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ORIGINAL PAPER

The introgression of resistance to *Tuta absoluta* in tomato based on glandular trichomes

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Abstract

Tomato (*Solanum lycopersicum* L.) is one of the most important and consumed vegetables worldwide both in fresh and processed form. Among the main pests, the South American tomato pinworm (*Tuta absoluta* Meyrick, Lepidoptera: Gelechiidae) stands out as one that causes great damage to its production. Control is based essentially on intensive chemical applications which increases the production cost, besides promoting ecological imbalances and possible health problems for consumers and farmers. Among the plant defense mechanisms against herbivores pests are the glandular trichomes and their secondary metabolites that together can be poisonous, repellent, or trap insects and other organisms. In order to develop a resistant cultivar to *T. absoluta*, we introgressed a trichome-based resistance trait from the wild tomato (*Solanum pennellii*) into cultivated tomato. The wild tomato has the type IV glandular trichomes, the major site of biosynthesis for acylsugars, which have been consistently linked to broad-spectrum resistance to herbivorous arthropods of tomato. The backcross genotypes (F_2BC_2) come from a cross between the species *S. pennellii* access LA-716 (donor genome with high acylsugar content) and the commercial cultivar *S. lycopersicum* cv. Redenção (background genome with low acylsugar content). Eight genotypes with high recovery of the background genome were selected to identify and quantify the glandular and non-glandular trichomes as well as to assess their resistance to the *T. absoluta*. Direct correlations between the acylsugar content, number of type IV glandular trichomes and resistance to the South American tomato pinworm were observed, indicating that the desired trait from *S. pennellii* is inherited throughout the backcrosses. Two groups were formed among the analyzed genotypes: high and low levels of acylsugars. In the high-level group, the higher acylsugar content led to a better control of the pinworm in a similar way to the wild parent (resistant). The overall analysis of our data shows that the genotypes RVTA-2010pl#232 and RVTA-2010pl#257 have the greatest potential to develop tomato lines with desirable commercial traits for the processing industry besides resistance to the South American tomato pinworm.

Keywords *Solanum pennellii* · *Solanum lycopersicum* · Acylsugar · ISSR · Plant breeding

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important and consumed vegetables worldwide, cultivated in tropical and subtropical regions with a production of 182.3 million tons in 4.8 million hectares (FAOSTAT 2020). The tomato is also one of the most demanding crops in terms of phytosanitary products, due to several pathogens and pests that compromise the yield and commercial fruit traits. The South American tomato pinworm (SATP) (*Tuta absoluta* Meyrick, Lepidoptera: Gelechiidae) is an insect pest that has a major economic impact on the tomato, especially in tropical and subtropical climate countries (Han et al. 2019). Originally from the Western Neotropical region of South

America, the pinworm was recently identified in Spain and has spread rapidly through Afro-Eurasia (Biondi et al. 2018).

The control of this pest is complex and depend on intensive chemical applications. This control strategy increases the production cost, besides promoting ecological imbalances and possible health problems for consumers and farmers (Krechemer and Foerster 2020; Reddy and Rajam 2016). In agroecological systems, the damage caused by the tomato pinworm is even more severe, mainly due to the lack of phytosanitary products allowed by the legislation (Fiaboe et al. 2020). Thus, the use of resistant tomato would be the most viable and environmental-friendly method to effectively reduce the damage caused by pinworm (Biondi et al. 2018; Giorgini et al. 2019; Silva et al. 2016). However, no resistant/tolerant commercial tomato varieties are yet available.

The lack of resistances within the current cultivated tomato varieties make difficult to find natural sources for breeding programs. Consequently, resistance traits have been explored from wild tomato relatives that can be introgressed into susceptible cultivars (Giorgini et al. 2019; Lima et al. 2015; Paudel et al. 2019; Zanin et al. 2018). Among these traits, the presence of glandular trichomes have been shown to confer a high level of resistance against a wide range of pests, especially in wild tomato species (Ali et al. 2019; Dias et al. 2016; Smeda et al. 2018).

Trichomes are epidermal bulges located on aerial parts of many higher plants and provide the first line of defense against insects, pathogens, and herbivores contributing to a plant's resistance (Werker 2000). Glandular trichomes produce and secrete secondary metabolites in a species- and cultivar-specific fashion that can be poisonous, repellent, or trap insects and other organisms (Samanani and Facchini 2006; Weinhold and Baldwin 2011; Lucini et al. 2015; Vosman et al. 2018). Non-glandular trichomes are thought to enhance plant defense increasing the thickness of the epidermis and acting as obstacles against external invasion (Rodrıguez-Lopez et al. 2011; Simmons et al. 2004; War et al. 2012).

