



# Evaluation of resistance in wild tomato accessions to the whitefly *Bemisia tabaci* and the invasive tomato leafminer *Tuta absoluta*

Sunil Kumaraswamy<sup>1,2</sup>, Paola Sotelo-Cardona<sup>3</sup>, Aparna Shivanna<sup>4</sup>,  
Muthugounder Mohan<sup>1</sup>, Ramasamy Srinivasan<sup>3,\*</sup>

<sup>1</sup> ICAR-National Bureau of Agricultural Insect Resources, Bengaluru 560024, India

<sup>2</sup> University of Agricultural Sciences, Bangalore 560065, India

<sup>3</sup> World Vegetable Center, 60 Yi-Min Liao, Shanhua, Tainan 74151, Taiwan, China

<sup>4</sup> World Vegetable Center, South and Central Asia, ICRISAT Campus, Hyderabad 502324, India

\* Corresponding author: srini.ramasamy@worldveg.org

With 2 figures and 2 tables

**Abstract:** The global tomato cultivation faces serious threats from the whitefly *Bemisia tabaci* (Gennadius) and the invasive tomato leafminer *Tuta absoluta* (Meyrick). Identifying new resistant sources and exploring resistance mechanisms is crucial for long-term management of these rapidly spreading insects. This study evaluated antixenosis and antibiosis resistance in wild tomato accessions of *Solanum cheesmaniae* and *Solanum galapagense* through trichome characteristics and various assays, revealing resistance to *B. tabaci* (Asia II 7 biotype) and *T. absoluta*. Wild accessions resulted in reduced oviposition, increased adult mortality of whiteflies, fewer whitefly developmental stages, disrupted leafminer developmental stages, and reduced adult emergence. Moreover, high densities of type-IV glandular trichomes in wild accessions correlated negatively with the resistance parameters of both insects, influencing their preference and performance. Variations in resistance parameters were identified between wild and cultivated accessions, with *S. galapagense* (VI063117-10) demonstrating superior resistance. This study emphasizes the significance of understanding resistance traits in wild accessions to manage pest insects with diverse feeding behaviors and utilize them in developing multiple insect-resistant tomato varieties.

**Keywords:** Cryptic species; South American tomato pinworm; antixenosis; antibiosis; trichome

## 1 Introduction

Tomato (*Solanum lycopersicum* L.) is a widely cultivated solanaceous vegetable crop valued for its edible fruits. However, its production faces significant challenges from various arthropod pests. Among these pests, the whitefly *Bemisia tabaci* (Gennadius) poses a global threat due to its adaptability and exhibits cryptic species complex, including Asia II 7 biotype, which primarily infests Solanaceae and Fabaceae plants (Kanakala & Ghanim 2019). *Bemisia tabaci* inflicts direct damage on tomato plants as a phloem feeder, causing physiological disorders and promoting the growth of sooty mold, ultimately reducing photosynthetic efficiency (Andrade et al. 2017). Additionally, it can act as a vector for tomato yellow leaf curl virus (TYLCV), leading to lower yields and reduced fruit quality (Czosnek & Laterrot 1997).

Another significant threat to tomato crops is the invasive tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera:

Gelechiidae). This pest is highly destructive, causing extensive damage to both leaves and fruits throughout the crop cycle, resulting in substantial yield losses (Desneux et al. 2022). Moreover, *T. absoluta* has spread globally from its origin, affecting the regions of Afro-Eurasia, Europe, and Asia (Biondi et al. 2018; Han et al. 2019a). The establishment of *T. absoluta* is also favored by the presence of alternate host plants such as potato, eggplant, tobacco, and wild tomatoes (Cherif & Verheggen 2019).

Crop resistance breeding utilizing antixenosis (tissue hardness, wax, spines, and trichomes) and antibiosis (metabolites/toxins) traits from plants is crucial for cost-effective and long-term integrated pest management. Utilizing resistant wild genotypes of *Solanum galapagense* (Darwin & Peralta) and *Solanum pimpinellifolium* (L.) in traditional breeding holds promise for developing insect-resistant tomato varieties, given their close relationship to *S. lycopersicum*, ensuring breeding compatibility (Andrade et al. 2017). Furthermore,

trichomes have been identified as plant epidermal extensions functioning as a protective barrier against herbivores (Zhang et al. 2020). *Solanum* species exhibit seven trichome types, including four glandular (I, IV, VI, and VII) and three non-glandular trichomes (II, III, and V) (Channarayappa et al. 1992; Luckwill 1943). Glandular trichomes in wild tomato species of *Solanum cheesmaniae* (Riley), *S. galapagense*, *S. pimpinellifolium*, *Solanum habrochaites* (Knapp & Spooner) and *Solanum pennellii* (Correll) act as a protective barrier and produce secondary metabolites, providing structural and chemical defense against herbivores including *B. tabaci* (Rakha et al. 2017a), *T. absoluta* (Rakha et al. 2017b), *Myzus persicae* Sulzer (da Silva et al. 2019) and *Tetranychus urticae* Koch (Rakha et al. 2017c).

