

Evaluation of wild tomato accessions (*Solanum* spp.) for resistance to two-spotted spider mite (*Tetranychus urticae* Koch) based on trichome type and acylsugar content

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Abstract Tomato wild relatives are important sources of resistance to many pests of cultivated tomato [*Solanum lycopersicum* L. (syn. *Lycopersicon esculentum* Mill.)]. Eleven wild tomato accessions previously identified at AVRDC—The World Vegetable Center as resistant to *Bemisia tabaci* were evaluated for resistance to the two-spotted spider mite [*Tetranychus urticae* (Koch.)] based on egg numbers using the leaf disc and Tanglefoot no-choice bioassays, and damage scores in choice bioassays. Highest resistance based on choice and no-choice bioassays was identified in AVRDC *S. galapagense* accessions VI057400, VI045262, VI037869 and VI037239, and *S. cheesmaniae* accession VI037240, all of which are new sources of *T. urticae* resistance. In addition, *S. pimpinellifolium* accession VI030462 exhibited resistance only in the no-choice bioassay based on egg

numbers. Resistance to *T. urticae* based on the number of eggs from the no-choice bioassays was positively correlated with density of type IV glandular trichomes and negatively correlated with densities of type V trichomes. All resistant accessions accumulated high levels of total acylsugars, which were positively associated with type IV trichomes. There was a significant negative relationship between acylsugar content and *T. urticae* egg numbers from the no-choice bioassays. There was high correlation between the results from the leaf disc test and the Tanglefoot no-choice bioassay. These findings support the possible presence of broad-based insect and mite resistance in accessions closely related to cultivated tomato.

Keywords Insect resistance · *Solanum cheesmaniae* · *S. galapagense* · *S. pimpinellifolium* · Trichomes

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Introduction

Tomato (*Solanum lycopersicum* L.) is widely grown and one of the most economically important vegetable crops worldwide (FAO 2015). The cultivated tomato is a host for many pathogens and pests. The two-spotted spider mite (*Tetranychus urticae* Koch (Acari: Tetranychidae) is a cosmopolitan agricultural pest, feeding on more than 140 different plant families and 1100 plant species including tomato (Dermauw

et al. 2012). Warm, dry conditions favor rapid spider mite population build-up and increased feeding which, if uncontrolled, causes severe yield losses up to 90 % in greenhouse and open field tomato crops in South-east and West Africa (Sibanda et al. 2000). Spider mites feed by inserting their stylets into the leaf tissue and removing cell contents; the loss of leaf chlorophyll and the subsequent reduction in net photosynthetic rate causes leaf discoloration often called bronzing, a decline in overall plant health, or even death (Sances et al. 1981; Tomczyk and Kropczynska 1985; Park and Lee 2002). Spider mites can feed directly on fruit at mature green and breaker stages, and this damage can increase unmarketable fruit and economic losses (Ghidu et al. 2006). Biological control of spider mites through natural enemies has only been widely practiced in closed greenhouses (Perdikis et al. 2008). Current spider mite control relies mainly on frequent applications of synthetic acaricides. However, spider mites have the capacity to quickly develop acaricide resistance (Van Leeuwen et al. 2004; Dermauw et al. 2012). Many aspects of spider mite biology, including rapid development, high reproductive ability of up to 25 generations per year, and haplo-diploid sex determination facilitate rapid evolution of pesticide resistance (Khajehali et al. 2011). Unfortunately, there are no tomato cultivars with resistance to spider mite. Identification of resistance sources and development of rapid screening methods are the first steps toward the development of spider mite resistant cultivars.

Resistance to spider mite and other insects has been found in some accessions of wild tomato species particularly *S. habrochaites* Knapp et Spooner (syn. *Lycopersicon hirsutum* Dunal), and *S. pennellii* Correll (syn. *L. pennellii* (Correll) d' Arcy) (de Azevedo et al. 2003; Resende et al. 2006). The resistance in these species was related to the presence of glandular trichomes (Frelichowski and Juvik 2001; Muigai et al. 2002; Oriani et al. 2011; Bleeker et al. 2012), which are reported to be absent in cultivated tomato (Luckwill 1943; Antonious 2001). Trichomes are considered the most important factor conferring pest resistance. The genus *Solanum* possesses seven types of trichomes of which types II, III and V are non-glandular, and types I, IV, VI and VII are glandular (Gurr and McGrath 2001; Simmons and Gurr 2005). Glandular trichomes secrete secondary metabolites including acylsugars, methylketones and sesquiterpenes that can be toxic, repellent, trap insects or act as a physical

barrier, and interfere with insect feeding and oviposition (Dimock and Kennedy 1983; Snyder and Carter 1984; Channaryappa et al. 1992; Sharma et al. 2009).

