

Inheritance of type IV glandular trichome density and its association with whitefly resistance from *Solanum galapagense* accession LA1401

Marcela Carvalho Andrade · Alex Antônio da Silva · Irã Pinheiro Neiva · Izabela Regina C. Oliveira · Evaristo Mauro De Castro · David Merrill Francis · Wilson Roberto Maluf

Received: 4 February 2016 / Accepted: 28 October 2016
© Springer Science+Business Media Dordrecht 2017

Abstract Tomato is affected by a large number of arthropod pests, among which the whitefly (*Bemisia tabaci*) is considered to be one of the most destructive. Several accessions of the wild species of *Solanum galapagense*, including accession LA1401, are considered resistant to whitefly (*B. tabaci*). This resistance has been associated with the presence of type IV glandular trichomes on the leaf surface. Our research aimed to study the inheritance of type IV glandular trichome density and its association with resistance to whitefly (*B. tabaci* biotype B) in populations derived from the interspecific cross *Solanum lycopersicum* × *S. galapagense* ‘LA1401.’ High estimates

for both broad-sense and narrow-sense heritabilities of type IV glandular trichome densities suggest that inheritance of this trait is not complex. Whitefly resistance was associated with high density of type IV glandular trichomes. F₂ (*S. galapagense* × *S. lycopersicum*) population plants selected for the highest densities of type IV glandular trichomes showed similar levels of resistance to those found in the donor of resistance LA1401.

Keywords Heritability · Negative binomial · Zero-inflated Poisson · *Bemisia tabaci* biotype B

Electronic supplementary material The online version of this article (doi:10.1007/s10681-016-1792-1) contains supplementary material, which is available to authorized users.

M. C. Andrade (✉) · A. A. da Silva · E. M. De Castro
Department of Biology, Federal University of Lavras,
Lavras 37200-000, Brazil
e-mail: andrade.mc0@gmail.com

I. P. Neiva · W. R. Maluf
Department of Agriculture, Federal University of Lavras,
Lavras 37200-000, Brazil

I. R. C. Oliveira
Department of Exact Sciences, Federal University of
Lavras, Lavras 37200-000, Brazil

D. M. Francis
Department of Horticulture and Crop Science, The Ohio
State University, Wooster, OH 44691, USA

Introduction

Whitefly (*Bemisia tabaci*) is considered one of the most important arthropod pests in tomato and other vegetable crops. It affects tomato production directly (resulting in physiological disorders leading to irregular ripening) and indirectly (virus transmission), resulting in yield losses (Byrne and Bellows 1991; Morales and Jones 2004).

Pest management in tomato through chemical products has often been inefficient and can result in chemical-resistant pest populations. Alternatively, breeding programs have been trying to develop resistant cultivars by transferring the resistance present in wild species to the cultivated tomato. Sources of resistance to whitefly and other arthropod pests

have been reported in *Solanum pennellii*, *Solanum habrochaites*, *Solanum pimpinellifolium* and *Solanum galapagense* (Maluf et al. 2001; Muigai et al. 2003; Maciel et al. 2011; Firdaus et al. 2012; Lucatti et al. 2013, 2014; Lucini et al. 2015).

In wild species, whitefly resistance is often associated with the presence of high densities of glandular trichomes (type IV and VI) and with the presence of allelochemicals (Freitas et al. 2002; Muigai et al. 2003; Rodríguez-López et al. 2011; Firdaus et al. 2012). In *S. pennellii*, *S. pimpinellifolium* and *S. galapagense*, the acylsugar content is associated with the presence of type IV trichomes, which play an important role in whitefly resistance (Goffreda et al. 1989; Rodríguez-López et al. 2011; Lucatti et al. 2013; Firdaus et al. 2013; Lucini et al. 2015). Among these wild species, *S. galapagense* is one of the most closely related to *S. lycopersicum* phylogenetically (Peralta et al. 2008). Recent studies have shown its accessions present high levels of resistance to whitefly. This resistance was associated with the presence of type IV glandular trichomes and acylsugar content (Firdaus et al. 2012, 2013; Lucatti et al. 2013). Those factors make the *S. galapagense* accessions important sources of resistance to be deployed in breeding programs.

Further studies using specific accessions of *S. galapagense* would be helpful in understanding the genetic factors involved in the presence of trichomes and their relation to whitefly resistance, which provide important information for accomplishing a successful trait introgression. Among the accessions of *S. galapagense*, the accession LA1401 can be highlighted because it bears at least three traits of economic importance: resistance to whitefly, salt tolerance and high soluble solids content. (Lucatti et al. 2013; Mahmoud et al. 1986; Shannon and Wrona 1992; Razdan and Mattoo 2006). In this work, we aimed to study the inheritance of type IV glandular trichome densities and their association with resistance to whitefly in populations derived from the interspecific cross *S. lycopersicum* ‘TOM-684’ × *S. galapagense* ‘LA1401.’

Materials and methods

This study was developed through a partnership between the Universidade Federal de Lavras (UFLA) in Lavras and the Hortiagro Sementes S.A. seed

company in Ijaci, both in the State of Minas Gerais, Brazil. The field experiments were carried out at the Hortiagro Sementes S.A. seed company. The analyses for identification and quantification of trichomes were performed at the Plant Anatomy Laboratory at the Biology Department, UFLA.

Plant material

Segregating populations were obtained from the interspecific cross *S. lycopersicum* ‘TOM-684’ × *S. galapagense* ‘LA1401’. LA1401 (=P₂) is a wild accession characterized by a high level of acylsugar, the presence of type IV trichomes and resistance to insects (Jouy et al. 1992; Lucatti et al. 2013). TOM-684 (=P₁) is susceptible to insects and has low acylsugar content. It is a proprietary fresh-market tomato inbred line from Hortiagro Sementes S.A. The parents LA1401 and TOM-684 were crossed to obtain the F₁ generation. F₁ plants were self-pollinated and backcrossed with both parents (accession LA1401 and line TOM-684) obtaining respectively the F₂ generation and the backcrosses: F₁BC₁₍₁₎ [(F₁ × TOM-684)] and F₁BC₁₍₂₎ [(F₁ × LA1401)].