Wild tomato species have been acknowledged for their resistance to various tomato herbivores. Yet, studies evaluating their resistance across different accessions involving insects with diverse feeding behaviors and specific cryptic species are lacking. With the increasing requirement of insect-resistant tomato varieties, there is a priority on introgressing resistance traits from wild accessions into cultivated varieties (Kortbeek et al. 2021). Identifying and comprehending the antixenosis and antibiosis resistance mechanisms in wild tomato accessions through trichome characteristics, choice and no-choice bioassays against *B. tabaci* and *T. absoluta* is crucial for the development of multi-resistant varieties that can withstand attacks from various insects, reducing reliance on pesticides (Han et al. 2019b).

This study investigates how two tomato herbivores choose and perform on different tomato accessions, the phloem-feeding *B. tabaci* and the leafminer *T. absoluta*. By analyzing their oviposition preference and overall performance, we aim to identify accessions that are resistant to both insects. We hypothesize that accessions with glandular trichomes may deter both pests better than those with fewer or no trichomes. In addition, wild accessions may have built-in defenses that adversely affect insect development. Finally, by analyzing insect interactions with different tomato accessions, we expect to identify promising resistant traits that provide broad-spectrum resistance to insects with different feeding behaviors.

## 2 Material and methods

### 2.1 Plant material and growth conditions

Three wild tomato accessions (*S. cheesmaniae* (VI037240-7), *S. galapagense* (VI057400-3 and VI063177-10)) and two cultivated accessions of *S. lycopersicum* (Hawaii-7996 and CLN-5915) (Fig. S1) were selected to assess resistance against *B. tabaci* and *T. absoluta*. The seeds of wild tomato accessions were provided by M/S I&B Seeds Pvt Ltd, Bengaluru, India, and the cultivated accessions were obtained from the World Vegetable Center, South and Central Asia, ICRISAT campus, India. Seedlings were initially raised in

coco peat-filled nursery trays, then grown in pots filled with a sterilized potting mixture (2:2:1 ratio of black soil, sand, and vermicompost) in a glasshouse ( $27 \pm 1$  °C,  $70 \pm 10\%$  RH, 12:12 light-dark cycles), and six-week-old plants were used for resistance evaluation.

### 2.2 Rearing and cryptic species identification of *B. tabaci*

The initial *B. tabaci* population, collected from cotton plants at ICRISAT, Hyderabad, India ( $17.5111^{\circ}\text{N}$ ,  $78.2752^{\circ}\text{E}$ ), was reared on eggplants inside a glasshouse ( $27 \pm 1$  °C,  $70 \pm 10\%$  RH, 12:12 hours light-dark cycles) within a cage ( $45 \times 45 \times 45$  cm). The whitefly population was identified as *B. tabaci* Asia II 7 (Fig. S2) through Bayesian analysis of mitochondrial cytochrome oxidase I (*mtCOI*) (Tay et al. 2012).

### 2.3 Rearing of *T. absoluta*

*Tuta absoluta* were initially collected from a tomato field at ICRISAT and the larvae were reared on tomato plants (cv. Vasudha, RK hybrid seeds, Secunderabad, Telangana, India) inside a glasshouse ( $27 \pm 1$  °C,  $70 \pm 10\%$  RH, 12:12 hours light-dark cycles). Pupae obtained from these plants were placed in a separate cage ( $75 \times 75 \times 75$  cm) for adult emergence. The emerged adults were fed *ad libitum* with 10% honey solution and allowed to mate and oviposit on potted tomato plants for colony multiplication.

### 2.4 Analysis of leaf trichome type and density

Trichomes were identified based on the description by Luckwill (1943) and revision by (Channarayappa et al. 1992; Glas et al. 2012) (Table S1). Trichome density on the adaxial (upper) and abaxial (lower) leaf surfaces was assessed using 5mm<sup>2</sup> leaf discs from 30 plants (6 per accession), as described by (Firdaus et al. 2012).

### 2.5 No-choice and choice oviposition assay of *B. tabaci*

The oviposition preference of *B. tabaci* on tomato accessions was assessed in no-choice and choice assays inside a glasshouse ( $27 \pm 1$  °C,  $70 \pm 10\%$  RH, 12:12 hours light-dark cycles). In the no-choice oviposition assay, ten adult female whiteflies were released into each clip cage (2.5 cm radius and 1.5 cm depth) installed on second and third fully expanded leaves of each tomato accession (Fig. S3A). After three days, dead/alive adults and eggs were counted using a stereo microscope (Magnus MSZ-TR, Olympus Opto Systems, India) as described by (Momotaz et al. 2010). In the choice oviposition assay, one hundred female whitefly adults were released into each cage ( $75 \times 75 \times 75$  cm) with five equidistantly arranged accessions (one plant per accession) (Fig. S3B). After three days, adults and eggs were counted on each accession, and subsequently, the adults were removed from the cage. The number of *B. tabaci* developmental stages was assessed over 10-, 15-, and 19-days post-introduction. Both

no-choice and choice assays were replicated ten times using 50 plants (ten per accession) in each assay.

