 (520.5–422.5)f	R
BTR-190, BTR-042, BTR-103, TO-937-15 , BTR-173, BTR-373, BTR-026, BTR-366, BTR-331, BTR-341	2.4 \pm 0.25 (363–108.5)g	R

Genotypes indicated in bold are the two parental lines.

¹Overall mean considering all genotypes within a group. Values in parenthesis indicate the extreme rank sums for the genotypes in the group. Means followed by different letters are significantly different (Friedman multiple pair-wise test, followed by sequential Holm adjustment: $P < 0.05$).

²Genotypes were classified to one of three resistance levels based on the 95% confidence interval (95% CI) for the number of eggs laid on the standard susceptible parental genotype LAM-148 ($= 5.46 \pm 1.11$ eggs cm^{-2}). R (resistant) = values below the lower limit of the 95% CI; S (susceptible) = values within the 95% CI; HS (highly susceptible) = values exceeding the upper limit of the 95% CI.

the relationship between trichome densities and acylsugar levels.

Preference of whitefly adults for the selected sub-group of tomato genotypes

The mean number of whitefly adults per plant displayed a significant variation among the 10 selected genotypes at the three evaluation times (Friedman test: $F_r = 36.41, 35.12, \text{ and } 33.47$, for 24, 28, and 72 h, respectively; all $P < 0.0001$, $n = 8$). An increase in number of whiteflies per plant over time was observed after 24 and 48 h (Table 3). This increase had a similar magnitude across all 10 selected genotypes. LAM-148 and the F_3 families BTR-063, BTR-343, and BTR-302 were the genotypes with the highest whitefly infestation. BTR-228 and BTR-142 exhibited intermediate levels of adult infestation, whereas TO-937-15 was least preferred, followed by BTR-331, BTR-042, and BTR-026, which were all classified as resistant.

Bemisia tabaci biotype B oviposition in no-choice test with a group of selected tomato genotypes

Whitefly adults caged on tomato plants laid significantly different numbers of eggs across the 10 selected genotypes

($F_{9,135} = 2.23$, $P = 0.024$) (Figure 1). Egg densities were similar on BTR-302, BTR-228, BTR-063, BTR-042, BTR-343, BTR-148, BTR-142, BTR-026, and LAM-148. Oviposition on BTR-331 and TO-937-15 was significantly lower than on LAM-148, supporting the notion that they are resistant against *B. tabaci*. Families BTR-026 and BTR-142 exhibited intermediate infestation levels.

Development and survival of *Bemisia tabaci* biotype B reared on a group of selected tomato genotypes

Egg viability was similar across the sub-group of selected genotypes ($F_{9,70} = 0.82$, $P = 0.57$) (Table 4). Survival of second ($F_{9,70} = 2.51$, $P = 0.015$) and third ($F_{9,70} = 3.174$, $P = 0.0028$) instars differed among genotypes. Nymphs in the second instar reared on BTR-042 survived significantly shorter when compared to nymphs reared on LAM-148. In the third instar, lower survival was observed for BTR-042 and BTR-142 compared to LAM-148. On the other hand, survival of first ($F_{9,70} = 0.58$, $P = 0.80$) and fourth ($F_{9,70} = 1.72$, $P = 0.33$) instars did not differ among the selected genotypes (Table 4). Overall, survival from egg hatching to adult eclosion was similar across genotypes ($F_{9,70} = 1.68$, $P = 0.11$). The times for egg, nymph, and

Table 2 Mean (\pm SE; n = 16) density of eggs (no. cm^{-2}) laid by *Bemisia tabaci* biotype B in free-choice tests on 57-day-old tomato plants of the parental lines and 99 BTR (*B. tabaci*-resistant) F_3 families derived from the interspecific cross *Solanum lycopersicum* LAM-148 \times *S. pimpinellifolium* TO-937-15. Eggs were counted 7 days after exposure to adult whiteflies