Parents (P₁ and P₂) and the populations F₁, F₂, F₁BC₁₍₁₎ and F₁BC₁₍₂₎ were sown in flat 128-well seed trays with commercial potting mix. After 1 month, the seedlings were transplanted to the greenhouse in a completely randomized design in order to be exposed to natural whitefly (*B. tabaci* biotype B) infestation. The plants were tied individually and irrigated by drip system. Fertilizing as well as phytosanitary management was performed according to the recommendation for the tomato crop (Alvarenga 2013). During the whitefly experiment, insect pest management was not performed. The experiment consisted of 40 plants from each parental line, 40 plants from the F₁, 268 individuals from the F₂ and 120 individuals from each backcross. Identification and quantification of trichomes types were made in a random sample of plants obtained from the populations. The sampled population consisted of 20 plants from each parental line, 20 plants from the F₁, 145 individuals from the F₂ and 60 individuals from each backcross.

Resistance to whitefly

Whitefly (*B. tabaci* biotype B) infestation occurred naturally in the greenhouse 40 days after transplanting.

The plants were evaluated relative to traits related to whitefly resistance 3 weeks after the infestation. This three-week period was necessary to allow the assessment of traits related to whitefly resistance in all stages of plant development. Insect pest management was not performed during the whitefly resistance experiment.

Incidence of whitefly was evaluated by measuring the number of eggs and the number of nymphs in the sampled leaf area. The number of eggs and number of nymphs were quantified on the abaxial surfaces of the fully expanded fourth leaf in five areas of 1 cm² using a 10× magnifying glass with a millimeter scale. The total number of eggs and total number of nymphs per 5 cm² leaf surface were considered for statistical analyses.

Identification and quantification of trichomes

Two months after transplanting plants to the greenhouse, the fully expanded fourth leaf from each plant was collected and stored in 70% ethanol in a beaker for 48 h. After storage, paradermal sections were prepared from the abaxial and adaxial surfaces of leaflets. The sections were cleared in 50% sodium hypochlorite for 20 s and washed three times in water to remove the excess of hypochlorite. After that, sections were placed in 0.1% safranin dye for 20 s and then washed twice in water to remove the excess of dye. Six paradermal sections per surface were used per plant to make slides. Semi-permanent slides were prepared as mounts in glycerinated water (Freitas et al. 2002).

The slides were viewed under a 10× optical microscope (Carl Zeiss, model AXIL1) with an attached camera (AxionCam ERc5s). Images were captured using the Axio Vision Rel. 4.8 program and analyzed using the Image Tool v. 3.00 software (Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX). Type IV and type II+III trichome densities were quantified in three different areas of 1 mm² in each epidermal surface of the leaf (abaxial and adaxial) for each trichome type. Trichome types were identified and classified based on Luckwill (1943). Because the number of trichomes on abaxial and adaxial surfaces were highly correlated for each type of trichome, the sum of the trichomes of both surfaces for each type of trichome was considered for statistical analyses. Therefore, trichome numbers were expressed in 6 mm² per leaf surface.

Statistical analysis

Statistical models

The traits trichome density, number of eggs and number of nymphs result in count data that do not follow a normal distribution. Poisson distribution is the natural choice for modeling count data, but such distribution imposes equality of mean and variance. In this study, the count outcomes exhibit overdispersion, that is, variance greater than the mean. In order to model this extra variability and obtain reliable estimates for the variances of each population and trait, negative binomial (NB) (Hilbe 2011) and zero-inflated Poisson (ZIP) (Zuur et al. 2009) models were fitted. A random variable Y follows a negative binomial distribution if its probability function is given by

$$\Pr(Y = y) = \frac{\Gamma(k^{-1} + y)}{\Gamma(k^{-1})y!} \left(\frac{k\mu}{1 + k\mu} \right)^y \left(\frac{1}{(1 + k\mu)} \right)^{1/k},$$

where k is the dispersion parameter of the distribution. This distribution approaches Poisson as $k \rightarrow 0$.

Negative binomial models were fitted to data on trichome density and number of eggs, considering each population separately. In all cases, there is only an overall effect, that is, no treatment effect. Then, assuming the logarithmic link function, we have that

$$y_{ij} = \mu_i + \varepsilon_{ij},$$

$$E(Y_i) = \mu_i = e^{\beta_i},$$

$$\text{Var}(Y_i) = \mu_i + k_i \mu_i^2 = e^{\beta_i} + k_i e^{2\beta_i},$$

where y_{ij} is the count outcome of the j th plant in the i th population, $i = 1, \dots, 6$ and $j = 1, \dots, J$; $E(Y_i)$ and $\text{Var}(Y_i)$ are the mean and the variance, respectively, of the i th population. The β_i and k_i estimates are obtained using, for example, the maximum likelihood method, which is available in statistical programs like R and SAS.

Zero-inflated Poisson models were fitted to the number of nymphs because of the high incidence of zeros in this trait. The probability function of a random variable Y , which follows a ZIP distribution, is

$$\Pr(Y = y) = \begin{cases} \pi + (1 - \pi) \exp(-\lambda) & y = 0 \\ \frac{(1 - \pi) \exp(-\lambda) \lambda^y}{y!} & y > 0 \end{cases}$$

In this case, the population is considered to have two types of individuals: the first type has count data always equal to zero, and the second type gives Poisson count data, which can be zero. In the probability function expression, λ refers to the mean of the Poisson distribution, and π (zero-inflation probability) represents the probability of zero counts.