## 2.6 No-choice and choice oviposition assay of *T. absoluta*

In the no-choice oviposition assay, each tomato accessions were placed in separate cages ( $65 \times 25 \times 25$  cm) in a glasshouse ( $27 \pm 1$  °C,  $70 \pm 10\%$  RH, 12:12 light-dark cycles) (Fig. S3C). Two pairs of two-day-old *T. absoluta* adults were introduced into each cage for oviposition. A cotton plug soaked in 10% honey solution served as an *ad libitum* food source for adults. After two days, the eggs laid on each accession were recorded following adult removal. For the choice oviposition assay, ten pairs of two-day-old *T. absoluta* adults were released into each cage ( $75 \times 75 \times 75$  cm) containing five equidistantly arranged tomato accessions (one plant per accession) in a glasshouse ( $27 \pm 1$  °C,  $70 \pm 10\%$  RH, 12:12 light-dark cycles) (Fig. S3B). After two days, the number of eggs on each accession was recorded following adult removal. Both no-choice and choice assays were replicated ten times using 50 plants (ten per accession) in each assay.

## 2.7 Larval no-choice assay of *T. absoluta*

A no-choice assay assessed the antibiosis resistance of tomato accessions on *T. absoluta* larvae under laboratory conditions ( $26 \pm 1$  °C, 70% RH). Ten second-instar larvae, with an average length of  $1.610 \pm 0.070$  mm, were placed in each Petri dish free from dust or contamination and were fed with leaves of each accession separately (Fig. S3D). The larval mortality was recorded at 3-, 6-, 9-, 12-, and 15 days post-introduction. Pupation parameters and adult emergence were also recorded, and the experiment was replicated ten times.

## 2.8 Statistical analysis

Count data on trichome densities, whitefly developmental stages, and *T. absoluta* eggs were square root-transformed ( $\sqrt{x+0.5}$ ), while larval mortality and adult emergence percentage underwent  $\text{Arcsin}(\sqrt{x})$  transformation before statistical analysis, addressing issues of non-normal distribution and heterogeneous standard deviations in biological variables to enhance assumption fit (McDonald 2014). Transformed data was subjected to ANOVA and Tukey-Kramer honestly significant difference (HSD) test ( $p = 0.05$ ). The difference between the adaxial and abaxial trichome densities was compared using a student's t-test ( $p = 0.05$ ). The statistical software SAS (v9.1; SAS Institute, Cary, NC) was used to analyze the trichome data, and R studio (v4.1.3) was used to assess resistance parameters. Pearson's correlation was used to assess the relationship between resistance parameters of *B. tabaci*, *T. absoluta*, and trichome densities. Partial least square discriminant analysis (PLS-DA) with variable importance plot (VIP) was performed to observe the differences in resistance parameters between wild and cultivated tomato accessions, highlighting the top param-

eters crucial for cluster separation (Macel et al. 2019). The top-ranked parameter in each assay was analyzed for logical relations using the Jvenn program to identify exclusive and shared resistance parameters among accessions (Bardou et al. 2014).

## 3 Results

### 3.1 Trichome type and density in wild tomato accessions

Tomato accessions exhibited three glandular (type-I, IV, and VI) and three non-glandular types of trichomes (type-II, III and V). The densities of type-IV, V, and VI trichomes varied significantly between wild and cultivated accessions on both adaxial ( $F_{4,20} = 33.56$ ,  $p < 0.001$ ) and abaxial ( $F_{4,20} = 94.06$ ,  $p < 0.001$ ) leaf surfaces, whereas, the densities of type-I, II, and III trichomes were consistently low and non-significant. Furthermore, the wild accessions (VI037240-7, VI057400-3, and VI063177-10) showed significantly higher densities of type-IV trichomes, while cultivated accessions (Hawaii-7996 and CLN-5915) had significantly higher densities of type-V and type-VI trichomes on both leaf surfaces (Table S2).

Additionally, trichome densities varied greatly between leaf surfaces. Abaxial leaf surfaces generally exhibited higher densities of type-IV and type-VI trichomes across accessions, except for VI057400-3. Hawaii-7996 and CLN-5915 showed significantly higher densities of type-V trichomes on their abaxial surfaces, while no significant differences were observed in wild accessions. Further, type-VI trichomes were more abundant on the abaxial leaf surface of VI063177-10 and the adaxial leaf surface of CLN-5915, with no significant difference in other accessions, suggesting an overall higher trichome density on the abaxial surface (Fig. S4).