The species of the genus Solanum possess eight different types of trichomes. Types I, IV, VI, and VII are glandular trichomes, and types II, III, V, and VIII are non-glandular trichomes. Wild tomato species such as *Solanum pennellii* have the type IV glandular trichomes, the major site of biosynthesis for acylsugars (McDowell et al. 2011; Zhang et al. 2020). Acylsugar consist of sucrose or glucose cores esterified with fatty acid acyl groups of variable length and branching pattern (Fig. 1) (Lybrand et al. 2020).

The acylsugars have been consistently linked to broad-spectrum resistance to herbivorous arthropods in *S. pennellii* and other Solanum species. Studies with acylsugars report deleterious effects on the life cycle of insects and mites (antibiosis resistance mechanism), reducing feeding, oviposition, and interfering in behavior (antixenosis resistance

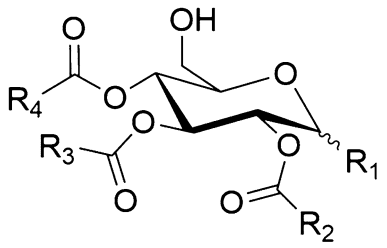

Fig. 1 Structure of acylglucose purified from *S. pennellii*. Fonte*:* (Lybrand et al. 2020)

mechanism) (Lucini et al. 2015; Vosman et al. 2018). Leckie et al. (2016) showed that it was possible to reduce the whitefly oviposition rate on susceptible tomato treated with purified acylsugars extracts from *S. pennellii* (LA-716). Likewise, introgression of these traits in tomato cultivated plants had reduced important pest infestation (Pereira et al. 2008; Su et al. 2018). Thus, increasing type IV glandular trichomes and acylsugars production has long been a target in tomato breeding programs (Paudel et al. 2019; Zanin et al. 2018; Dias et al. 2016; Lucini et al. 2015).

Genetic inheritance studies on acylsugars in tomatoes demonstrate that the achievement of higher levels of this allelochemical depends mainly on the action of a major gene, with additive effect of minor genes (Resende et al. 2002; Smeda et al. 2018). The genetic inheritance type (one major gene) of this characteristic can facilitate the obtention of genotypes with a satisfactory level of resistance to some pests through successive crosses followed by selection for high levels of acylsugars in the leaves (Oliveira et al. 2018; Resende et al. 2009).

The crossing of commercial cultivars with wild tomatoes produces hybrids with resistance to some pests, but with unsuitable market characteristics. To reestablish market traits in the resistant hybrids, backcross methods can be applied to recover the background genome; however, they require a long time and present relatively high cost (Kabelka et al. 2004; Mesquita et al. 2005; Osei et al. 2018). To minimize this problem, marker-assisted selection has been successfully integrated into breeding programs (Collard and Mackill 2008; Dreher et al. 2003; Lenaerts et al. 2019). Molecular markers are uninfluenced by the environment and can be applied in the early stages of crop development, reducing costs and time for the selection process. In addition, it is possible to select a greater number of alleles of the background genome, since the donor has non-market characteristics, bringing greater gains in selection (Bishwas et al. 2016).

Among the available markers, the ISSR (inter-simple sequence repeat) and the expressed sequences tags (EST)-based markers are widely used in breeding since they are efficient, relatively simple, and highly reproducible

techniques, in addition to the high rates of polymorphism (Foolad and Panthee 2012; Kiani and Siahchehreh 2018; Rai et al. 2013). Moreover, the ISSR markers have a uniform distribution throughout the entire plant genome, thus being excellent for studies of recurrent genome recovery in backcross programs (Lenaerts et al. 2019). On the other hand, EST-based markers are within coding regions, which can accelerate the recovery of contrasting characteristics among parents.

Tomato lines for fresh consumption with high levels of acylsugars and with resistance to important pests such as striped mite (*Tetranychus urticae*)(Oliveira et al. 2018), green peach aphid (*Myzus persicae*) (Silva et al. 2019), whitefly (*Bemisia tabaci*)(Resende et al. 2009; Dias et al. 2016) including South American tomato pinworm (Maluf et al. 2010) have been developed. However, few studies have been carried out to incorporate pest resistance genes in tomato genotypes destined for industrial processing (Oliveira et al. 2018, 2020) or targeting sustainable systems. Thus, in this study, we describe the development of a resistant cultivar to the South American tomato pinworm. To achieve this goal, we introgressed a trichome-based resistance trait from the wild tomato *S. pennellii* into cultivated tomato, *S. lycopersicum*. F_2BC_2 tomato materials present a wide distribution of glandular trichomes and high content of acylsugars contributing to resistance to South American tomato pinworm.