Recent studies of wild relatives closely related to tomato found *Bemisia tabaci* resistance in some accessions of *S. cheesmaniae* (L. Riley) Fosberg (formerly *L. cheesmaniae* L. Riley), *S. galapagense* S. C. Darwin et Peralta (formerly *L. cheesmaniae* f. *minor* (Hook. f.) C. H. Müll.) and *S. pimpinellifolium* L. (formerly *L. pimpinellifolium* (L.) Mill.) (Firdaus et al. 2012, Lucatti et al. 2013, Rakha et al. 2015), all of which can be easily crossed with *S. lycopersicum* (Rick 1971; Peralta et al. 2008). Furthermore, *S. pimpinellifolium* accession TO-937 provided acceptable resistance to *T. urticae* (Fernández-Muñoz et al. 2000). Eleven wild tomato accessions including *S. cheesmaniae*, *S. galapagense*, and *S. pimpinellifolium* were previously identified at AVRDC—The World Vegetable Center as resistant to *B. tabaci* (Rakha et al. 2015). Identification of additional insect resistance in some or all these accessions would help to prioritize accessions for breeding cultivars with broad pest resistance. The objectives of this study were to evaluate selected whitefly-resistant accessions identified at AVRDC for spider mite resistance, assess whether trichomes and total acylsugars were related to mite resistance, and evaluate different spider mite screening methods for efficiency and accuracy.

Materials and methods

Plant materials

Eleven accessions including *S. galapagense*, *S. cheesmaniae*, and *S. pimpinellifolium* and a susceptible tomato check (*S. lycopersicum*) were evaluated in greenhouse assays (Table 1). In a previous work (Rakha et al. 2015), these accessions were classified as abundant or sparse for type IV trichomes and evaluated for whitefly resistance based on number of adults, eggs, nymphs, and pupa from choice bioassays, and for adult mortality and egg number in no-choice bioassays (Rakha et al. 2015). Ten of these accessions exhibited high whitefly resistance, except *S. cheesmaniae* VI037238. AVRDC tomato line CL5915-93D4-1-0-3 (CL5915) was included as a spider mite susceptible check. Seeds of accessions were obtained from the AVRDC Genetic Resources and Seed Unit

Table 1 Mean numbers of *T. urticae* eggs on wild tomato accessions compared to the tomato check CL5915 evaluated in Tanglefoot and leaf disc no-choice bioassays

Taxa and AVRDC accessions code	Other codes	Tanglefoot test		Leaf disc test
		Eggs (no.)/10 females		Eggs (no.)/female
		Abaxial	Adaxial	Abaxial
<i>Solanum galapagense</i>				
VI057400	LA 483	0.0 b	1.7 cd	0.1 c
VI045262	LA 1141	0.0 b	4.0 bcd	0.2 c
VI037869	LA 1408	0.0 b	5.3 bcd	0.3 c
VI037239	LA 436	0.0 b	7.0 bc	0.3 c
VI063177	LA 0530	1.0 b	6.3 bcd	0.3 c
VI037241	LA 526	0.2 b	7.0 bc	0.5 c
VI063174	LA 0438	0.2 b	10.2 b	0.9 c
VI037340	LA 1408	0.8 b	9.8 b	0.7 c
<i>Solanum cheesmaniae</i>				
VI037240	LA 483	0.0 b	0.0 d	0.2 c
VI037238	LA 429	20.0 a	17.5 a	8.2 a
<i>Solanum pimpinellifolium</i>				
VI030462	PI 390519	0.0 b	6.6 bc	0.3 c
<i>Solanum lycopersicum</i>				
CL5915		20.0 a	21.6 a	5.1 b

Means followed by different letters within columns are different according to least significant difference (LSD) test at $p = 0.05$

(GRSU). Accessions and check were first tested in no-choice and later in choice spider mite bioassays at AVRDC.

Spider mite bioassays

The two-spotted spider mite (*T. urticae*) colony multiplied for choice and no-choice bioassays was kindly provided by the Tainan District Agricultural Research and Extension Station. The colony was reared and maintained on 2–3-week-old bean (*Phaseolus vulgaris* L.) plants in a growth room at 23–30 °C. Bean plants were replaced every 10 days by cutting an infested plant and placing it on top of a new plant for several days to allow spider mites to move onto the new plants. Adult female spider mites were transferred by fine brush onto new bean plants and allowed to oviposit. Two days after infestation, all mites were removed from the bean leaf and the deposited eggs hatched to become first-generation adults after approximately 10 days. Two-day-old female spider mites were used for no-choice bioassays.