Genotype	Egg density ¹ (no. eggs cm^{-2})	Classification ²
BTR-343, BTR-099, BTR-118, BTR-297, BTR-252, BTR-222, LAM-148 , BTR-190, BTR-242, BTR-232, BTR-357, BTR-124	27.5 \pm 1.75 (1294.5–1036.5)a	S
BTR-324, BTR-013, BTR-273, BTR-026, BTR-301, BTR-339, BTR-294, BTR-346, BTR-309, BTR-156, BTR-280, BTR-173, BTR-045, BTR-182, BTR-341, BTR-011, BTR-373, BTR-366, BTR-313, BTR-165, BTR-248, BTR-041, BTR-211, BTR-107, BTR-091, BTR-300, BTR-057, BTR-015, BTR-312, BTR-141	18.6 \pm 0.44 (1029–846.5)b	S
BTR-263, BTR-148, BTR-306, BTR-315, BTR-331, BTR-227, BTR-275, BTR-369, BTR-352, BTR-152, BTR-067, BTR-010, BTR-103, BTR-055, BTR-051, BTR-034, BTR-255, BTR-188, BTR-206, BTR-290, BTR-074, BTR-078, BTR-104, BTR-039, BTR-354, BTR-279, BTR-094, BTR-042, BTR-046, BTR-217, BTR-292, BTR-017, BTR-006, BTR-001, BTR-285, BTR-327, BTR-268, BTR-022, BTR-111, BTR-261, BTR-237, BTR-363, BTR-229, BTR-299, BTR-238, BTR-323, BTR-077, BTR-179, BTR-063, BTR-289, BTR-244, BTR-066, BTR-216, BTR-302, BTR-254, BTR-228, BTR-235, BTR-142, TO-937-15	9.7 \pm 0.47 (727–160.5)c	R

Genotypes indicated in bold are the two parental lines.

¹Overall mean considering all genotypes within a group. Values in parenthesis indicate the extreme rank sums for the genotypes in the group. Means followed by different letters are significantly different (Friedman multiple pair-wise test, followed by sequential Holm adjustment: $P < 0.05$).

²Genotypes were classified to resistance level based on the 95% confidence interval (95% CI) for the number of eggs laid on the standard susceptible parental genotype LAM-148 (= 26.09 \pm 12.74 eggs cm^{-2}). R (resistant) = values below the lower limit of the 95% CI; S (susceptible) = values within the 95% CI.

Table 3 Mean (\pm SE; n = 8) number of *Bemisia tabaci* biotype B adults on abaxial tomato leaf surface of the parental lines and eight selected BTR (*B. tabaci*-resistant) F_3 families derived from the interspecific cross *Solanum lycopersicum* LAM-148 \times *S. pimpinellifolium* TO-937-15 at 24, 48, and 72 h after the start of infestation in a free-choice test conducted under glasshouse conditions

Genotype	No. adults/plant ¹			Classification ²
	24 h	48 h	72 h	
LAM-148 , BTR-063, BTR-343, BTR-302	42.3 \pm 5.43 (392–306)a	48.8 \pm 2.74 (433–330)a	58.7 \pm 5.57 (538–406)a	S
BTR-142, BTR-228	29.5 \pm 3.15 (269–208)ab	32.1 \pm 3.4 (284–269)ab	44.3 \pm 1.09 (369–339)ab	S
BTR-026, BTR-042, BTR-331	20.9 \pm 1.06 (200–110)b	25.3 \pm 1.37 (230–147)b	28.4 \pm 4.98 (293–156)b	R
TO-937-15	1.4 \pm 1.12 (11)c	1.8 \pm 0.77 (15)c	2.0 \pm 1.20 (18)c	R

Genotypes indicated in bold are the two parental lines.

¹Values in parenthesis indicate the extreme rank sums for the genotypes in the group. Means within a column followed by different letters are significantly different (Friedman multiple pair-wise test, followed by sequential Holm adjustment: $P < 0.05$).

²Genotypes were classified to resistance level based on the 95% confidence interval (95% CI) for the number of adults/plant after 72 h on the standard susceptible parental genotype LAM-148 (= 57.9 \pm 14.4 adults/plant). R (resistant) = values below the lower limit of the 95% CI; S (susceptible) = values within the 95% CI.

egg-to-adult development were similar across the 10 selected genotypes ($P > 0.05$). Thus, the observed developmental time and survival of *B. tabaci* egg and nymphal stages did not allow us to infer that antibiosis is the resistance mechanism associated with these selected genotypes.