Materials and methods

Segregating populations from the crossing between *S. pennelli* LA-716 and cultivar Redenção

The tomato genotypes used in this study came from a F_2BC_1 population that resulted from a crossing between *S. lycopersicum* cv. Redenção and *S. pennelli* LA-716: RVTA-2010-31-pl#177, RVTA-2010-31-pl#310, RVTA-2010-31-pl#319, and RVTA-2010pl#345. They were selected by Dias et al. (2016) for resistance to whitefly (*Bemisia tabaci*), with high levels of acylsugars. These genotypes were again crossed with the cv. Redenção (background genome) to obtain the F_1BC_2 population, which was self-fertilized, originating the F_2BC_2 population. The backcross was carried out at full flowering stage of the genotypes selected for high levels of allelochemical (pollen source) and the cv. Redenção (female parent with desirable traits for industrial processing). For this, 20 plants of the cv. Redenção and 10 of each genotype with high allelochemical content were grown in a greenhouse. First, these plants were seeded in 128-cell polystyrene trays with a commercial substrate based on pine bark and vermiculite in a 3:1 ratio. The wild parent was sown 15 days before the commercial genotype, considering the slow initial development of the species. When they reached approximately 10 to 15 cm in height, they were transplanted into polypropylene pots with a capacity of 5 dm^3 of substrate containing subsurface soil and commercial substrate in a 1:1 ratio. To correct the pH, 25.3 g of Calcium Carbonate were added per pot. For fertilization, 30 g of the formula 4-14-8 containing N (nitrogen), P(P2O5), K (K2O) were applied. At 30 days after transplanting, 12 g of urea per pot were applied in the pre-flowering period. At flowering, but before anthesis, flowers of the cv. Redenção were emasculated and pollinated with pollen from the flowers of the parents selected in the previous generation.

Quantification of acylsugars and genotype selection

To quantify the levels of acylsugars, the F_2BC_2 tomato genotypes were grown in a greenhouse in pots with 2 dm^3, as described above. A total of 420 genotypes were evaluated, with 105 plants from each crossing, 40 plants from the cv. Redenção (control of low acylsugars), and 40 plants of *S. pennellii* (control of high acylsugars).

In the pre-flowering phase, 50 to 60 days after the transplantation of the genotypes, six leaf disks (5.65 cm^2) were removed in triplicate from the upper third part of each plant, using a cork borer of 3/8' in diameter. Leaf disks were placed in test tubes containing 2 mL of dichloromethane for acyl sugar extraction. The tubes were shaken for 30 s and the leaf disks were removed for evaporation by the extractor. To promote the saponification of the acylated groups, sodium hydroxide dissolved in methanol at 0.1 M concentration was added. Afterward, the solvent was evaporated and 0.4 ml of water added to the residue. For sucrose hydrolysis, 0.1 ml of 0.04 M hydrochloric acid solution was added and heated for 5 min until boiling. After the sequence of reactions proposed by Resende et al. (2002), adapted from the tests for determining reducing sugars by Somogy–Nelson, the samples were read in a spectrophotometer. The absorbances were determine at 540 nm (Nelson 1944) and the sugar concentration was calculated in nmols cm^{-2} of leaf area.

Based on the levels of acylsugars, contrasting F2BC2 plants were selected (high and low allelochemical plants). Then, they were used in the bioassays with the SATP. Only the 13 high-level acylsugar genotypes and the parents were used to determine genetic similarity using ISSR and EST molecular markers.

Molecular analyses and behavior assessments regarding the response to the SATP were performed with plants grown into 10 dm^3 pots, filled with a mixture of 1:1 soil and pin bark substrate. The pH of the soil was corrected to 5.6 ($CaCl_2$) with base saturation at 75% with the application of 3.8 g of calcium carbonate. For each pot, 27.3 g of Nitrogen, Phosphorus, and Potassium (N-P-K) as a 0-20-20 formula was applied. The pots were kept in a greenhouse

with an average temperature of 28 ± 3 °C, relative humidity of 75 ± 4%, and photophase of 13 h of light and 11 h of dark.