As before, there is only an overall effect, and each population was considered separately. In this case,

$$\lambda_i = e^{\beta_i},$$

that is, the mean of the Poisson count data, and

$$\pi_i = \frac{e^{\alpha_i}}{1 + e^{\alpha_i}}.$$

From these terms, we have

$$E(Y_i) = \mu_i = (1 - \pi_i)\lambda_i,$$

$$\text{Var}(Y_i) = \mu_i + \left(\frac{\pi_i}{1 - \pi_i}\right)\mu_i^2,$$

where $E(Y_i)$ and $\text{Var}(Y_i)$ are the mean and variance, respectively, of the i th population, $i = 1, \dots, 6$. The β_i and α_i estimates are obtained through the maximum likelihood method, implemented in statistical programs.

The aforementioned models were fitted to the trichomes and whitefly (eggs and nymphs) count data using the NLMIXED procedure of the SAS 9.3 program (SAS Institute 2011). Some codes are presented in Online Resource 1. In all traits, the outcomes refer to the total values per plant instead of the average, because these models are used for count data.

Variance estimate and heritability

From the parameters estimates of the fitted model, the variances of parents (P_1 and P_2), populations F_1 , F_2 , backcrosses $F_1BC_{1(1)}$ [= ($F_1 \times \text{TOM-684}$)] and $F_1BC_{1(2)}$ [= ($F_1 \times \text{LA1401}$)] were estimated considering each trait. The heritability values were then estimated by ratios of genotypic variance $V(G)$ or additive variance $V(A)$ and phenotypic variance $V(P)$ (Wright 1968):

$$V(P) = VF_2$$

$$V(E) = \frac{VP_1 + VP_2 + (2 \times VF_1)}{4}$$

$$V(G) = V(P) - V(E)$$

$$V(A) = (2 \times VF_2) - (VF_1BC_{1(1)} + VF_1BC_{1(2)}),$$

where $V(E)$ is environmental variance; VP_1 is the environmental variance among plants of parent TOM-684; VP_2 is the environmental variance among plants of parent LA1401; VF_1 is the environmental variance among plants of F_1 population; VF_2 is the variance among individuals of F_2 population; $VF_1BC_{1(1)}$ is the variance among individuals of the first backcross to the parent TOM-684; $VF_1BC_{1(2)}$ is the variance among individuals of the first backcross to the parent LA1401.

Associative analysis between type IV glandular trichomes and whitefly resistance

The 20 plants with the highest densities and the 20 plants with the lowest densities of type IV trichomes were selected from the F_2 sampled population. These two groups, as well as the parents (P_1 and P_2) and the F_1 population, were considered as covariates (treatment effects) in the estimation of contrasts. Then, the negative binomial model is

$$y_{ij} = \mu_i + \varepsilon_{ij},$$

$$\mu_i = e^{\beta_0 + \beta_i},$$

where y_{ij} is the count outcome of the j th plant in the i th group (high F_2 , low F_2 , P_1 , P_2 and F_1), $i = 1, \dots, 5$, and $j = 1, \dots, J$, where $J = 20, 20, 20, 20, 20$, for the groups high F_2 , low F_2 , P_1 , P_2 and F_1 , respectively.

The model specification is similar for the ZIP model, where

$$\lambda_i = e^{\beta_0 + \beta_i},$$

and

$$\pi_i = \frac{e^{\alpha_0 + \alpha_i}}{1 + e^{\alpha_0 + \alpha_i}}.$$

Contrasts of means between the groups were tested based on the asymptotic χ^2 distribution of the likelihood ratio statistic using the GENMOD procedure of the SAS 9.3 program (SAS Institute 2011).

Spearman correlations between the traits type IV glandular trichomes, types II+III non-glandular trichomes, number of eggs and number of nymphs were calculated using the F_2 population.

Results

Trichome densities and whitefly incidence

The average total values for density of type IV glandular trichomes, types II+III non-glandular trichomes, number of eggs and number of nymphs for P_1 , P_2 , F_1 , F_2 , $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ are shown in Table 1. Type IV glandular trichomes were found in high density in the parent LA1401 (*S. galapagense*), absent in the parent TOM-684 (*S. lycopersicum*) and in low density in the F_1 population. Contrasting results were observed for types II+III non-glandular trichomes, for which the density in the parent LA1401 was close to zero (0.9) (Table 1). The backcross $F_1BC_{1(1)}$ [= ($F_1 \times$ TOM-684)] had low mean density of type IV trichomes. In the backcross $F_1BC_{1(2)}$ [= ($F_1 \times$ LA1401)] the mean densities of type IV trichomes were comparable to the densities present in the F_2 population.

The parental accession LA1401, considered resistant to whitefly, had a lower number of eggs and nymphs in both the total population and the sampled population of the experiment (Table 1). The F_1 population had values for the number of eggs and number of nymphs close to those of the susceptible parent TOM-684. In the sampled population used for quantification of trichome densities, the F_2 plants selected for highest density of type IV glandular trichomes had lower numbers of eggs and nymphs than the F_2 plants selected for lowest density of type IV glandular trichomes. In addition, the numbers of eggs and nymphs in the plants selected for highest density of type IV trichomes were similar to those found in LA1401 (Table 1).

Type V non-glandular trichomes showed low variation among the parents and segregating populations (Table 1). Densities of this trichome type were similar for both parents (LA1401 and TOM-684) and the F_2 population. Even the F_2 plants selected for the highest density of type IV glandular trichomes and F_2 plants selected for the lowest density of type IV glandular trichomes had a similar average for densities of the type V non-glandular trichomes, which also was comparable with the average of the parents (Table 1). Type V trichomes were not correlated to the other trichome types or to the whitefly incidence (Table 3), suggesting that their presence it is not associated to whitefly resistance in these populations; therefore, this

trichome type was not considered for further investigation in this study.