No-choice bioassays were carried out in April and May 2015 using Tanglefoot tests (East et al. 1992) and leaf disc tests (Bleeker et al. 2012). Seed was sown in 14 cm pots with potting soil in an AVRDC greenhouse

(26 ± 4 °C, 10/14 h day/night). Plants were watered daily and fertilized weekly with NPK 15-15-15. Five weeks after sowing, five seedlings per accession and the susceptible check were moved from the plastic house to growth rooms. Plants were arranged following a completely randomized design with one plant per experimental unit. The experiment was conducted on stainless steel benches with 18 plants (two rows of nine plants) on each bench. Plant spacing was 20 cm between and within accessions. The growth room temperature was decreased slowly from 30 to 26 °C to allow plants to adjust to the lower optimal temperatures (26 ± 2 °C) and conditions (70 % RH, 16/8 h day/night) for spider mites. For the Tanglefoot tests, plants were infested with spider mites 6 weeks after sowing. Ten female mites were placed on the adaxial side of the lateral leaflet of the third leaf from the apex of each plant. Mites were prevented from escaping from the leaflet by a band of Tanglefoot (Tanglefoot Co., Grand Rapids, Michigan, USA) according to the method described by East et al. (1992). All plants were inspected twice daily to ensure no leaflets touched the surface, which would allow mites to escape from the leaf. Two days after infestation the number of eggs on the adaxial and abaxial leaflets were counted. The leaflets were cut

from plants to facilitate egg counting under a Leica S8 APO stereo microscope (10×). For leaf disc tests, two young leaflets at the 2nd and 3rd leaf from the apex were cut from each tested plant of the accessions and check. Two leaf discs were cut from the middle of each leaflet using a 25 mm diameter circular punch and placed on wet cotton in plastic boxes (3 cm high and 6 cm wide, diameter = 6 cm) with their abaxial side up. One female mite was placed on each leaf disk. Two days after infestation the number of eggs was counted on each leaf disc as described above.

Choice bioassays were carried out in June and July 2015 using seedling trays. Seed was sown in 72-plug seedling trays with 40 ml peat moss per cell. The accessions and check were grown at 26 ± 4 °C, 6/18 h day/night and fertilized and watered as described above. Three weeks after sowing, ten seedlings per accession and 180 plants from the check were transplanted in small pots (7 cm). These plants were arranged according to a randomized complete block design in 35-plug seedling trays (5 rows \times 7 columns) with one plant per accession and 18 plants per check in each experimental unit. The 11 accessions were randomly assigned to cells of the 2nd and 4th rows, and the check CL5915 occupying the 1st, 3rd and 5th row of each tray, so that there were 29 plants per tray. The trays were moved from the plastic house to growth rooms. The growth room temperature was decreased slowly to the optimal temperatures for spider mite as described above. One week after moving to the growth rooms, the plants were covered by a net (60 mesh/cm²) and mass infested with a very high density of spider mites from bean leaves. Each plant was inoculated with about 100 to 150 spider mite adults, eggs and nymphs. When the bean leaves wilted the net was removed from the plants, because the spider mites had moved onto the tomato plants. Leaf damage was scored 5 days after spider mite infestation using a 0–3 visual scale, where 0 = no damage, 1 = few small feeding patches (<20 % of leaf area); 2 = large feeding patches (20–49 % of leaf area); 3 = entire leaf with feeding marks (\geq 50 % of leaf area).

Trichome and acylsugar analyses

During the no-choice bioassays, plants were simultaneously assessed for trichome type and density using a Leica S8 APO stereo microscope (10×). Densities

were determined from the interior middle section of the abaxial and adaxial surfaces of the third leaf from the apex in the Tanglefoot tests. Trichome type classification was carried out according to Luckwill (1943) and determined by morphology and presence/absence of trichome glands. The trichomes were identified and counted on a 1 mm² area.

The 11 accessions and control plants were tested for acylsugar content 8 weeks after sowing in the plants used for no-choice bioassays. Three samples of 3 lateral leaflets were taken from each plant, placed in polyethylene vials (20 ml), and placed in a drying oven at 30 °C for 3 days. Fully dried leaf samples were washed with 3 ml of methanol. Acylsugar levels in the rinsate were measured as described by Leckie et al. (2012), in which the Nelson reaction originally used to measure sugar was replaced by a modified peroxidase/glucose oxidase assay (Setter et al. 2001). This modified assay provides improved results and eliminates use of arsenic compounds. Total acylsugar data were reported as micromoles of acylsugars per gram of dried leaf tissue (μ mol/g).

Statistical procedures were performed using the statistical software SAS (version 9.1; SAS Institute, Cary, NC). Egg numbers from the no-choice bioassay based on Tanglefoot and leaf disc tests were transformed by natural logarithm (1n) before analysis. Data of spider mite resistance parameters in both choice and no-choice bioassays, densities of type I, IV, V and VI trichomes, and acylsugar content were subjected to one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test ($p = 0.05$). Linear correlations were calculated among densities of type I, IV, V and VI trichomes, and the number of eggs in the no-choice bioassay based on the Tanglefoot test and acylsugar content.