Assessment of recurrent genome recovery by molecular markers

In this work, 13 high-level acylsugars genotypes were used (RVTA-2010-31-177pl#28 = RVTA-28H, RVTA-2010-31-177pl#39=RVTA-39H, RVTA-2010-31 177pl#113=RVTA-113H, RVTA-2010-31-177pl#177=RVTA-177H, RVTA-2010-31 177pl#180=RVTA-180H, RVTA-2010-31-177pl#232=RVTA-232H, RVTA-2010-83-347pl#257=RVTA-257H, RVTA-2010-83-347pl#359=RVTA-359H, RVTA-2010-83-347pl#61=RVTA-61H, RVTA-2010-31-177pl#152=RVTA-152H, RVTA-2010-31-177pl#155=RVTA-155H, RVTA-2010-83-347pl#214=RVTA-214H, and RVTA-2010-31-177PL#160=RVTA-160H) along with the parents *S. lycopersicum* cv. Redenção and the wild access *S. pennellii* LA-716.

Leaflets of each genotype were collected and immediately stored in liquid nitrogen for subsequent DNA extraction, following the method described by Sharma et al. (2008). First, 100 mg of plant material was ground in liquid nitrogen. Then, 1 mL of extraction buffer (100 mM Tris–HCl pH 8.0, 200 mM EDTA, 2 M NaCl, 2% PVP, 2% CTAB, 2% β-mercaptoethanol) was added to the samples, which were incubated in a water bath for 30 min at 65 °C. Then the DNA was separated from other cellular components using phenol:chloroform:isoamyl alcohol in a 25:24:1 ratio followed by centrifugation. After this step, the DNA was precipitated with PEG (Polyethylene Glycol) and washed with ethanol to obtain high purity. At the end of the extraction, the DNA was resuspended in TE (10 mM Tris–HCl; pH 8.0; 1 mM EDTA) with RNAse, incubated at 37 °C for 30 min, and stored at −20 °C until use. The DNA samples were quantified by electrophoresis on a 0.8% agarose gel, stained with ethidium bromide (0.5 μg mL^{-1}), by comparison with Lambda phage DNA (50 ng, 100 ng, and 200 ng).

Molecular analyses

The DNA amplification reactions via PCR (polymerase chain reaction) were performed with six ISSR primers and six EST primers (Table 1). These primers were previously selected by Secco et al. (not published) as the most informative considering the parents used in this study. The PCR reactions for the ISSR analyses were prepared with a final volume of 12.5 μL containing 20 ng of DNA, 1X PCR buffer, 0.2 μM of primer, 200 μM of dNTP, 1.5 mM of $MgCl_2$, and 1 U of Taq DNA Polymerase. The thermal cycler was programmed for initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing temperature (Table 1) for 45 s, and extension at 72 °C for 90 s; and a final extension step at 72 °C for 5 min. The amplification products were separated by agarose gel (1.8%) electrophoresis stained with ethidium bromide (0.5 μg mL^{-1}). To determine the size of the amplified fragments, the 100 bp DNA ladder was used. The fragments

Table 1 Inter-simple sequence repeat (ISSR) and expressed sequence tags (EST) primers used to evaluate the recovery of the background genome in the tomato genotypes with high acylsugar content

Chromosome	Primer	Forwad/Reverse	Sequence (5′–3′)	T °C
–	807	–	(AG)8 T	52°
–	809	–	(AG)8G	55°
–	835	–	(AG)8YC	54°
–	864	–	(ATG)6	50°
–	873	–	(GACA)4	50°
–	878	–	(GGAT)4	54°
1	TES1444	TES1444F	GATTTTTGTTTTGTCGCGTGC	68°
		TES1444R	AAAGCTAACCGTGCAAAGGA	
1	TES1242	TES1242F	CCACCGCAACAAACCTTATT	68°
		TES1242R	GGGTGGTGAGAAGGATCTGA	
2	TES1925	TES1925F	GAGCTTTTAACATGGCGGATG	68°
		TES1935R	CCACAGCTGAATCTCCAACA	
3	TES1935	TES1935F	GTTCAAAGGAGTCCTCATGGC	68°
		TES1935R	CCACAGCTGAATCTCCAACA	
3	TES1203	TES1203F	GAAGACTGCAGGCGATCCTTA	68°
		TES1203R	CCTCACAAACAGGTTTCGGT	
8	TES1884	TES1884F	GCCTTCCTTACTTCGCCACTG	68°
		TES1884R	TTCCGTGAAATACTTTGCCC	

T °C Annealing temperature

were visualized in UV light and photo-documented by a digital system.