Quantification of trichomes and acylsugars in a group of selected tomato genotypes

The type IV glandular trichomes were the most frequent and abundant among the eight selected F_3 families, as well as in TO-937-15. The parental line LAM-148 did not have

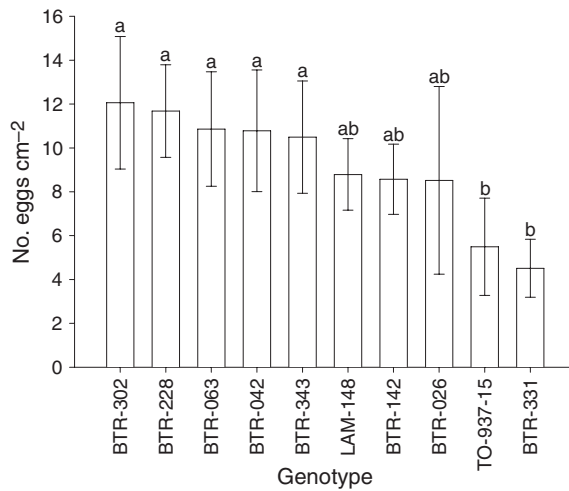


Figure 1 Mean (\pm SE; $n = 16$) number of *Bemisia tabaci* biotype B eggs laid on the parental lines and eight selected BTR (*Bemisia tabaci*-resistant) F₃ families derived from the interspecific cross *Solanum lycopersicum* 'LAM-148' \times *S. pimpinellifolium* 'TO-937-15' under no-choice test conditions conducted in a greenhouse. Means capped with the same letter do not differ significantly (Tukey HSD test after Bonferroni and Šidák correction: $P < 0.005$).

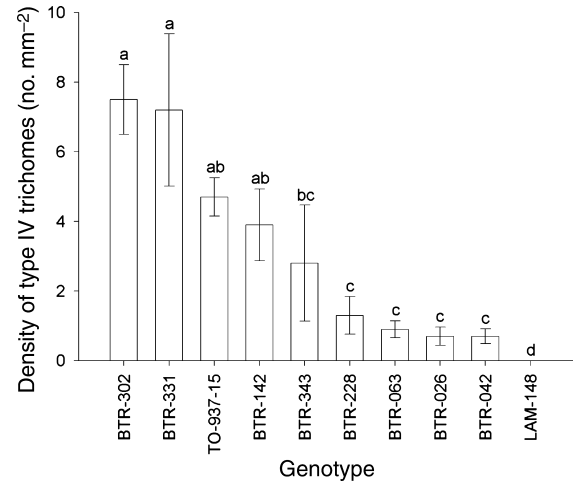


Figure 2 Mean (\pm SE; $n = 8$) density of type IV glandular trichomes on both sides of the leaflet in the parental lines and eight selected F₃ families derived from the interspecific cross *Solanum lycopersicum* 'LAM-148' \times *S. pimpinellifolium* 'TO-937-15'. Means capped with the same letter do not differ significantly (Tukey HSD test after Bonferroni and Šidák correction: $P < 0.005$).

this type of trichome (Figure 2). The type IV glandular trichomes occurred on both leaflet sides, but they were more abundant on the abaxial surface. Significant density variation was observed among the genotypes for type IV glandular trichomes (abaxial + adaxial surfaces: $F_{9,70} = 14.80$, $P < 0.0001$) (Figure 2).