Results

Spider mite resistance in no-choice and choice bioassays

Two types of no-choice tests, Tanglefoot and leaf disc, were used to screen tomato accessions for spider mite resistance. These accessions were compared to the susceptible control for the number of eggs 2 days after infestation (Table 1). The Tanglefoot test revealed highly significant differences between accessions and

the check for number of eggs on the abaxial and adaxial leaf surfaces ($p < 0.001$). Eggs were deposited mostly on the adaxial surface except for *S. cheesmaniae* accession VI037238 and the susceptible check CL5915. Mean number of eggs on the abaxial and adaxial surfaces of leaflets increased 2 to 20-fold on CL5915 and *S. cheesmaniae* accession VI037238 compared to all *S. galapagense* accessions, *S. cheesmaniae* accession VI037240 and *S. pimpinellifolium* accession VI030462. Interestingly, no eggs were found on the abaxial and adaxial surfaces of leaflets of *S. cheesmaniae* accession VI037240 and very few eggs were found on *S. galapagense* accession VI057400. All *S. galapagense* displayed significantly higher spider mite resistance compared to the susceptible check and egg number dropped to zero on the abaxial surface for four of eight *S. galapagense* accessions along with *S. cheesmaniae* accession VI037240 and *S. pimpinellifolium* accession VI030462. In the leaf disc test, highly significant differences between accessions and the check were found for egg number ($p < 0.001$). High levels of resistance were observed in all *S. galapagense*, *S. cheesmaniae* accession VI037240, and *S. pimpinellifolium* accession VI030462. Conversely, the number of eggs increased 5 to 55-fold on CL5915 and 8 to 88-fold on *S. cheesmaniae* accession VI037238, which were similar to results of the Tanglefoot test (Table 1). This was also evident from high correlations between the leaf disc and Tanglefoot tests ($r = 0.92$ $p < 0.0001$ for abaxial surface and $r = 0.72$ $p < 0.0001$ for adaxial surface).

Selected tomato accessions were evaluated for spider mite damage in the choice bioassay 5 days after infestation (Fig. 1). The ANOVA revealed highly significant differences among accessions and the check for the damage ($p < 0.001$). All CL5915 plants, *S. cheesmaniae* accession VI037238, and *S. pimpinellifolium* accession VI030462 had severe damage. None of the accessions were immune to spider mite but all *S. galapagense* accessions and *S. cheesmaniae* accession VI037240 sustained significantly less damage than CL5915.

Trichome diversity and its relationship to spider mite resistance

Trichome types I, IV, V and VI in selected tomato accessions and the check were evaluated on both the

abaxial and adaxial leaf surfaces in Tanglefoot tests; types II and III were ignored because they have not been associated with insect resistance. The analysis of variance revealed highly significant differences ($p < 0.001$) between accessions and the check for density of trichome types IV, V and VI (Table 2). Type IV trichomes were abundant on the abaxial and adaxial leaf surfaces of all *S. galapagense* accessions and *S. cheesmaniae* accession VI037240, whereas the check CL5915 and *S. cheesmaniae* accession VI037238 lacked type IV. Conversely, type V trichomes were absent in all *S. galapagense* accessions and *S. cheesmaniae* accession VI037240, whereas the check and *S. cheesmaniae* accession VI037238 contained relatively high densities of type V trichomes. It is noteworthy that type IV trichomes on *S. pimpinellifolium* accession VI030462 were low on the adaxial leaf surface but were relatively high on the abaxial surface. Most accessions were devoid of type VI trichomes or developed sparse densities of lobe-shaped type VI trichomes; notably, *S. pimpinellifolium* accession VI030462 developed relatively high densities of spherical-shaped type VI trichomes. Type I trichomes were present on all accessions and the check, but uniformly low on the abaxial and adaxial leaf surfaces with a mean density rating of 1. All resistant accessions produced high densities of type IV trichomes and no type V trichomes. Correlations were subjectively categorized as low (± 0.18 – 0.30), moderate (± 0.31 – 0.50), high (± 0.51 – 0.75) and very high ($\pm \geq 0.76$). Type IV trichome density was negatively correlated with egg number on both leaf surfaces. In contrast, significant positive correlations were detected between type V trichome density and number of eggs on both leaf surfaces. Furthermore, correlations between number of eggs on the abaxial and adaxial leaf surfaces and type I trichomes and type VI trichomes were either weakly statistically significant or not significant; only the correlation between number of eggs on the abaxial leaf surface showed a moderate negative correlation.