For the markers based on expressed sequence tags (Table 1), the DNA amplification reactions via PCR were prepared in a final volume of 10 µL containing: 0.5 ng of DNA, 1X PCR buffer, 0.8 µM of each primer, 0.2 mM of dNTP, 3 mM of $MgCl_2$, and 0.4U of Taq DNA polymerase. For amplification, the thermal cycler was programmed for an initial denaturation at 94 °C for 3 min, followed by 9 cycles of 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 30 s; 21 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 10 min. The amplification products were separated by electrophoresis on a 12% polyacrylamide gel run at 2000 V for 4 h. The electrophoresis gel was visualized by silver nitrate staining.

Pinworm resistance bioassay

Adults of the South American tomato moth were collected from commercial tomato production areas in a protected environment in the municipality of Faxinal, PR during the spring (located at 824 m of altitude and Latitude: 23° 59′ 59″ South, Longitude: 51° 19′ 15″ West) to start a controlled rearing in the laboratory. The moths were released on tomato plants of the cv. Santa Clara (susceptible) placed in wooden cages (C150 × L150 × H100 cm) with anti-aphid netting. The cages were kept in a greenhouse with an average temperature of 28 ± 3 °C, average humidity of 75 ± 4%, and photophase of 13/11 h light/dark regime. After 72 h of the release of adult moths on tomato plants, leaflets with eggs were collected and transferred to tomato plants in another cage placed in the same environment, aiming to standardize the age of the insects.

The bioassay was conducted in a greenhouse with a randomized block design with 12 treatments and 2 controls in 4 repetitions. Each plot was composed of a pot with a plant. Each cage was considered a block. The treatments consisted of eight F_2BC_2 tomato genotypes with greater genetic similarity to the recurrent parent, that is, with genotypic constitution closer to do commercial and, with also high levels of acylsugar (RVTA-28H, RVTA-39H, RVTA-113H, RVTA-177H, RVTA-180H, RVTA-232H, RVTA-257H, and RVTA-359H); or low levels of acylsugar (RVTA-205L, RVTA-227L, RVTA-376L, and RVTA-385L), along with the parents *S. lycopersicum* cv. Redenção and the wild accession *S. pennellii* LA-716, as controls of susceptibility or resistance to the South American tomato pinworm, respectively. The insects used in the bioassays were bred on tomato plants, cv. Santa Cruz (susceptible) inside wooden cages covered with veil. To ensure age-matched adults at the time of infestation, moths were transferred to other cages for oviposition. Afterward, the adults were removed, leaving only the tomato plants with eggs. The caterpillars empupated and gave rise to adults that were later sexed in the laboratory.

At the pre-flowering stage of the plants (60 days after transplanting), adult moths of the same age were sexed at the laboratory and released into the cages at the rate of 1.23 females for each male per plant. The methodology proposed by Resende et al. (2006) reports that the infestation of each plant in the pre-flowering stage with 1 male for each 1.23 females is sufficient to cause economic damage to the tomato plant in cages. With a stereomicroscope (Olympus SZ61), eggs (area of 20 mm^2), and caterpillars (total area of the leaflet) were counted, at all instars, on the adaxial and abaxial surfaces of two opposing leaflets, in lower, middle, and upper thirds of the plant at 07, 14, 21, and 28 days after infestation. For this, the leaflets were detached from the plants and taken to the laboratory for evaluation. The values corresponding to eggs and pinworms were related to the averages obtained from the four evaluations, in the two leaflets, harvested in the three thirds of the tested plants.

The severity of the damage caused by the South American tomato pinworm was assessed at 14, 21, 28, and 35 days after infestation using a grading scale (Labory et al. 1999) as follows:

(A) **Intensity of plant damage (IPD)**: 0- no damage; 1- small lesions (0.1% to 5% damage); 2- small, non-coalescing lesions (5.1% to 20% damage); 3- medium to large lesions (20.1% to 50% damage); 4- large and coalescing lesions (50.1% to 80% damage); and 5- totally destroyed plants (over 80% damage).

(B) **Types of leaflet lesions (TLL)**: 0- no lesions; 1- few and minor lesions; 2- small and medium lesions; 3- medium-sized lesions, without coalescence; 4- large and coalescing lesions, deformed leaflets; and 5- totally destroyed leaflet.