The highest type IV trichome densities were observed in BTR-331 and BTR-302. These values were higher than that of the resistant parental line TO-937-15. Genotypes BTR-228, BTR-063, BTR-026, and BTR-042 had the lowest type IV trichome densities; BTR-343 displayed an intermediate density. Density on BTR-142 was close to that on the resis-

Table 4 Mean (\pm SE; $n = 8$) of egg, nymph, and nymph to adult time period viability of *Bemisia tabaci* biotype B reared on the parental lines and eight selected BTR (*B. tabaci* Resistance) F₃ families derived from the interspecific cross *Solanum lycopersicum* 'LAM-148' \times *S. pimpinellifolium* 'TO-937-15' under greenhouse conditions ($n = 120$ eggs)

Genotype	Egg viability (%) ^{NS}	Nymph viability per instar (%)				Nymphal stage viability (%) ^{2NS}
		1st instar ^{NS}	2nd instar ¹	3rd instar	4th instar ^{NS}	
LAM-148	85.2 \pm 3.92	84.6 \pm 2.66	97.1 \pm 2.62 a	89.9 \pm 4.88 a	60.8 \pm 9.64	82.4 \pm 4.05
BTR-331	82.8 \pm 7.41	86.1 \pm 3.75	80.8 \pm 3.62 ab	84.1 \pm 5.91 ab	86.8 \pm 11.47	69.3 \pm 3.64
BTR-063	79.7 \pm 6.30	83.9 \pm 4.25	83.1 \pm 4.97 ab	71.0 \pm 4.16 ab	63.4 \pm 10.56	70.3 \pm 6.60
BTR-302	77.2 \pm 5.35	85.3 \pm 4.07	86.7 \pm 3.39 ab	68.0 \pm 6.98 ab	60.4 \pm 8.29	74.0 \pm 4.72
BTR-142	77.2 \pm 6.87	85.1 \pm 7.38	86.5 \pm 4.21 ab	63.7 \pm 6.39 b	76.0 \pm 7.84	74.2 \pm 7.74
BTR-026	76.2 \pm 6.00	82.9 \pm 4.00	90.2 \pm 3.54 ab	77.4 \pm 7.57 ab	59.9 \pm 7.27	75.0 \pm 5.11
BTR-228	75.6 \pm 5.35	86.9 \pm 4.31	84.0 \pm 4.85 ab	84.5 \pm 4.88 ab	76.2 \pm 9.95	79.8 \pm 4.57
BTR-343	70.5 \pm 9.02	74.7 \pm 5.23	83.8 \pm 7.03 ab	68.7 \pm 4.03 ab	72.6 \pm 8.21	74.6 \pm 4.77
TO-937-15	69.2 \pm 7.13	81.0 \pm 4.18	75.6 \pm 7.55 ab	71.9 \pm 4.42 ab	76.2 \pm 8.49	63.0 \pm 8.50
BTR-042	65.6 \pm 8.23	84.5 \pm 3.84	73.0 \pm 2.96 b	60.1 \pm 3.52 b	85.8 \pm 8.85	62.0 \pm 4.54

Genotypes indicated by black bold letters are the two parental lines.

^{NS} = non-significant; ANOVA ($P > 0.05$).

¹Means followed by the same letter within a column do not differ by Tukey HSD test (after Bonferroni and Šidák correction: $P > 0.005$).

²Nymphal stage viability (%) = (number of nymphs reaching 4th instar/number of nymphs entering 1st instar) \times 100.

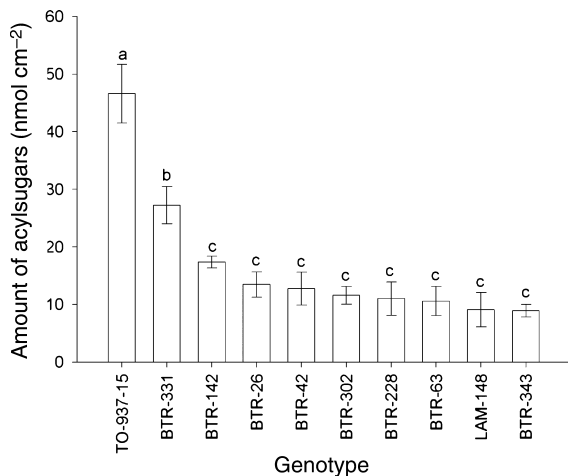


Figure 3 Mean (\pm SE; $n = 8$) level of acylsugars on leaves of the parental lines and eight selected BTR (*Bemisia tabaci*-resistant) F_3 families derived from the interspecific cross *Solanum lycopersicum* 'LAM-148' \times *S. pimpinellifolium* 'TO-937-15'. Means capped with the same letter do not differ significantly (Tukey HSD test after Bonferroni and Šidák correction: $P < 0.005$).