Acylsugar content and its relationship to spider mite resistance and trichomes

Large differences ($p < 0.001$) in total acylsugar content were found among accessions and the check (Fig. 2). All *S. galapagense* accessions accumulated 9 to 22-fold higher levels of acylsugars ($\mu\text{mol acylsugar/}$

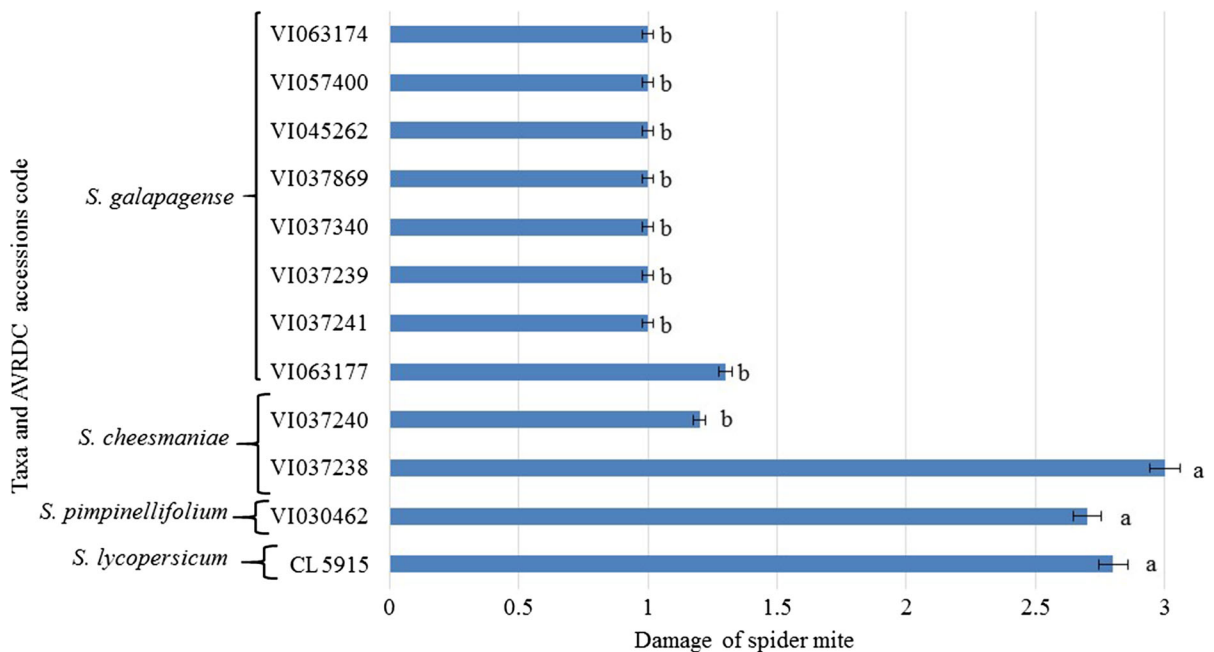


Fig. 1 Spider mite damage in selected wild tomato accessions (*Solanum* spp.) and tomato check (CL5915) in the growth room 5 days after spider mite infestation. Ratings assessed visually 5 days after spider mite infestation, and are based on the leaf damage using a 0–3 scale, where 0 = no damage, 1 = few

small feeding patches (<20 % of leaf area); 2 = large feeding patches 20–49 % of leaf area; 3 = entire leaf with feeding marks (≥50 % of leaf area). Mean ± SE ($n = 10$) followed by different letters are significantly different according to least significant difference (LSD) test at $p < 0.05$

g dry leaf tissue) compared to CL5915. Total acylsugar content in *S. cheesmaniae* accession VI037240 and *S. pimpinellifolium* accession VI030462 were also significantly greater (7 to 12-fold increase) than the check. In contrast, CL5915 and *S. cheesmaniae* accession VI037238 accumulated low acylsugar levels (<4.2 μmol acylsugar/g dry leaf tissue). Correlation analysis indicated strong associations between acylsugar accumulation, spider mite resistance based on egg number, and trichome types. Highly significant negative correlations were obtained between total acylsugar content and number of eggs from the Tanglefoot test ($r = -0.73$ for abaxial surface and $r = -0.68$ for adaxial surface). Type IV trichome density on the abaxial and adaxial leaf surfaces was positively correlated with total acylsugar content (Table 3). Conversely, negative and highly significant correlations were found between type V trichome density on the abaxial and adaxial leaf surfaces and total acylsugar content (Table 3). Furthermore, correlations between acylsugar content and type I trichomes and type VI trichomes on the abaxial

and adaxial leaf surfaces were either weakly statistically significant or not significant.

Discussion

The two spotted spider mite, *T. urticae*, is one of the most economically damaging pests of cultivated tomato, particularly in Southeast Africa, West Africa, South Asia, Southeast Asia, Europe and Mediterranean countries. Sustainable chemical control of spider mites is difficult because of its capacity to rapidly develop resistance to new acaricides (Dermauw et al. 2012). Spider mite resistant tomato cultivars, if available, would offer growers an inexpensive and easier method of pest control.