(C) **Percentage of attacked leaflets (PAL)**: 0- no attacked leaflets; 1- 0.1% to 5% of leaflets attacked; 2- 5.1% to 20% of attacked leaflets; 3- 20.1% to 50% of leaflets attacked; 4- 50.1% to 80% of leaflets attacked; and 5- more than 80% of the leaflets attacked. The evaluations were performed at 28 days after the infestation.

In the bioassay with *T. absoluta*, the attractiveness index was estimated by AI = 2 T/(T + C), where AI = attractiveness index; T (treatment) = number of insects attracted to the evaluated genotype; and C (control) = number of insects attracted to the susceptible control genotype, *S. lycopersicum* cv. Redenção. The AI value range between 0 and 2, with AI = 1 indicating similar attraction between the evaluated genotypes (repellent test plant) and the control (attractant test plant), AI < 1 corresponding to less attraction (greater repellency) by the evaluated genotype and AI > 1 showing greater attraction for the tested genotype compared with the

control. This index is an adaptation of the formula mentioned by Lin et al. (1990) and used by Baldin et al. (2000).

The oviposition preference index was also calculated using the formula proposed by Fenemore (1980): OPI = [(T–C)/(T + C)] × 100, where T (treatment) = number of eggs counted in the evaluated treatment and C (control) = number of eggs counted in the susceptible control genotype, *S. lycopersicum* cv. Redenção. The index ranges from +100 (very stimulating) to −100 (total deterrence), with a value of 0 (zero) indicating neutrality. The classification of genotypes (stimulant/deterrent) was carried out by comparing the mean of treatment eggs with the mean of the control *S. lycopersicum* cv. Redenção.

Identification and quantification of leaf trichomes

To evaluate the types and number of trichomes, newly expanded leaflets from the upper third of the plants in the pre-flowering stage (45–60 days) were collected. Subsequently, these samples were sent to the Electron Microscopy and Microanalysis Laboratory (LMEM) of the Londrina State University (UEL), in Londrina, Paraná, Brazil.

To identify and quantify the glandular and non-glandular trichomes, paradermical cuts of approximately 12 mm^2 were performed on the abaxial and adaxial faces of each leaflet, using eight replicates. Then the plant material was fixed on microscope slides with carbon tape on a metallic support (Goldstein et al. 1992), with subsequent analysis of 300 μm^2 of the leaf surface in a scanning electron microscope (SEM) Tescan® Veja3 HV at 30kv with attached camera, to quantify and classify the trichomes, observing the presence or absence of the gland shape at the apical extremity.

Data analysis

The oviposition data and number of pinworms did not meet the assumption of normality. Therefore, after the analysis of variance, these data were transformed using the formula $(x + 1)^{0.5}$. Subsequently, the Scott–Knott cluster analysis was performed ($P < 0.05$). Orthogonal contrasts between groups of genotypes with different acylsugar contents were verified using the SISVAR statistical program (Ferreira 2011). The correlations between the variables analyzed were estimated by Spearman's rank test ($P < 0.05$) and the significance was verified by the *t*-test ($P < 0.05$). The means of the genotypes for each response variable were normalized and visualized in a heatmap, and, for the hierarchical grouping, Euclidean distance and the UPGMA method were used.

In the molecular characterization using ISSR and EST markers, the genotyping of the materials was performed according to the presence (1) or absence (0) of bands and a binary matrix was generated to analyze the genetic similarity by the Jaccard coefficient using the NTSYS 2.2 software.

Results

Molecular marker-assisted recurrent genome recovery

ISSR primers amplified 40 polymorphic fragments between the cv. Redenção (background genome) and the wild access *S. pennellii* LA-716 (donor genome). EST-based primers amplified 10 polymorphic bands. The joint evaluation of the 50 polymorphic fragments allowed to establish the genetic similarity between the evaluated genotypes (Table 2). The plants that showed higher similarity with the recurrent cv. (Redenção) and, consequently, greater recovery of the background genome, were RVTA-39H (0.64), RVTA-257H (0.64), RVTA-152H (0.62), RVTA-28H (0.62), RVTA-232H (0.60), RVTA-113H (0.54), RVTA-177H (0.52), and RVTA-180H (0.50) (Table 2). Genotypes with less than 50% of similarity to the recurrent parent were not used in the South American tomato pinworm resistance bioassay.

Pinworm resistance bioassay

Eight acylsugar-rich genotypes with high genetic similarity to the recurrent parent (RVTA-28H, RVTA-39H, RVTA-113H, RVTA-177H, RVTA-180H, RVTA-232H, RVTA-257H, and RVTA-359H), four low-acylsugar genotypes (RVTA-205L, RVTA-227L, RVTA-276L, and RVTA-385L), and the parents were used to the resistance test.