tant parent (Figure 2). A negative and significant correlation was observed between type IV glandular trichome density and number of whitefly adults per plant (free-choice test: $r = -0.32$, $P = 0.003$) and with egg density (no. eggs cm^{-2} ; no-choice test: $r = -0.24$, $P = 0.0026$).

The acylsugar levels (Figure 3) varied among the selected genotypes ($F_{9,50} = 2.54$, $P = 0.019$). TO-937-15 displayed the highest level of acylsugars, ca. five-fold higher than those of the susceptible standard LAM-148. The highest level of acylsugars among the selected F_3 families was found in BTR-331. Average acylsugar level of the other selected F_3 families did not differ from LAM-148. Acylsugar levels also correlated negatively with the number of whitefly adults per plant (free-choice test: $r = -0.85$, $P = 0.0009$) and with egg density (no. eggs cm^{-2} ; no-choice test: $r = -0.68$, $P = 0.014$).

A significant and positive correlation was observed between type IV glandular trichome density and the level of acylsugars ($r = 0.27$, $P = 0.020$), whereas the correlation between non-glandular trichomes and acylsugar levels was negative ($r = -0.73$, $P = 0.008$). Type IV glandular trichome density on both sides of the leaves (Figure 2) did not necessarily indicate either high overall acylsugar levels in the leaf (Figure 3) or higher resistance levels to whitefly. One illustrative example was provided by the families BTR-302 and BTR-142, which displayed high density of type IV glandular trichomes but low acylsugar levels. In addition, under no-choice test conditions, these F_3 families

were classified as susceptible to *B. tabaci* based on all evaluation criteria.

Horticultural traits of the parental lines and of the group of selected tomato genotypes

Flowers of each plant within the selected families and parental lines were observed for the presence/absence of style stigma extrusion out of the antherial-cone. The F_3 families BTR-063, BTR-142, BTR-228, BTR-302, and BTR-331 displayed stigma extrusion, a trait also observed in parental line *S. pimpinellifolium* TO-937-15. This segregating trait was absent in BTR-026, BTR-042, BTR-343, as well as in the parental line *S. lycopersicum* LAM-148.

Fruit weight significantly differed among the selected genotypes ($F_{9,104} = 445.63$, $P < 0.0001$). In the selected families, fruit weight ranged from 4.92 ± 0.81 (BTR-228) to 14.52 ± 1.39 g (BTR-142). These values were small compared to that of the commercial line LAM-148 (139.52 ± 1.45 g), but significantly higher than in the resistant parental line TO-937-15 (1.34 ± 0.1 g). BTR-331 showed the highest levels of resistance to whitefly as well as transgressive segregation for type IV glandular trichomes, and had fruits significantly larger (7.19 ± 0.89 g) than fruits of TO-937-15.

Discussion

Tomato resistance to *B. tabaci* is a highly desirable trait, as it can reduce insect infestations while reducing both the frequency of insecticide sprayings and production costs (Freitas et al., 2002). In the present study, we were able to select promising sources of *B. tabaci* biotype B resistance after screening 99 F_3 families derived from an interspecific cross between one commercial 'Santa Cruz' line and one accession of the wild species *S. pimpinellifolium*. Differences in the classification of a given genotype were observed across the two initial free-choice and no-choice screening assays for whitefly oviposition. These results might be explained by the contrasting environmental conditions in both tests (plants kept in polystyrene trays vs. plants cultivated in soil beds) as well as by the distinct phenological stages of the plants. However, it is important to point out that, despite these quite dissimilar experimental conditions, an overall convergence of the tomato genotype performances was observed, allowing for the selection of F_3 families with contrasting resistance to *B. tabaci*.