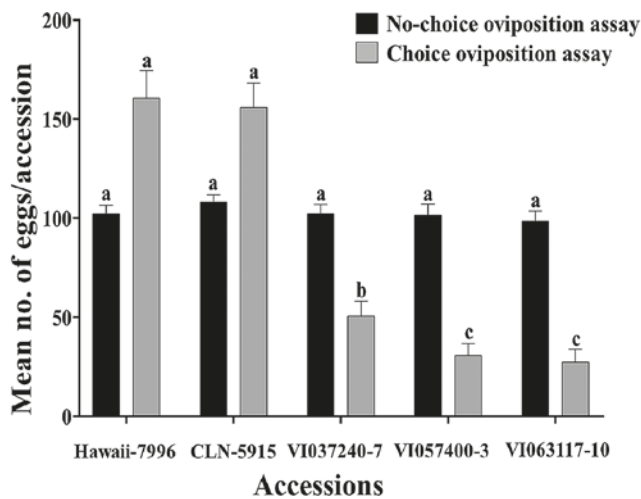
### 3.2 No-choice and choice oviposition assay of *B. tabaci*

In the no-choice oviposition assay, whiteflies deposited significantly more eggs on Hawaii-7996 and CLN-5915 compared to wild accessions ( $F_{4,49} = 104.8$ ,  $p < 0.001$ ), with VI057400-3 and VI063177-10 recording the lowest egg counts. Moreover, wild accessions showed significantly higher adult mortality (94–97%) of whiteflies ( $F_{4,49} = 71.82$ ,  $p < 0.001$ ) (Table 1).

In the choice oviposition assay, adults showed a strong preference for cultivated accessions, depositing significantly more eggs on CLN-5915 and Hawaii-7996 ( $F_{4,49} = 262.8$ ,  $p < 0.001$ ), with VI063177-10 being least preferred for oviposition ( $F_{4,49} = 290.5$ ,  $p < 0.001$ ). Moreover, Hawaii-7996 and CLN-5915 exhibited significantly higher numbers of nymphs ( $F_{4,49} = 304.7$ ,  $p < 0.001$ ) and pupae ( $F_{4,49} = 423.4$ ,  $p < 0.001$ ) compared to wild accessions. Among wild acces-

**Table 1.** Resistance parameters of *B. tabaci* on tomato accessions evaluated in no-choice and choice oviposition assays (glasshouse conditions). Data (means  $\pm$  SE) followed by different letter(s) within a column are significantly different ( $P < 0.05$ , ANOVA followed by HSD posthoc test). DWF (days after whitefly infestation)-whitefly adults were counted 3 and 19 days after whitefly infestation.

Accessions	No-choice oviposition assay			Choice oviposition assay			
	No. of eggs	Adult mortality (%)	Adults at 3 DWF	No. of eggs	No. of nymphs	No. of pupae	Adults at 19 DWF
Hawaii-7996	277.10 $\pm$ 18.03 <sup>a</sup>	46.00 $\pm$ 4.45 <sup>b</sup>	13.80 $\pm$ 1.00 <sup>b</sup>	166.20 $\pm$ 9.39 <sup>b</sup>	106.70 $\pm$ 9.83 <sup>b</sup>	72.40 $\pm$ 5.15 <sup>b</sup>	42.50 $\pm$ 3.70 <sup>b</sup>
CLN-5915	297.80 $\pm$ 10.95 <sup>a</sup>	33.00 $\pm$ 2.70 <sup>b</sup>	24.30 $\pm$ 0.53 <sup>a</sup>	219.80 $\pm$ 15.95 <sup>a</sup>	161.80 $\pm$ 14.63 <sup>a</sup>	103.80 $\pm$ 8.07 <sup>a</sup>	80.70 $\pm$ 8.84 <sup>a</sup>
VI037240-7	36.20 $\pm$ 11.49 <sup>b</sup>	94.00 $\pm$ 3.92 <sup>a</sup>	1.10 $\pm$ 0.31 <sup>c</sup>	26.90 $\pm$ 3.59 <sup>c</sup>	3.90 $\pm$ 1.03 <sup>c</sup>	0.50 $\pm$ 0.30 <sup>c</sup>	0 <sup>c</sup>
VI057400-3	11.80 $\pm$ 3.82 <sup>c</sup>	97.00 $\pm$ 1.33 <sup>a</sup>	1.40 $\pm$ 0.33 <sup>c</sup>	6.10 $\pm$ 1.28 <sup>d</sup>	0 <sup>d</sup>	0 <sup>c</sup>	0 <sup>c</sup>
VI063117-10	18.50 $\pm$ 5.38 <sup>c</sup>	95.50 $\pm$ 1.38 <sup>a</sup>	0.40 $\pm$ 0.16 <sup>c</sup>	1.50 $\pm$ 0.52 <sup>e</sup>	0 <sup>d</sup>	0 <sup>c</sup>	0 <sup>c</sup>
F value	104.81	71.82	262.83	290.55	304.71	423.46	222.43
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
df	4, 49	4, 49	4, 49	4, 49	4, 49	4, 49	4, 49