Heritability

The negative binomial model was fitted for type IV glandular trichomes, types II+III non-glandular trichomes and number of eggs, because count data with overdispersion were observed for these traits. The number of nymphs had count data with overdispersion due the high incidence of zero; therefore, the best statistical model fitted was a zero-inflated Poisson. After fitting the appropriate model for each trait, the variances were estimated for the parents (LA1401 and TOM-684) and each of the populations F_1 , F_2 , $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ (Table 2).

The density of type IV glandular trichomes had a high estimate for both broad-sense heritability (0.93) and narrow-sense heritability (0.60). For this type of trichome, the additive variance estimate represented 64% of the genotypic variance and for non-additive variance represented 36%. The estimates of broad-sense heritability were relatively high for types II+III trichome density ($h_a^2 = 0.81$), number of eggs ($h_a^2 > 0.42$) and number of nymphs ($h_a^2 > 0.61$) (Table 2).

Association between glandular trichomes type IV and whitefly resistance

The measured traits were significantly correlated (Table 3). Type IV glandular trichome densities were negatively correlated with type II+III non-glandular trichomes ($r = -0.56$), number of eggs ($r = -0.22$) and number of nymphs ($r = -0.24$). Positive correlations were found with type II+III non-glandular trichomes, number of eggs and number of nymphs (Table 3).

Parents LA1401 and TOM-684 were statistically different ($p < 0.05$) for type IV trichome densities, type II+III trichome densities and number of eggs (Table 4). Even though the parents were not significantly different for the number of nymphs (Table 4), the number of nymphs was nonetheless higher in the TOM-684 and lower in LA1401 (Table 1).

F_2 plants with the highest average of type IV trichome density had a significantly lower number of eggs and number of nymphs than the F_2 plants with the

Table 1 Average total values for density of type IV glandular trichomes, density of types II+III non-glandular trichomes, density of type V non-glandular trichomes, number of eggs and number of nymphs per leaf of the populations derived from interspecific cross *S. galapagensis* LA1401 × *S. Lycopersicum* TOM-684

Populations	Sampled population			Total population		
	Type IV trichomes ^a	Type II+III trichomes ^a	Type V trichomes ^a	Eggs ^b	Nymphs ^b	Nymphs ^b
TOM-684 (<i>P</i> ₁)	0.0 (±0)	313.9 (±14.4)	13.6 (±1.6)	12.0 (±2.6)	0.8 (±0.2)	10.7 (±1.7)
LA1401 (<i>P</i> ₂)	82.3 (±5.7)	0.9 (±0.3)	16.1 (±1.4)	1.6 (±0.6)	0.2 (±0.1)	2.9 (±1.2)
<i>F</i> ₁	4.1 (±1.1)	207.3 (±7.5)	9.7 (±1.1)	7.7 (±2.0)	0.6 (±0.3)	12.9 (±2.4)
<i>F</i> ₂	21.0 (±2.8)	150.4 (±6.7)	14.0 (±0.7)	8.9 (±1.4)	0.9 (±0.1)	11.4 (±1.3)
<i>F</i> ₂ plants selected						
<i>F</i> ₂ highest density (IV)	94.1	57.6	15.2	1.2	0.1	–
<i>F</i> ₂ lowest density (IV)	0.0	194.1	13.5	5.8	0.7	–
<i>F</i> ₁ BC ₁₁ (<i>F</i> ₁ × TOM-684)	0.4 (±0.2)	283.7 (±9.2)	6.8 (±0.9)	8.2 (±1.2)	1.0 (±0.2)	12.2 (±1.6)
<i>F</i> ₁ BC ₁₂ (<i>F</i> ₁ × LA1401)	25.3 (±5.3)	160.4 (±13.3)	11.3 (±1.0)	8.7 (±1.4)	0.9 (±0.2)	9.3 (±1.2)

Mean followed by standard error of mean

Total population Includes all the plants used in the experiment, which consisted of 40 plants from each parental line, 40 plants from the *F*₁, 268 individuals from the *F*₂ and 120 individuals from each backcross

Sampled population A random sample of plants obtained from the total population, which consisted of 20 plants from each parental line, 20 plants from the *F*₁, 145 individuals from the *F*₂ and 60 individuals from each backcross

*F*₂ highest density (IV) Average total density of the 20 plants that showed the highest density of type IV glandular trichomes selected in the *F*₂ population sampled

*F*₂ lowest density (IV) Average total density of the 20 plants that showed the lowest density of type IV glandular trichomes selected in the *F*₂ population sampled

^a Average total density of trichomes per 6 mm²

^b Average total number of eggs or nymphs per 5 cm²

Table 2 Estimates of variance in the populations derived from interspecific cross *S. galapagense* LA1401 × *S. Lycopersicum* TOM-684 and heritability of the type IV glandular trichomes, types II+III non-glandular trichomes, number of eggs and number of nymphs

Populations	Sampled population				Total population	
	Type IV trichomes	Types II+III trichomes	Eggs	Nymphs	Eggs	Nymphs
TOM-684 (P_1)	0.0	4129.3	121.9	0.6	87.8	0.6
LA1401 (P_2)	532.7	2.0	6.5	0.3	32.0	0.2
F_1	21.1	984.9	104.7	1.1	215.9	1.6
F_2	1940.8	8017.5	155.1	2.1	235.8	4.1
$F_1BC_{11}(F_1 \times \text{TOM-684})$	2.8	5127.0	71.6	1.8	172.6	2.1
$F_1BC_{12}(F_1 \times \text{LA1401})$	2723.9	16735.8	118.4	2.6	145.9	2.5
Broad-sense heritability	0.93	0.81	0.46	0.61	0.42	0.76
Narrow-sense heritability	0.60	– ^a	– ^a	– ^a	– ^a	– ^a
$s (h_a^2)$	0.07	0.09	0.13	0.10	0.09	0.06

Total population Includes all the plants used in the experiment, which consisted of 40 plants from each parental line, 40 plants from the F_1 , 268 individuals from the F_2 and 120 individuals from each backcross