Additional free-choice and no-choice tests, carried out with the parental lines and this sub-group of selected F_3 families, indicated that the whitefly adults were able to lay eggs on all the genotypes. However, significantly lower oviposition levels were observed in

some genotypes, such as TO-937-15 (resistant parental line) and BTR-331, in both tests, reinforcing the consistency of the resistance.

The selected tomato genotypes varied in resistance, with some genotypes ranked as resistant in the oviposition assay, but susceptible in the adult attractiveness test. This singularity has also been found in other studies with *S. pimpinellifolium* where the expression of resistance to *B. tabaci* was variable among genotypes (Fancelli et al., 2003). These results indicate the occurrence of distinct defense mechanisms within accessions of the same wild tomato species, which can be associated with distinct morphological and biochemical traits influencing the attractiveness and acceptance of the host plant by the insect (Rodríguez-López et al., 2011).

Despite differences in adult attractiveness and oviposition preference among the selected tomato genotypes, they exhibited similar effects on the developmental characteristics of *B. tabaci*. Previous results using a distinct *S. pimpinellifolium* accession showed significant negative effects on *B. tabaci* development (Fancelli & Vendramim, 2002). Therefore, we cannot exclude the absence of an antibiosis effect based upon only the outcome of our study, which describes the analysis of only one developmental generation. Perhaps antibiosis is expressed in further generations and detected, for instance, through population growth parameters and life-history traits.

Selection of whitefly resistant tomato genotypes may be more efficient if resistance mechanisms are completely understood (Fancelli et al., 2005). Pest resistance in wild *Solanum* (section *Lycopersicon*) species has been found associated with trichome density and type. Leaf trichomes may confer resistance by restraining access of the insect to the plant surface (Tingey & Gibson, 1978; Toscano et al., 2003). Further, the presence of glandular trichomes has been considered the most important morphological characteristic related to leaf resistance against arthropods. It is well known that glandular trichomes can disrupt oviposition and feeding activities of small sucking insects such as whiteflies (Sippell et al., 1987; Heinz & Zalom, 1995; McAuslane, 1996; Baldin & Beneduzzi, 2010). In accordance with our study, Aragão et al. (2000) found that type IV trichome density is the most relevant indirect selection criterion in arthropod resistance breeding programs.

The impact of the acylsugar levels on *B. tabaci* in our study is in accordance with previous work where acylsugar played an important role as a resistance factor against whitefly (Liedl et al., 1995), red spider mite (Resende et al., 2008), and tomato leaf miner (Gonçalves-Neto et al., 2010; Maciel et al., 2011). Similar variation on the level of acylsugars and their differential response to the red

spider mite was also detected in hybrids from the cross *S. lycopersicum* × *S. pimpinellifolium* TO-937-15 in Spain (Alba et al., 2009).

Type IV glandular trichome densities were negatively correlated with *B. tabaci* oviposition levels and they were positively correlated with acylsugar levels. This matches previous results with *S. pimpinellifolium* (Alba et al., 2009) and *S. pennellii* (Goffreda et al., 1989) accessions. The density of glandular trichomes was not in all cases positively correlated with acylsugar levels, indicating that cellular differentiation leading to the formation of glandular trichome type IV and the biochemical pathways resulting in acylsugar synthesis and transport to trichome vesicles are genetically and physiologically independent traits. Therefore, in our F₃ families it should be possible to identify segregating individuals exhibiting high densities of type IV glandular trichomes and low levels of acylsugars and vice versa. In this context, selection of individual plants based solely on high density of type IV glandular trichomes might sometimes not be effective in increasing the *B. tabaci* biotype B resistance levels in applied tomato breeding programs. These recombinant lines might also be of interest in genetic studies of whitefly resistance in tomatoes aiming to dissect the individual genetic components of this polygenic trait.