**Fig. 1.** Number of *T. absoluta* eggs laid (Mean  $\pm$  SE) on tomato accessions in no-choice and choice oviposition assays. Different letters across the similar colored bars indicate significant differences at a 5% significance level.

sions, VI057400-3 and VI063117-10 showed no nymphs or pupae, and VI037240-7 had the negligible number of nymphs and pupae. At 19 days after whitefly infestation (DWF), Hawaii-7996 and CLN-5915 had significantly more adults, while no adults emerged from wild accessions ( $F_{4,49} = 222.4$ ,  $p < 0.001$ ) (Table 1).

### 3.3 No-choice and choice oviposition assay of *T. absoluta*

In the no-choice oviposition assay, the number of eggs laid by *T. absoluta* did not differ significantly between wild and cultivated accessions ( $F_{4,49} = 0.59$ ,  $P = 0.67$ ). However, in the choice oviposition assay, a significant preference was observed with higher egg deposition on Hawaii-7996 and CLN-5915 compared to wild accessions ( $F_{4,49} = 42.53$ ,  $P < 0.001$ ) (Table S3, Fig. 1).

### 3.4 Larval no-choice assay of *T. absoluta*

In the larval no-choice assay, wild accessions induced significantly higher larval mortality (62–72%) than cultivated accessions (28–32%) ( $F_{4,49} = 38.30$ ,  $p < 0.001$ ), with VI063117-10 showing the highest mortality (72%) and CLN-5915 the lowest (28%). Furthermore, extended larval development was observed in wild accessions, with VI063117-10 displaying the longest duration (11.4 days) ( $F_{4,49} = 15.35$ ,  $p < 0.001$ ). Additionally, wild tomato accessions demonstrated extended pupal duration, with VI057400-3 exhibiting the longest duration (8.7 days) ( $F_{4,49} = 2.91$ ,  $p = 0.031$ ), while no significant differences were found in pupal mortality ( $F_{4,49} = 1.64$ ,  $p = 0.178$ ) between wild and cultivated accessions. However, pupal weight ( $F_{4,49} = 9.86$ ,  $p < 0.001$ ), length ( $F_{4,49} = 10.86$ ,  $p < 0.001$ ), and width ( $F_{4,49} = 28.87$ ,  $p < 0.001$ ) were significantly lowest on wild accessions. Adult emergence was also lowest on wild accessions (20–26%) compared to cultivated accessions (49–52%) ( $F_{4,49} = 22.32$ ,  $p < 0.001$ ), with the least emergence observed in VI037240-7 (20%) (Table 2).

### 3.5 Correlation of resistance parameters of *B. tabaci* and *T. absoluta* with trichome densities

The resistance parameters of *B. tabaci* such as the number of nymphs and pupae exhibited a significant negative correlation with type-I (correlation coefficients ( $r$ ) =  $-0.76$ ,  $-0.67$  respectively, all with  $p < 0.001$ ), type-IV ( $r = -0.67$  ( $p < 0.001$ ),  $-0.55$  ( $P = 0.004$ ) respectively) and type-VI ( $r = -0.63$  ( $p < 0.001$ ),  $-0.47$  ( $P = 0.017$ ) respectively) trichomes. While the number of nymphs was positively correlated with type-V ( $r = 0.54$ ,  $P = 0.005$ ) trichomes. Additionally, the number of eggs in the choice assay was negatively correlated with type-IV ( $r = -0.40$ ,  $P = 0.042$ ) and type-VI ( $r = -0.48$ ,  $P = 0.013$ ) trichomes. In contrast, adult mortality and number of adults at 19 DWF were positively correlated with type-I ( $r = 0.77$  ( $P < 0.001$ ),  $0.48$  ( $P = 0.014$ ) respectively), type-IV ( $r = 0.97$  ( $P < 0.001$ ),  $0.79$  ( $P < 0.001$ ) respectively)

**Table 2.** Antibiosis effect of wild tomato accessions on larval mortality, duration, pupal mortality, duration, weight, size, adult emergence of *T. absoluta* (lab conditions). Data (means  $\pm$  SE) followed by different letter(s) within a column are significantly different ( $P < 0.05$ , ANOVA followed by Tukey–Kramer posthoc test).