Sampled population This is a random sample of plants obtained from the total population, which consisted of 20 plants from each parental line, 20 plants from the F_1 , 145 individuals from the F_2 and 60 individuals from each backcross

$s (h_a^2)$ Estimate of associate error to broad-sense heritability by Vello and Vencovsky (1974)

^a Estimate of the narrow-sense heritability higher than the estimate of the broad-sense heritability, or negative estimate of the narrow-sense heritability, in which the real estimate probably is zero

Table 3 Correlation between the traits type IV glandular trichomes, types II+III non-glandular trichomes, type V non-glandular trichomes, number of eggs and number of nymphs in the F_2 sampled population

	Type IV trichomes	Types II+III trichomes	Type V trichomes	Number of eggs
Type II+III trichomes	–0.56**			
Type V trichomes	0.06 ^{ns}	0.14 ^{ns}		
Number of eggs	–0.22**	0.24**	–0.13 ^{ns}	
Number of nymphs	–0.24**	0.34**	0.02 ^{ns}	0.37**

** Significant at the 0.01 level

^{ns} Non-significant at the 0.05 level

lowest average of type IV trichome density (Table 4). Considering type IV glandular trichomes, the number of eggs and number of nymphs, the contrasts between LA1401 and F_2 plants with the highest density of type IV trichomes were not significant, nor were the contrasts significant between TOM-684 and F_2 plants with the lowest density of type IV trichomes.

Discussion

Inheritance of type IV glandular trichome density

Type IV glandular trichomes were observed in abundance in the parent LA1401 (*S. galapagense*) and in

the plants selected for the highest density of type IV trichomes in the F_2 population. *S. galapagense* accessions are characterized by having high densities of this type of trichome (Simmons and Gurr 2004; Firdaus et al. 2012; Lucatti et al. 2013). The frequency distribution graphs (Fig. 1) associated with the average total values for the density of type IV glandular trichomes found in the populations F_1 , F_2 , $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ (Table 1) are indicative that a high density of type IV trichomes may be under the control of incompletely recessive alleles from LA1401. In fact, the F_2 population had average total values for the density of type IV glandular trichomes approximately four times lower than the density found in the parent LA1401 (Table 1). Apparently, F_2 plants that are

Table 4 Contrasts between populations with different densities of type IV glandular trichomes to the traits type IV glandular trichomes, types II+III non-glandular trichomes, number of eggs and number of nymphs

Estimated contrasts	Type IV trichome		Types II+III trichome		Eggs		Nymphs	
	Contrast ^a	Pr > χ^2 ^b						
P_1 (=TOM-684)– P_2 (=LA1401)	–82.3	<0.0001	313.1	<0.0001	10.4	<0.0001	0.6	0.0855
F_2 lowest density (IV)– F_2 highest density (IV)	–94.1	<0.0001	136.6	<0.0001	4.6	0.0004	0.7	0.0006
P_1 – F_1	–4.1	<0.0001	106.6	0.0007	4.3	0.2634	0.2	0.7134
P_2 – F_1	78.2	<0.0001	–206.5	<0.0001	–6.1	0.0004	–0.4	0.0597
P_1 – F_2 lowest density (IV)	0.0	1	119.8	<0.0001	6.2	0.0626	0.1	0.1454
P_2 – F_2 lowest density (IV)	82.3	<0.0001	–193.3	<0.0001	–4.2	0.0026	–0.6	0.0096
P_1 – F_2 highest density (IV)	–94.1	<0.0001	256.4	<0.0001	10.8	<0.0001	0.7	0.0073
P_2 – F_2 highest density (IV)	–11.8	0.236	–56.7	<0.0001	0.4	0.5158	0.1	0.3116

F_2 highest density (IV) Average total density of the 20 plants that showed the highest density of type IV glandular trichomes selected in the F_2 population sampled

F_2 lowest density (IV) Average total density of the 20 plants that showed the lowest density of type IV glandular trichomes selected in the F_2 population sampled

^a Contrasts using the observed values

^b Probability considering the test of the likelihood ratio

heterozygous and homozygous for the TOM-684 alleles negatively affect the average of the F_2 population since a large proportion of those plants probably has type IV trichomes either in lower density or absent, respectively (Fig. 1). Their behaviors were similar to what was expected in the F_1 population and in the parent TOM-684. F_2 plants selected for the highest density of type IV glandular trichomes are probably recessive homozygous for the alleles inherited from LA1401, and they had average values similar to those found in LA1401 for type IV trichomes (Table 4). In addition, the F_1 population and the backcross toward TOM-684 [= ($F_1 \times$ TOM-684)] had low densities of type IV trichomes, with values close to zero, which was the value found for TOM-684 (Table 1; Fig. 1).

High estimates for both broad-sense and narrow-sense heritabilities are indicative that inheritance of type IV glandular trichome may not be complex. High broad-sense heritability for type IV trichomes was also reported in *S. galapagense* accession PRI195004 by Firdaus et al. (2013). These authors concluded that the inheritance of this trichome type is relatively simple in this *S. galapagense* source. Freitas et al. (2002) studied the inheritance of glandular trichomes in a segregating population from the interspecific cross *S. habrochaites* accession PI 127826 and *S. lycopersicum* TOM-556.

For *S. habrochaites*, high broad-sense heritability for type IV trichomes was also demonstrated. Furthermore, research into the *S. habrochaites* source shows that densities of type IV glandular trichomes appear to be under the control of an incompletely recessive allele at a major locus. In *S. pennelli*, Saeidi (2012) suggested that two independent dominant genes were responsible for the presence of type IV trichomes. Additive and non-additive effects are involved in the expression of type IV trichomes. Furthermore, the high estimate for the additive variance suggests that it should be rather easy to practice selection for this trait and supports the idea that the inheritance of type IV trichomes is not complex.

Types II+III non-glandular trichomes, number of eggs and number of nymphs also had high estimates of broad-sense heritability (Table 2), which indicates that genetic effects are more important than environmental effects in the expression of these traits.