The F₃ family BTR-331 behaved as a promising genotype, combining high levels of resistance to *B. tabaci* biotype B, low adult attractiveness, high type IV glandular trichome density, and high foliar acylsugar levels. The acylsugar level of BTR-331 (27.3 nmol cm⁻²) was close to that reported by Rodríguez-López et al. (2011) in ABL 14-8 (29.3 nmol cm⁻²), an advanced breeding line derived from an interspecific cross between *S. lycopersicum* and *S. pimpinellifolium* TO-937-15. ABL 14-8 displayed high levels of resistance to *B. tabaci* biotype Q and had a significant impact in reducing primary and secondary *Tomato yellow leaf curl virus* transmission rates (Rodríguez-López et al., 2011). Therefore, additional studies of the potential effects of BTR-331 on *B. tabaci* population growth would be valuable. Furthermore, this information would be useful as a phenotypic marker for early identification of whitefly resistant individuals in subsequent breeding generations derived from crosses between Santa Cruz/Chonto lines and *S. pimpinellifolium* TO-937-15. So far no experiment using *S. pimpinellifolium* TO-937 and derived genotypes was able to determine an effective threshold of foliar acylsugar concentration that would negatively affect the behavior of *B. tabaci* to guide tomato breeding programs aiming for high levels of resistance to this pest. Our results indicate that effective acylsugar levels against *B. tabaci* biotype B would be in the range observed in BTR-331 (24–30 nmol cm⁻²).

Genetic resistance to whiteflies would be the most comprehensive strategy to control the whitefly/begomovirus complex due to its dual impact on their biology/behavior (Heinz & Zalom, 1995) as well as their efficiency as a virus vector (Rodríguez-López et al., 2011). Our results indicated that both the density of type IV glandular leaf trichomes and the concentration of leaf acylsugars are important components of plant resistance to whitefly. Phenotypic expression of resistance to whiteflies was displayed irrespective of plant age. Indeed, resistance expression at the seedling stage (i.e., 23-day-old plants) is relevant to whitefly management, as it may impact the virus vectoring ability of this insect (Toscano et al., 2002a, b; Fancelli et al., 2008; Oriani et al., 2011; Rodríguez-López et al., 2011). Early begomovirus infection via viruliferous whiteflies at the seedling stage may result in severe tomato fruit yield losses, as was shown under tropical conditions (Giordano et al., 2005a).

In this study, at least five well-characterized polygenic tomato traits were detected segregating in our *S. pimpinellifolium* TO-937-15-derived population: fruit size, fruit mass, type IV trichome density, stigma extrusion, and acylsugar concentration. This large number of genetic factors involved will demand the use of strategies based upon recurrent selection breeding schemes and/or large segregating populations to allow recombination of all desirable traits, as well as the breakdown of potential repulsion phase linkages (Moreno-Gonzalez & Cubero, 1993). Likewise, introgression into commercial cultivars of whitefly resistance detected in distinct sources, such as *S. pennellii* and *S. habrochaites* accessions, has been difficult due to the polygenic nature of the trait (Lawson et al., 1997; Freitas et al., 2002). Repulsion phase linkages observed in *S. pennellii* and *S. habrochaites* accessions are difficult to break because recombination frequencies are often greatly reduced in taxonomically distant tomato species (Bonema et al., 1997). From a breeding standpoint, the identification of a useful source of whitefly resistance in *S. pimpinellifolium* TO-937-15 is very important, because this wild species is closely related to the cultivated tomato (genetic diversity at the nucleotide level is only 0.6%; Tomato Genome Consortium, 2012). This genomic similarity could allow a more efficient incorporation of this trait into elite tomato lines by facilitating recombination and elimination of undesirable linkage drag that is potentially associated with the resistance genes in this genetic background.

In summary, our results reinforce the hypothesis that resistance to *B. tabaci* in *S. pimpinellifolium* TO-937-15 and in their progenies is based upon a positive combination of high levels of acylsugar and high densities of type IV glandular trichomes, as also previously

observed in populations and lines derived from *S. pennellii* (Freitas et al., 2002; Neiva et al., 2013). Resistance of *S. pimpinellifolium* TO-937-15 to the New World biotype B population found in this study suggests that it is also a source of wide spectrum resistance against distinct members of the *B. tabaci* complex. These results highlight the potential of *S. pimpinellifolium* as a source for resistance to whitefly in tomato breeding programs.

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