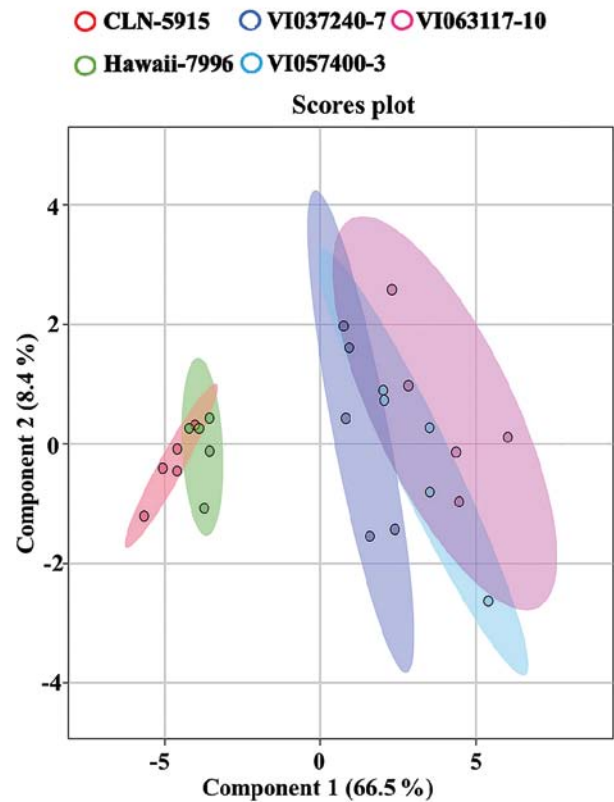
Accessions	Larval mortality (%)	Larval duration (Days)	Pupal mortality (%)	Pupal duration (Days)	Pupal weight (mg)	Pupal length (mm)	Pupal width (mm)	Adult emergence (%)
Hawaii-7996	32.00 $\pm$ 3.59 <sup>b</sup>	10.12 $\pm$ 0.16 <sup>b</sup>	24.37 $\pm$ 4.96 <sup>a</sup>	7.75 $\pm$ 0.11 <sup>b</sup>	4.23 $\pm$ 0.05 <sup>a</sup>	4.64 $\pm$ 0.02 <sup>a</sup>	1.41 $\pm$ 0.01 <sup>a</sup>	52.00 $\pm$ 4.89 <sup>a</sup>
CLN-5915	28.00 $\pm$ 2.49 <sup>b</sup>	10.85 $\pm$ 0.13 <sup>ab</sup>	31.48 $\pm$ 3.91 <sup>a</sup>	8.00 $\pm$ 0.14 <sup>ab</sup>	4.13 $\pm$ 0.03 <sup>a</sup>	4.67 $\pm$ 0.01 <sup>a</sup>	1.41 $\pm$ 0.01 <sup>a</sup>	49.00 $\pm$ 2.76 <sup>a</sup>
VI037240-7	68.00 $\pm$ 3.88 <sup>a</sup>	10.90 $\pm$ 0.12 <sup>ab</sup>	35.50 $\pm$ 6.89 <sup>a</sup>	7.80 $\pm$ 0.16 <sup>ab</sup>	3.75 $\pm$ 0.17 <sup>b</sup>	4.41 $\pm$ 0.08 <sup>b</sup>	1.35 $\pm$ 0.03 <sup>b</sup>	20.00 $\pm$ 2.58 <sup>b</sup>
VI057400-3	62.00 $\pm$ 3.26 <sup>a</sup>	10.30 $\pm$ 0.08 <sup>ab</sup>	31.50 $\pm$ 4.04 <sup>a</sup>	8.70 $\pm$ 0.37 <sup>a</sup>	3.25 $\pm$ 0.14 <sup>c</sup>	4.29 $\pm$ 0.04 <sup>bc</sup>	1.31 $\pm$ 0.02 <sup>b</sup>	26.00 $\pm$ 2.66 <sup>b</sup>
VI063117-10	72.00 $\pm$ 2.49 <sup>a</sup>	11.40 $\pm$ 0.12 <sup>a</sup>	19.16 $\pm$ 7.24 <sup>a</sup>	8.10 $\pm$ 0.19 <sup>a</sup>	3.33 $\pm$ 0.21 <sup>c</sup>	4.14 $\pm$ 0.11 <sup>c</sup>	1.16 $\pm$ 0.03 <sup>c</sup>	22.00 $\pm$ 2.49 <sup>b</sup>
<i>F</i> value	38.30	15.35	1.64	2.91	9.86	10.86	28.87	22.32
<i>P</i> value	< 0.001	< 0.001	0.178	0.031	< 0.001	< 0.001	< 0.001	< 0.001
df	4, 49	4, 49	4, 49	4, 49	4, 49	4, 49	4, 49	4, 49

and type-VI ( $r = 0.92$ ,  $0.77$  respectively, all with  $p < 0.001$ ) trichomes, but negatively correlated with type-V ( $r = -0.52$  ( $P = 0.007$ ),  $0.40$  ( $P = 0.047$ ) respectively) trichomes (Table S4, Fig. S5A).