In general, some wild tomato species are far less attractive to pests than cultivated tomato due to their glandular trichomes and production of effective defense compounds. Most previous studies focused on identification of spider mite resistance from distant wild tomato species including accessions of *S.*

Table 2 Mean numbers of trichome type densities on wild tomato accessions compared to the tomato check CL5915 evaluated in the Tanglefoot no-choice bioassay

Taxa and AVRDC accessions code	Other codes	Trichomes density/mm ^{2z}							
		Type I		Type IV		Type V		Type VI	
		Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Abaxial	Abaxial	Adaxial
<i>Solanum galapagense</i>									
VI057400	LA 483	1.0	1.0	15.3 bc	11.0 ab	0.0 c	0.0 d	2.3 bc	2.0 cd
VI045262	LA 1141	1.0	1.0	15.5 bc	10.3 ab	0.0 c	0.0 d	2.3 bc	2.3 cd
VI037869	LA 1408	1.0	1.0	19.0 abc	12.8 a	0.0 c	0.0 d	1.8 cd	1.5 cd
VI037239	LA 436	1.0	1.0	16.0 bc	7.6 b	0.0 c	0.0 d	3.0 bc	3.8 bc
VI063177	LA 0530	1.0	1.0	22.0 ab	10.0 ab	0.0 c	0.0 d	2.3 bc	3.3 bc
VI037241	LA 526	1.0	1.0	17.8 abc	8.6 ab	0.0 c	0.0 d	2.6 bc	2.4 bcd
VI063174	LA 0438	1.0	1.0	14.6 c	7.2 b	0.0 c	0.0 d	2.8 bc	3.4 bc
VI037340	LA 1408	1.0	1.0	13.5 c	11.3 ab	0.0 c	0.0 d	2.3 bc	1.8 cd
<i>Solanum cheesmaniae</i>									
VI037240	LA 483	1.0	1.0	23.5 a	12.5 a	0.0 c	0.0 d	4.5 b	5.3 b
VI037238	LA 429	1.0	1.0	0.0 d	0.0 c	5.0 b	2.0 b	0.0 d	0.0 d
<i>Solanum pimpinellifolium</i>									
VI030462	PI 390519	1.0	1.0	4.6 d	1.4 c	1.0 c	1.0 c	8.2 a	8.8 a
<i>Solanum lycopersicum</i>									
CL5915		1.0	1.0	0.0 d	0.0 c	9.0 a	5.0 a	3.4 bc	2.4 bcd

Means followed by different letters within columns are different according to least significant difference (LSD) test at $p < 0.05$

^z Type—trichome densities were evaluated in a 1 mm² area during no-choice bioassay based on Tanglefoot method as described in Materials and Methods

habrochaites f. *glabratum* (PI 134417, PI 251304, PI 134418 and PI 126449), *S. habrochaites* (PI 127826, LA1777, LA 1740 and LA 2860) and *S. pennellii* (LA 716, LA 2963 and LA 2580) (Gentile et al. 1969; Snyder and Carter 1984; Antonious and Snyder 2006; Saeidi and Mallik 2006; Onyambus et al. 2011; Bleeker et al. 2012; Lucini et al. 2015). More than 250 wild tomato accessions were recently screened at AVRDC including *S. galapagense*, *S. cheesmaniae* and *S. pimpinellifolium* and high levels of whitefly (*B. tabaci*) resistance were identified in one or more accessions of each species (Rakha et al. 2015). These species are closely related to cultivated tomato and introgression of resistance into cultivated tomato from these species should be easier and faster compared to the green-fruited species *S. pennellii*, and *S. habrochaites* (Rick, 1971; Peralta et al. 2008).

In this study we evaluated the previously identified whitefly-resistant accessions of *S. galapagense*, *S. cheesmaniae* and *S. pimpinellifolium* for spider

mite resistance based on oviposition in no-choice bioassays and damage scores in choice bioassays. The previous whitefly resistance study indicated resistance in these accessions was based on the presence of high densities of glandular type IV trichomes resulting in high foliar acylsugar content, and absence or low densities of type V trichomes. Our purpose was to determine if some or all of these accessions were also resistant to spider mite, and if this resistance was based on the same mechanisms. We found that most whitefly-resistant accessions were also resistant to spider mite, the most resistant accessions being *S. galapagense* VI057400, VI045262, VI037869 and VI037239, and *S. cheesmaniae* VI037240. To our knowledge, this is the first report of resistance to *T. urticae* in these accessions. Spider mite resistance in these accessions was expressed in early plant stages (4–6 weeks old) and resulted in relatively low leaf damage and egg number; early trichome development is advantageous because tomato seedlings are often