Resistance to whitefly

Negative correlations among type IV glandular trichomes and both the number of eggs and number of nymphs are indicative that this trichome type is associated with the resistance to whitefly (Table 3), because populations that had higher numbers of type

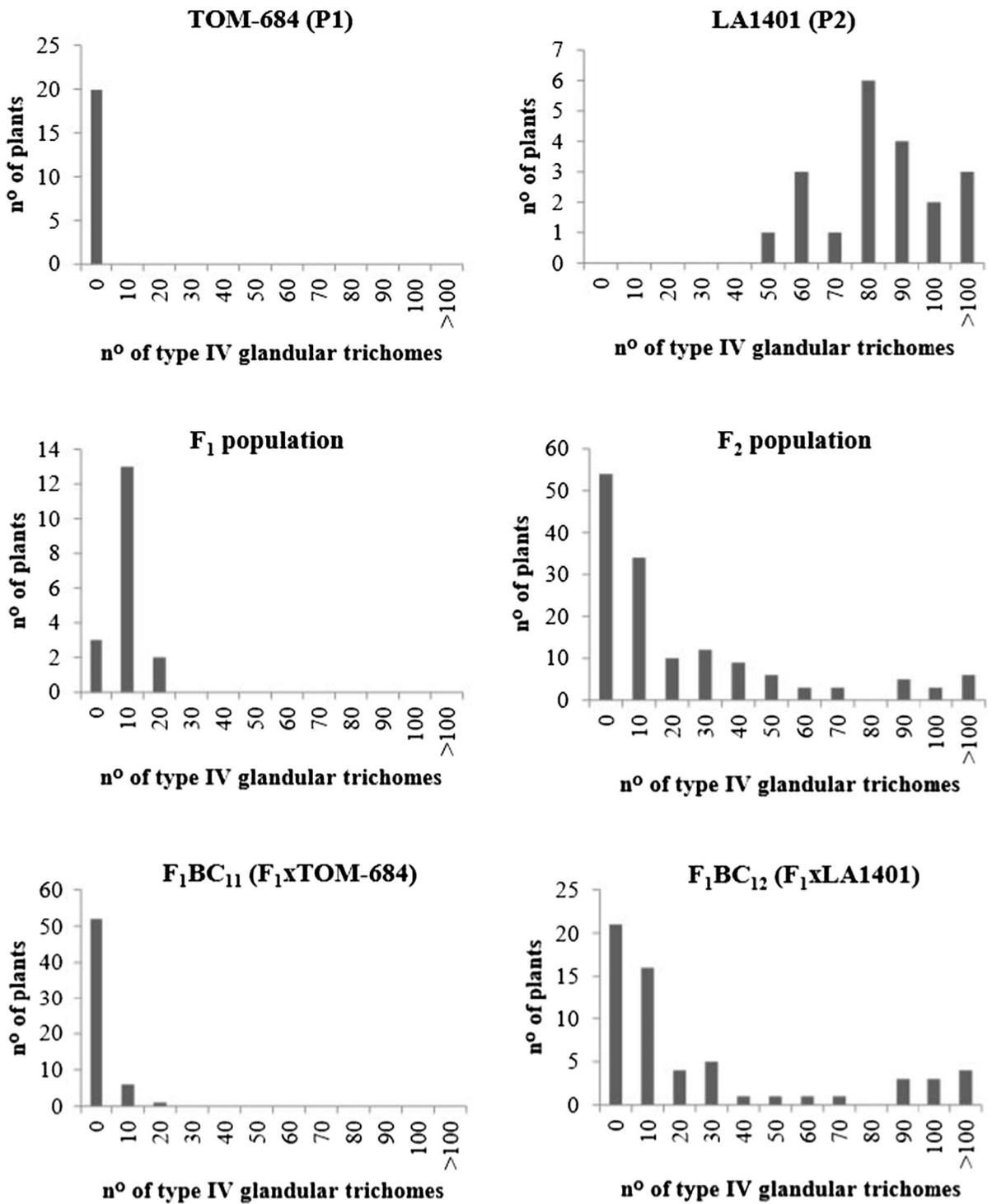


Fig. 1 Frequency distributions of the number of type IV glandular trichomes per 6 mm² in populations derived from interspecific cross *S. galapagense* LA1401 × *S. Lycopersicum* TOM-684

IV trichomes had low incidence of number of eggs and nymphs (Table 1). Estimates of this correlation were low, but highly significant. A possible explanation for these low estimates would be the expected high environmental variation associated with natural whitefly infestation. Other factors could be that not only type IV glandular trichome density may be responsible for whitefly resistance, but also their association with acylsugar contents, as reported by Firdaus et al. (2012, 2013) and Lucatti et al. (2013). Firdaus et al. (2012) evaluated whitefly resistance in different accessions of several wild tomato species. They reported that resistant accessions showed a high correlation between type IV trichomes and whitefly resistance components.

In fact, this association between type IV trichomes and whitefly resistance is supported by the results of the estimated contrasts (Table 4). F_2 plants selected for the highest density of type IV trichomes behaved similarly to the resistant parent LA1401, whereas in the F_2 plants selected for the lowest density of type IV trichomes, the incidences of eggs and nymphs were similar to those found in the susceptible parent TOM-684. The relation between whitefly resistance and type IV trichomes has been reported in several wild species of tomato, such as *S. galapagense*, *S. habrochaites* and *S. pennelli* (Muigai et al. 2002; Freitas et al. 2002; Saeidi 2012; Firdaus et al. 2012, 2013; Lucatti et al. 2013). Presence of type IV trichomes has also been associated with resistance to other arthropod pests such as spider mite and aphids (Goffreda et al. 1989; Maluf et al. 2007; Saeidi 2012; Lucini et al. 2015).