In the preference test, the feeding choice was significantly influenced by the levels of acylsugars present in the F_2BC_2

Table 2 Genetic similarity among tomato F_2BC_2 genotypes from the cross between the wild genotype S. pennellii and the cultivar Redenção (S. lycopersicum)

	LA-716	'Redenção'
LA-716	1.00	0.00
'Redenção'	0.00	1.00
RVTA-28H	0.23	0.62
RVTA-39H	0.33	0.64
RVTA-61H	0.24	0.47
RVTA-113H	0.32	0.54
RVTA-152H	0.23	0.48
RVTA-155H	0.29	0.44
RVTA-177H	0.40	0.52
RVTA-180H	0.32	0.50
RVTA-214H	0.14	0.46
RVTA-232H	0.22	0.60
RVTA-257H	0.21	0.64
RVTA-359H	0.12	0.55
RVTA-160H	0.18	0.46

genotypes for all evaluated characteristics. The average of eggs observed in 20 mm^2 of leaflets allowed to separate the genotypes in six groups, with average values ranging from 1.00 (*S. pennellii*) to 2.85 (cv. Redenção). Among the different groups, the genotype with less preference for the pest was the wild access *S. pennellii* LA-716. The genotypes selected with high levels of acylsugars, RVTA-39H, RVTA-177H, RVTA-232H, RVTA-257H, and RVTA-359H, obtained lower values of oviposition when compared with the low-acylsugar genotypes (RVTA-205L, RVTA-205L, RVTA-376L, and RVTA-385L) and the cv. Redenção. The presence of pinworms was observed in greater numbers in genotypes classified as low in acylsugars, differing from the genotypes with high content of the allellochemical. Among the high-acylsugar genotypes, RVTA-232H, RVTA-257H, and RVTA-359H stood out, showing no difference from the resistant control *S. pennellii* LA-716 (Table 3).

Concerning the plant damage intensity (PDI), types of leaflet lesions (TLL), and percentage of attacked leaflets (PAL), three groups were formed. The resistant parent, *S. pennellii* LA-716, had significant lower PDI, TLL, and PAL, compared with the other genotypes. An intermediate group was composed of plants with high levels of acylsugars (RVTA-28H, RVTA-39H, RVTA-113H, RVTA-177H, RVTA-232H, RVTA-257H, and RVTA-359H). A third group was formed by plants that were greater attacked by the South American tomato pinworm: the commercial cv. Redenção and the genotypes with low levels of acylsugars (RVTA-205L, RVTA-227L, RVTA-376L, and RVTA-385L), including the RVTA-180H genotype that was selected for high levels of this allelochemical compound (Table 3).

The C1 contrast demonstrates the comparison between high- and low-acylsugar genotypes. The negative values for NE (number of eggs), NP (number of pinworms, PDI, TLL, and PAL (−0.40, −0.90, −1.33, −1.32, and −1.33, respectively), indicate that the South American tomato pinworm is affected regarding all traits analyzed in high-acylsugar genotypes, resulting in a decrease in the number of eggs and pinworms, as well as in the plant damage intensity. The C2 contrast also shows the effectiveness of genotypes with high allelochemical levels in comparison to the cv. Redenção, which has a low content of acylsugars and presents values of −0.65, −0.94, −1.05, −1.04, and −1.14 for NE, NP, PDI, TLL, and PAL, respectively. When the high-acylsugar genotypes were contrasted with *S. pennellii* LA-716, no

Table 3 Acylsugar content (AC, nmols cm^2), average number of eggs (NE, 20 mm^{-2}), average number of pinworm per leaflet (NP), plant damage index (PDI, %), types of leaflet lesions (TLL, %), percentage of attacked leaflets (PAL, %), attractiveness index (AI), oviposition preference index (OPI), in F_2BC_2 tomato genotypes with different levels of acylsugars, *S. pennellii* access LA-716, and *S. lycopersicum* cv. Redenção subjected to infestation with the South American tomato pinworm