Among resistance parameters of *T. absoluta*, the number of eggs in the choice assay exhibited a significant negative correlation with type-I ( $r = -0.7$ ,  $p < 0.001$ ) and type-IV ( $r = -0.82$ ,  $p < 0.001$ ) trichomes but positively correlated with type-V ( $r = 0.85$ ,  $p < 0.001$ ) and type-VI ( $r = 0.65$ ,  $p < 0.001$ ) trichomes. The consumed leaf area was negatively correlated with type-IV ( $r = -0.48$ ,  $p = 0.014$ ) trichomes but positively with type-V ( $r = 0.40$ ,  $p = 0.043$ ) trichomes. The larval mortality was positively correlated with type-I ( $r = 0.65$ ,  $p < 0.001$ ) and type-IV ( $r = 0.86$ ,  $p < 0.001$ ) trichomes but negatively with type-V ( $r = -0.64$ ,  $p < 0.001$ ) trichomes. Furthermore, the larval duration was positively correlated with type-IV ( $r = 0.49$ ,  $p = 0.012$ ) trichomes. The pupal duration was positively correlated with type-I ( $r = 0.45$ ,  $p = 0.023$ ) and type-IV ( $r = 0.49$ ,  $p = 0.012$ ) trichomes, while pupal weight, length and width were positively correlated with type-VI ( $r = 0.55$  ( $p = 0.003$ ),  $0.45$  ( $p = 0.023$ ),  $0.46$  ( $p = 0.018$ ) respectively) trichomes. Adult emergence showed a positive correlation with type-V ( $r = 0.57$ ,  $p = 0.002$ ) and type-VI ( $r = 0.68$ ,  $p < 0.001$ ) trichomes (Table S5, Fig. S5B).

### 3.6 Comparison of resistant parameters among tomato accessions

The wild accessions formed a well-separated cluster from cultivated accessions in the PLS-DA scores plot, indicating differences in the expression of resistance (Fig. 2). The top seven parameters, including adult mortality of *B. tabaci*, larval mortality, and larval duration of *T. absoluta*, number of *B. tabaci* eggs in choice oviposition assay, pupal duration of *T. absoluta*, type-IV trichomes and number of *B. tabaci* nymphs, identified by VIP plot, drive the observed separation. The color map on the right indicates the relative values of these parameters across accessions (Fig. S6). Among wild accessions, VI063117-10 emerged as the top performing



**Fig. 2.** PLS-DA scores plot depicting the variability in *B. tabaci* and *T. absoluta* resistance parameters among tomato accessions.

accession in seven resistant parameters, outperforming other resistant wild accessions, while VI057400-3 demonstrated superiority in three parameters compared to VI037240-7, which was topped in one parameter. However, all three accessions performed equally in one parameter, with VI057400-3 and VI063117-10 showing equal performance in two parameters (Table S6, Fig. S7).

## 4 Discussion

In the present study, wild tomato accessions with higher densities of type-IV glandular trichomes exhibited resistance against *B. tabaci* in both the no-choice and choice oviposition assays while exhibiting antixenosis and antibiosis resistance to *T. absoluta* in the choice oviposition and larval no-choice assays, respectively. However, no accessions were resistant to *T. absoluta* in the no-choice oviposition assay. In contrast, cultivated accessions with higher densities of type-V and type-VI trichomes were found to be susceptible.

Host plant resistance has been well documented in wild tomato species, and this resistance is mainly attributed to glandular trichomes. Type-IV glandular trichomes and associated acylsugars in wild accessions of *S. cheesmaniae*, *S. galapagense*, *S. habrochaites*, *S. pennellii*, and *S. pimpinellifolium* have demonstrated effective resistance to *B. tabaci* (Rakha et al. 2017a), including MEAM1 (Narita et al. 2023), as well as *T. absoluta* (Rakha et al. 2017b). In contrast, tomatoes lacking type-IV trichomes are susceptible to *B. tabaci* (Rakha et al. 2017a) and *T. absoluta* (Rakha et al. 2017b). Glandular trichomes induce mortality, impede probing, stylet pathway, phloem-sap uptake, and virus transmission of whitefly via acylsugar secretion (Fortes et al. 2020).

Additionally, leaf characteristics and high phenol content govern tomato resistance to whiteflies and ToLCV (Pal et al. 2021). High fecundity and rapid reproduction of *B. tabaci* facilitate efficient colonization and infestation of host plants (Bellotti & Arias 2001). Similarly, type-V non-glandular trichomes lacking secondary metabolites in *S. lycopersicum* promote whitefly reproduction (Zhang et al. 2020). *Bemisia tabaci* lays eggs on both upper and lower leaf surfaces, and its life cycle, from egg to adult, is approximately 16 to 31 days, depending on the host plants (Sani et al. 2020).