Type II+III non-glandular trichomes were positively correlated to the number of eggs and nymphs, which suggests that their presence is associated with an increase in the rate of eggs and nymphs. Hence, we can infer that types II+III non-glandular trichomes do not confer resistance to whiteflies. Types II+III and type IV trichomes were negatively correlated; therefore, the selection of plants with high density of type IV trichomes results in an indirect selection of plants resistant to whitefly with a low density of types II+III trichomes. A negative correlation between type III and type IV trichomes was also found by Firdaus et al. (2013).

The resistance provided by glandular trichomes seems to be associated with the allelochemical contents that are present inside of the trichome glands (Goffreda et al. 1989; Maluf et al. 2001; Muigai et al.

2003; Firdaus et al. 2012, 2013; Lucini et al. 2015). These allelochemicals may provide antibiosis and/or antixenosis plant response to the pests (Simmons and Gurr Simmons and Gurr 2004). Lucatti et al. (2013) evaluated whitefly resistance in accessions of the *S. galapagense* and *S. cheesmanii*. In their study, all the *S. galapagense* accessions showed resistance to whitefly. This resistance was accompanied by a high density of type IV trichomes as well as high acylsugar accumulation. In contrast, *S. cheesmanii* accessions were susceptible, even though they had acylsugar levels comparable to those found in *S. galapagense*. This fact could probably be explained by the absence of type IV trichomes in *S. cheesmanii*. In conclusion, the authors suggest that a minimum level of acylsugars and the presence of type IV glandular trichomes are needed to achieve an effective level of resistance. Firdaus et al. (2013) also found that the presence of type IV trichomes and acylsugar content were related to whitefly resistance.

In *S. pennelli*, the relation between acylsugar content and densities of glandular trichomes were initially reported by Fobes et al. (1985) and Goffreda et al. (1989). In contrast, Gonçalves (2006) and Costa (2013) did not find any correlation between glandular trichomes and acylsugar accumulation. Both authors evaluated advanced populations, selected for the high level of acylsugar, from the cross *S. lycopersicum* × *S. pennellii* accession LA0716. Because these advanced populations were selected solely for their acylsugar content, but not for densities of glandular trichomes, the authors suggest that acylsugar may also be present in other parts of the plant, such as the cells of the leaf epidermis.

Our study suggests that the inheritance of type IV trichomes is relatively simple and it appears to be associated with the resistance to whitefly in *S. galapagense*. However, other mechanisms that were not evaluated in our study, such as the acylsugar content, also seem to be involved in this resistance. Based on the progenies from the plants selected for highest and lowest densities of type IV trichomes, further studies can be carried out to confirm the relation between type IV trichomes and acylsugar content in populations derived from *S. lycopersicum* ‘TOM-684’ × *S. galapagense* ‘LA1401.’

Acknowledgements The authors wish to acknowledge the universities, agencies and company that supported this research

project: Universidade Federal de Lavras (UFLA), The Ohio State University (OSU), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and HortiAgro Sementes S.A.