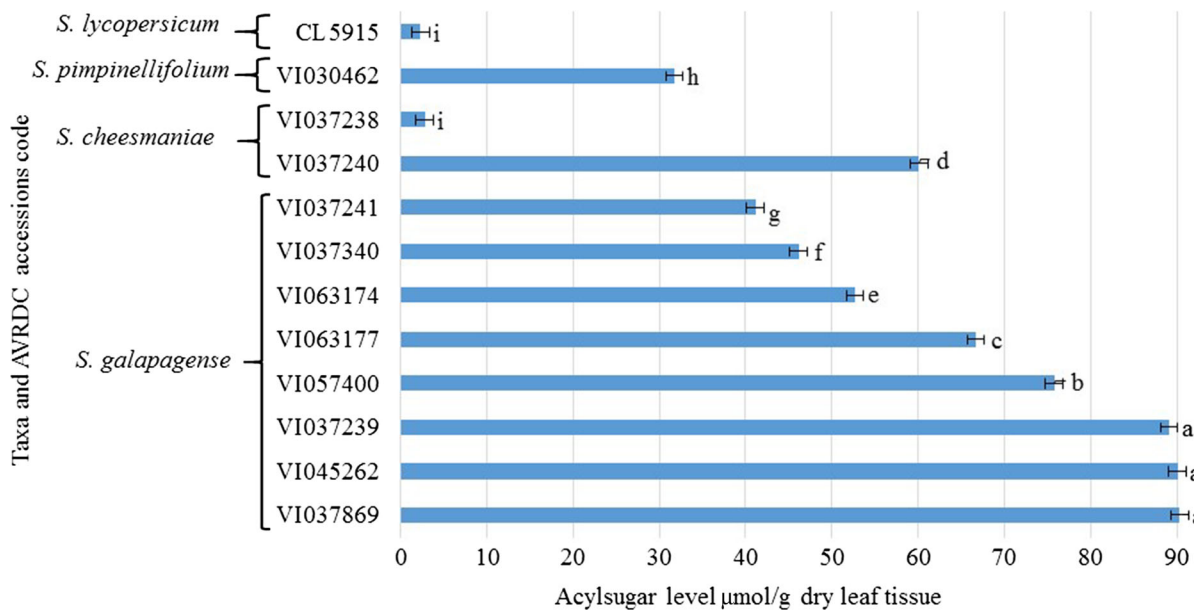


Fig. 2 Total acylsugars levels (μmol acylsugar/g dry leaf tissue) measured in selected wild tomato (*Solanum* spp.) accessions and tomato check (CL5915) 6 weeks after sowing.

Mean \pm SE ($n = 5$) followed by different letters according to least significant difference (LSD) test at $p < 0.05$

Table 3 Linear correlations between trichome types, total acylsugar and *T. urticae* egg numbers of selected wild tomato accessions evaluated in the Tanglefoot no-choice bioassay

Trichomes density/mm ^{2z}	Total acylsugar ($\mu\text{mol/g}$ dry leaf tissue)	Eggs (no.) ^y	
		Adaxial	Abaxial
Type I (adaxial)	0.00 ^{ns}	-0.00 ^{ns}	-0.00 ^{ns}
Type I (abaxial)	0.08 ^{ns}	-0.25 ^{ns}	-0.06 ^{ns}
Type IV (adaxial)	0.68**	-0.57**	-0.64**
Type IV (abaxial)	0.69**	-0.55**	-0.69**
Type V (adaxial)	-0.71**	0.52**	0.87**
Type V (abaxial)	-0.68**	0.53**	0.87**
Type VI (adaxial)	0.04 ^{ns}	-0.09 ^{ns}	-0.33*
Type VI (abaxial)	0.05 ^{ns}	-0.13 ^{ns}	-0.25 ^{ns}

^{ns} Indicates not significant

^z Type—trichome densities were evaluated in a 1 mm² area during no-choice bioassay by Tanglefoot method as described in Materials and Methods

^y *T. urticae* egg numbers were counted on the abaxial and adaxial leaf surfaces of selected wild tomato accessions evaluated in no-choice bioassay by Tanglefoot method

*, ** Indicate significance at $p < 0.05$ and $p < 0.001$, respectively

exposed to spider mite infestation in greenhouses before field transplanting. Fortunately, densities of glandular trichomes also increased with the plant age (data not shown) on resistant accessions.

We found high densities of type IV trichomes and acylsugar content in the *S. galapagense* accessions (VI057400, VI045262, VI037869 and VI037239) and *S. cheesmaniae* accession VI037240. The reduction in