Whiteflies prefer to lay eggs on leaves with non-glandular trichomes for protection from natural enemies (Firdaus et al. 2012). This preference is consistent with the present results, where whiteflies strongly preferred *S. lycopersicum* accessions with high-density type-V non-glandular trichomes for oviposition. Additionally, type-VI glandular trichomes are less effective in repelling insects on tomato plants (Zhang et al. 2020). However, type-VI trichomes in *S. habrochaites* var. *hirsutum* produce sesquiterpenes that confer resistance to *M. persicae*, *T. urticae*, *B. tabaci*, and *T. absoluta* (da Silva et al. 2013; de Oliveira et al. 2020).

*Tuta absoluta* typically lays eggs on the undersides of tomato leaves, and the larvae feed on the leaves, creating characteristic mines and completing their life cycle within 30–35 days (Desneux et al. 2022). Additionally, its diverse feeding habits on Solanaceae crops and its invasive nature complicate control efforts (Biondi et al. 2018). The availability of *T. absoluta*-resistant tomato cultivars is limited; however, trichome-based resistance has been reported in the accessions of *S. cheesmaniae*, *S. galapagense*, *S. pennellii* and *S. pimpinellifolium* (Rakha et al. 2017b). Insects

typically choose the most susceptible host to maximize the survival of their offspring, and fecundity serves as a reliable parameter to assess the impact of the host plant on lepidopteran herbivores (Razmjou et al. 2014).

This study revealed consistent oviposition preferences of *T. absoluta* across all accessions in the no-choice oviposition assay. This suggests that in the absence of the preferred host, *T. absoluta* will oviposit on any available host, regardless of offspring survival, which is supported by the antibiosis resistance observed in *S. cheesmaniae* and *S. galapagense* accessions in the larval no-choice assay. Similar results have been reported previously where accessions of *S. galapagense*, *S. cheesmaniae*, *S. chilense*, *S. pennellii*, and *S. habrochaites* associated with increased production of acylsugars and higher content of zingiberene resulted in high larval mortality, longer developmental time and underdeveloped pupae of *T. absoluta* (Ghosh et al. 2023). Prolonged larval development could increase the insect's susceptibility to natural enemies, potentially enhancing plant resistance (Benrey & Denno 1997), underscoring the importance of considering these indirect effects in resistance breeding programs.

The reduced oviposition preference (i.e., antixenosis) of *T. absoluta* on wild accessions in the choice oviposition assay is attributed to a high density of type-IV glandular trichomes. Similarly, wild tomato species with a high density of glandular trichomes resulted in reduced oviposition and leaf damage by *T. absoluta* (Ghosh et al. 2023). On the other hand, the strong oviposition preference of *T. absoluta* in cultivated accessions may be due to its evolutionary adaptation to *S. lycopersicum*, allowing females to choose optimal sites for offspring survival (da Silva Galdino et al. 2015). Moreover, commercial species lack disruptive allelochemicals and have sparse glandular trichomes, which promote insect development (de Oliveira et al. 2020). The enhanced contact stimulatory effects of host plant volatiles also increase landing and oviposition on host plants compared to non-host plants (Proffit et al. 2011). Additionally, the susceptibility of domesticated tomatoes to pests has been linked to the loss of defensive genes during domestication (Whitehead et al. 2017).

Wild tomato species possess trichomes and various secondary metabolites, including acylsugars and zingiberene, which confer resistance to *B. tabaci* (Rakha et al. 2017a) and *T. absoluta* (Ghosh et al. 2023). Acylsugars consist of glucose and sucrose esters of fatty acids, giving leaves a sticky texture that hinders insect oviposition, feeding and development (Andrade et al. 2017). Additionally, the *Woolly* (Wo) and *Hair* genes, the transcription factor SIMYC1 and specific loci on chromosome 2, along with jasmonic acid, regulate the development of type-I, type-IV, type-VI trichomes and acylsugar synthesis in wild tomato species (Vosman et al. 2018; Xu et al. 2018). Therefore, understanding these regulatory mechanisms is crucial for transfer of insect resistance from wild to cultivated varieties, unlocking significant potential for developing insect-resistant varieties (Zeist et al. 2019).

This study found higher densities of type-IV glandular trichomes, antixenosis and antibiosis resistance categories in wild tomato species that govern insect resistance. This supports the potential use of resistance parameters for the development of insect-resistant varieties. Plant resistance plays a crucial role in shaping tri-trophic interactions and, consequently, influencing pest management (Han et al. 2022). Therefore, further investigations are needed to explore the dynamics of plant-insect interactions and multi-trophic interactions, focusing on the potential role of plant volatile organic compounds (VOCs) in plant resistance. Additionally, the studies on the responses of wild tomato species to insect herbivory by different feeding guilds, with emphasis on the synthesis of secondary metabolites, genes, proteins, and pathways associated with insect resistance, are crucial. Such efforts will provide valuable insights into the successful transfer of resistance traits from wild species to cultivated tomatoes.

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**Figure S1–S7, Table S1–S7**