References

- Alvarenga M (2013) Tomate: produção em campo, casa de vegetação e hidroponia. rev. Editora Universitária de Lavras, Lavras
- Byrne DN, Bellows JRTS (1991) Whitefly biology. *Annu Rev Entomol* 36:431–457. doi:[10.1146/annurev.en.36.010191.002243](https://doi.org/10.1146/annurev.en.36.010191.002243)
- Costa EMR (2013) Relação entre densidade de tricomas foliares e teores de zingibereno e de acilacúcares em tomates resistentes a pragas. Dissertation, Universidade Federal de Lavras
- Firdaus S, Heusden AWW, Hidayat N, Supena EDJ, Visser RGF, Vosman B (2012) Resistance to *Bemisia tabaci* in tomato wild relatives. *Euphytica* 187:31–45. doi:[10.1007/s10681-012-0704-2](https://doi.org/10.1007/s10681-012-0704-2)
- Firdaus S, Van Heusden AW, Hidayati N et al (2013) Identification and QLT mapping of whitefly resistance components in *Solanum galapagense*. *Theor Appl Genet* 126:1487–1501. doi:[10.1007/s00122-013-2067-z](https://doi.org/10.1007/s00122-013-2067-z)
- Fobes JF, Mudd JB, Marsden MPF (1985) Epicuticular lipid accumulation on the leaves of *Lycopersicon pennellii* (Corr.) D'Arcy e *Lycopersicon esculentum* Mill. *Plant Physiol* 77:567–570. doi:[10.1104/pp.77.3.567](https://doi.org/10.1104/pp.77.3.567)
- Freitas JA, Maluf WR, Das Graças Cardoso M, Gomes LA, Bearzotti E (2002) Inheritance of foliar zingiberene contents and their relationship to trichome densities and whitefly resistance in tomatoes. *Euphytica* 127:275–287. doi:[10.1023/A:1020239512598](https://doi.org/10.1023/A:1020239512598)
- Goffreda JC, Mutschler MA, Avé DA, Tingey WM, Steffens JC (1989) Aphid deterrence by glucose esters in glandular trichome exudate of the wild tomato, *Lycopersicon pennellii*. *J Chem Ecol* 15:2135–2147. doi:[10.1007/BF01207444](https://doi.org/10.1007/BF01207444)
- Gonçalves LD (2006) Herança do teor de acilacúcares em genótipos de tomateiro e sua relação com tricomas foliares e repelência ao ácaro *Tetranychus evansi*. Dissertation, Universidade Federal de Lavras
- Hilbe JM (2011) Negative binomial regression. Cambridge University, New York
- Jouy N, Bordat D, Bessiere JM (1992) Identification of (2,3,4-tri-*O*-acyl)- α -D-glucopyranosyl-(3-*O*-acyl)- β -D-fructofuranoside, responsible of the high level of leafminer resistance in *Lycopersicon cheesmanii*. *Rep Tomato Genet Coop* 42:22
- Lucatti AF, Van Heusden AW, De Vos RCH, Visser RGF, Vosman B (2013) Differences in insect resistance between tomato species endemic to the Galapagos Islands. *BMC Evol Biol* 13:175. doi:[10.1186/1471-2148-13-175](https://doi.org/10.1186/1471-2148-13-175)
- Lucatti AF, Meijer-Dekens FR, Mumm R, Visser RG, Vosman B, Van Heusden S (2014) Normal adult survival but reduced *Bemisia tabaci* oviposition rate on tomato lines carrying an introgression from *S. habrochaites*. *BMC Genet* 15:142. doi:[10.1186/s12863-014-0142-3](https://doi.org/10.1186/s12863-014-0142-3)
- Lucini T, Faria MV, Rohde C, Resende JTV, De Oliveira JRF (2015) Acylsugar and the role of trichomes in tomato genotypes resistance to *Tetranychus urticae*. *Arthropod-Plant Interact* 9:45–53. doi:[10.1007/s11829-014-9347-7](https://doi.org/10.1007/s11829-014-9347-7)
- Luckwill LC (1943) The genus *Lycopersicon*: an historical, biological and taxonomic survey of wild and cultivated tomatoes. Aberdeen University, Aberdeen
- Maciel GM, Maluf WR, Silva VF, Gonçalves NA, Gomes LAA (2011) Híbridos pré-comerciais resistentes a *Tuta absoluta* obtidos de linhagem de tomateiro rica em acilacúcares. *Hortic Bras* 29:151–156. doi:[10.1590/S0102-05362011000200003](https://doi.org/10.1590/S0102-05362011000200003)
- Mahmoud MH, Jones RA, El-Beltagy AS (1986) Comparative responses to high salinity between salt-sensitive and salt-tolerant genotypes of the tomato. *Acta Hort* 190:533–544. doi:[10.17660/ActaHortic.1986.190.62](https://doi.org/10.17660/ActaHortic.1986.190.62)
- Maluf WR, Campos GA, Cardoso MG (2001) Relationships between trichome types and spider mite (*Tetranychus Evansi*) repellence in tomatoes with respect to foliar zingiberene contents. *Euphytica* 121:73–80. doi:[10.1023/A:1012067505361](https://doi.org/10.1023/A:1012067505361)
- Maluf WR, Inoue IF, Ferreira RPD, Gomes LAA, Castro EM, Cardoso MG (2007) Higher glandular trichome density in tomato leaflets and repellence to spider mites. *Pesqui Agropecu Bras* 42:1227–1235. doi:[10.1590/S0100-204X2007000900003](https://doi.org/10.1590/S0100-204X2007000900003)
- Morales FJ, Jones PG (2004) The ecology and epidemiology of whitefly-transmitted viruses in Latin America. *Virus Res* 100:57–65. doi:[10.1016/j.virusres.2003.12.014](https://doi.org/10.1016/j.virusres.2003.12.014)
- Muigai SG, Schuster DJ, Snyder JC, Scott JW, Bassett MJ, McAuslane HJ (2002) Mechanisms of resistance in *Lycopersicon* germplasm to the whitefly *Bemisia argentifolii*. *Phytoparasitica* 30:347–360. doi:[10.1007/BF02979682](https://doi.org/10.1007/BF02979682)
- Muigai SG, Bassett MJ, Schuster DJ, Scott JW (2003) Greenhouse and field screening of wild *Lycopersicon* germplasm for resistance to the whitefly *Bemisia Argentifolii*. *Phytoparasitica* 31:27–38. doi:[10.1007/BF02979764](https://doi.org/10.1007/BF02979764)
- Peralta IE, Spooner DM, Knapp S (2008) Taxonomy of wild tomatoes and their relatives (*Solanum* sect. *Lycopersicoideae*, sect. *Juglandifolium*, sect. *Lycopersicon*; Solanaceae). *Syst Bot Monogr* 84:1–186. doi:[10.2307/25027972](https://doi.org/10.2307/25027972)
- Razdan MK, Mattoo AK (2006) Genetic improvement of solanaceous crops: tomato. CRC, Boca Raton
- Rodríguez-López MJ, Garzo E, Bonani JP, Fereres A, Fernández-Muñoz R, Moriones E (2011) Whitefly resistance traits derived from the wild tomato *Solanum pimpinellifolium* affect the preference and feeding behavior of *Bemisia tabaci* and reduce the spread of Tomato yellow leaf curl virus. *Phytopathology* 101:1191–1201. doi:[10.1094/PHTO-01-11-0028](https://doi.org/10.1094/PHTO-01-11-0028)
- Saeidi Z (2012) Inheritance of type IV glandular trichomes and two-spotted spider mite resistance in an interspecific cross of *Lycopersicon esculentum* \times *L. pennellii* ‘LA2580’. *J Res Agric Sci* 8:157–163
- SAS Institute (2011) SAS[®] 9.3 system options: reference. SAS Institute, Cary
- Shannon MC, Wrona AF (1992) Salt tolerance of a selected line from the cross between a wild, tolerant tomato species

- (*Lycopersicon cheesmanii*, ecotype LA1401) and a cultivated species (*L. esculentum* mill.). HortScience 27:597
- Simmons AT, Gurr GM (2004) Trichome-based host plant resistance of *Lycopersicon* species and the biocontrol agent *Mallada signata*: are they compatible? Entomol Exp Appl 113:95–101. doi:[10.1111/j.0013-8703.2004.00210.x](https://doi.org/10.1111/j.0013-8703.2004.00210.x)
- Vello NA, Vencovsky R (1974) Variâncias associadas às estimativas da variância genética e o coeficiente de herdabilidade. ESALQ, Piracicaba
- Wright S (1968) The genetics of quantitative variability. In: Wright S (ed) Evolution and genetics of populations, 2nd edn. University of Chicago, Chicago, pp 373–420
- Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer Science & Business Media, Berlin