egg number in the no-choice bioassay was highly correlated with high densities of type IV trichomes and total acylsugar content, suggesting the presence of host non-preference (antixenosis) and/or preference (antibiosis). All released adult females on these accessions stuck on the sticky exudate from glandular trichomes on the adaxial leaf surface and died (data not shown). This exudate blocked the mobility of the spider mites on the leaf surface to crawl from leaf to leaf (Van Haren et al. 1987; Lucini et al. 2015). Ample evidence links glandular trichomes, acylsugars and resistance to many arthropod pests including spider mite (Good and Snyder 1988; Maluf et al. 2007; Saeidi et al. 2007; Onyambus et al. 2011; Lucini et al. 2015), whitefly (Firdaus et al. 2012, Lucatti et al. 2013) and other arthropod pests (Maluf et al. 2001; Resende et al. 2006). Foliar application of acylsugars from *S. pennellii* LA716 reduces feeding of aphids *Myzus persicae* and *Macrosiphum euphorbiae* (Goffreda et al. 1989; Rodriguez et al. 1993) and sharply reduced oviposition and feeding of the leafminer, *Liriomyza trifolii* (Hawthorne et al. 1992) and whitefly, *B. tabaci* (Liedl et al. 1995). Lucini et al. (2015) also showed that acylsugars hampered the mobility of the mite on the leaf surface, ultimately causing death. It is highly likely that acylsugars accounted entirely or partly for the spider mite resistance in the *S. galapagense* accessions. The production of acylsugars and determination of acylsugar composition in tomato is a complex trait involving many loci. Previous research has identified multiple QTL associated with acylsugar production in populations derived from *S. pennellii* LA716 (Liedl et al. 1995; Leckie et al. 2012). It is noteworthy that *S. cheesmaniae* accession VI037240 developed similar densities of type IV trichomes but accumulated much less acylsugars per gram of dry leaf tissue than the *S. galapagense* accessions. Lucatti et al. (2013) also confirmed that *S. galapagense* accessions in general accumulate higher levels of acylsugars than *S. cheesmaniae* accessions. Thus, the presence of high type IV trichome numbers does not necessarily result in high acylsugars. *S. pimpinellifolium* accession VI030462 exhibited resistance in the no-choice bioassay based on the number of eggs, whereas the same accession was severely damaged in the choice bioassay. The low density of type IV trichomes on the adaxial leaf surface could explain the severe damage observed on this accession in the choice bioassay. However, most of the mites that fed on this accession

eventually died (data not shown), which could be due to the presence of some specific toxic compound(s) in or on VI030462 leaves that cause antibiosis. Only VI030462 developed a high density of spherical-shaped type VI trichomes while the other accessions had lobe-shaped type VI trichomes. Differences in type VI trichome shape may be related with differences in the kinds and concentrations of chemicals associated with insect resistance. Ben-Israel et al. (2009) found that spherical-shaped type VI trichomes accumulated higher concentrations of methylketone compared to lobe-shaped type VI trichomes in F₂ interspecific populations derived from *S. habrochaites* f. *glabratum* C. H. Müll. Similar associations were found between the presence of spherical-shaped type VI trichomes and whitefly resistance of VI030462 based on numbers of whitefly adults, nymphs, pupa, and eggs in the choice bioassay, and adult mortality and number of eggs from the no-choice bioassays (Rakha et al. 2015), suggesting the presence of similar mechanisms of resistance to spider mite and whitefly in this accession. Besides high type IV trichome density, whitefly resistance has been associated with sparsity or absence of type V trichomes (Butter and Vir 1989; Firdaus et al. 2012; Lucatti et al. 2013; Rakha et al. 2015). In this study only the susceptible check and one *S. cheesmaniae* accession, VI037238, produced type V trichomes, so the relationship of type V trichomes to spider mite resistance cannot be assessed here. In this study, type VI trichomes were not correlated with spider mite resistance, which agrees with the findings of Good and Snyder (1988) and Onyambus et al. (2011).

Leaf disc techniques frequently have been used to screen for spider mite resistance on tomato (Onyambus et al. 2011; Bleeker et al. 2012), strawberry (Giménez-Ferrer et al. 1993) and muskmelon (East et al. 1992). We found high and significant correlations between results of the leaf disc and Tanglefoot tests in the no-choice bioassay, indicating that the leaf disc test provided equivalent results. Tanglefoot tests were used successfully to screen spider mite resistance in muskmelon (East et al. 1992), but this method is very time consuming, labor intensive and not easily applicable to screening large plant populations. Compared to the Tanglefoot test, leaf disc tests are easier to conduct under controlled conditions and a small growth room is sufficient to screen large numbers of accessions/populations. An additional advantage of

the leaf disc is the option to carry out the test in a choice situation for spider mite resistance as described by Bleeker et al. (2012). Similar results from in vitro leaf disc tests and in vivo no-choice tests were observed in whitefly (Firdaus et al. 2012) and thrips resistance assessments (Maharijaya et al. 2011).

It is possible that the identified whitefly/spider mite-resistant accessions could offer resistance to other important tomato pests and future studies are planned to assess their resistance to South American tomato leafminer, (*Tuta absoluta*), other leafminer species (e.g. *L. trifolii*), tomato fruit worm (*Helicoverpa armigera*) and armyworm (*Spodoptera litura*). Resistance to each insect may depend on the susceptibility of the targeted insect to acylsugars and the levels and types of acylsugars produced, presence of other, non-acylsugar based insect resistance, glandular trichomes, coverage of foliar tissue (abaxial and adaxial leaf surfaces), effects of plant age, temperature, light intensity and other environmental factors on accumulation of acylsugars (Gurr and McGrath 2001; Kang et al. 2010, Lucatti et al. 2013; Puentes and Ågren, 2013). Although breeders would be interested to know the minimum acylsugar levels required for spider mite resistance, these cannot be determined from this study. Answers to these questions will inform decisions on the choice of accessions to introgress resistance and overall insect resistance strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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