Genotype	AC	NE	NP	PDI	TLL	PAL	AI	OPI
LA-716 (H)	145.57 a	1.00 a*	1.00 a*	0.25 c*	0.03 c*	0.08 c*	0.56	−48.05
RVTA-28 (H)	136.14 a	2.38 e	1.55 b	1.62 b	1.62 b	1.67 b	0.75	−8.98
RVTA-39 (H)	145.64 a	1.74 b	1.60 b	1.37 b	1.39 b	1.41 b	0.77	−24.18
RVTA-113 (H)	131.19 b	2.34 e	1.48 b	1.19 b	1.24 b	1.32 b	0.73	−9.82
RVTA-177 (H)	122.51 b	2.02 d	1.82 b	1.50 b	1.54 b	1.67 b	0.83	−17.04
RVTA-180 (H)	134.78 b	2.43 e	1.52 b	2.49 a	2.42 a	2.36 a	0.74	−7.95
RVTA-232 (H)	139.69 a	1.76 b	1.25 a	1.53 b	1.54 b	1.64 b	0.65	−23.64
RVTA-257 (H)	131.12 a	1.68 b	1.32 a	1.21 b	1.21 b	1.23 b	0.68	−25.83
RVTA-359 (H)	121.07 b	1.80 c	1.20 a	1.21 b	1.27 b	1.29 b	0.64	−22.58
RVTA-205 (L)	43.24 d	2.43 e	2.35 c	2.90 a	2.88 a	1.95 a	0.96	−7.95
RVTA-227 (L)	80.07 c	2.55 e	2.56 d	2.61 a	2.56 a	2.67 a	1.00	−5.55
RVTA-376 (L)	52.87 d	2.34 e	2.21 c	2.63 a	2.69 a	2.70 a	0.92	−9.82
RVTA-385 (L)	55.93 d	2.38 e	2.51 d	2.70 a	2.76 a	2.79 a	0.99	−8.98
Redenção (L)	44.76 d	2.85 f	2.57 d	2.59 a	2.57 a	2.69 a	1.00	0
CV (%)		6.14	10.09	14.37	20.41	20.54	9.45	26.51
C1—Genotypes (H) x (L)		−0.40*	−0.90**	−1.33**	−1.32**	−1.33**		
C2—Genotypes (H) x (Redenção)		−0.65*	−0.94**	−1.05**	−1.04**	−1.14**		
C3—Genotypes (H) x (LA716)		0.19 ns	0.12 ns	0.04 ns	0.06 ns	0.09 ns		

Means followed by the same letter in the column belong to the same group by the Scott–Knott test at 5% of probability

*Significant at 5% of probability

**Significant at 1% of probability

ns Not significant at 5% or 1% of probability

significance was identified for the orthogonal contrasts estimated for all characteristics (Table 3).

The attractiveness and preference for oviposition and feeding of the South American tomato pinworm for the different tomato genotypes demonstrated that *S. lycopersicum* cv. Redenção and RVTA-227L, with a low content of acylsugars, were the most attractive, followed by RVTA-385L, RVTA-376L, and RVTA-205L (all with low content of acylsugars), differing from *S. pennelli* LA-716 and other genotypes selected for high levels of the allelochemical (RVTA-28H, RVTA-39H, RVTA-113H, RVTA-177H, RVTA-180H, RVTA-232H, RVTA-257H, RVTA-359H, and RVTA-143H) (Table 3).

According to the attractiveness index calculated after infestation, none of the high-acylsugar genotypes were attractive to the Tomato South American pinworm when compared with the susceptible control *S. lycopersicum* cv. Redenção and the low-acylsugar genotypes. In addition, the oviposition preference index classifies all materials with a high content of acylsugar as not preferred for oviposition when compared with the susceptible control *S. lycopersicum* cv. Redenção and the low-acylsugar genotypes (Table 3).

Considering the density of trichomes in the different genotypes (Fig. 2), *S. pennelli* LA-716 and *S. lycopersicum* cv. Redenção had the highest density of total trichomes. However, acylsugar-rich genotypes, including the wild accession *S. pennelli,* had a higher number of total glandular trichomes and type IV glandular trichomes (Fig. 3A) compared with low-content acylsugar genotypes and the cv. Redenção. Non-glandular trichomes (Fig. 3B, C, and G) were observed in higher densities in genotypes with low content of acylsugars, with emphasis on the cv. Redenção (Fig. 2). The RVTA-232H genotype (Fig. 3D) and the wild accession *S. pennelli* (Fig. 3E) presented higher density of type IV glandular trichomes and total glandular trichomes on the leaflet surface. In the RVTA-385L genotype (Fig. 3F), non-glandular trichomes predominated, as well as in the other genotypes with a low content of acylsugars.

The Spearman rank correlation analysis (Fig. 4) indicated that the acylsugar content is inversely proportional to the average number of eggs (−0.65), average number of pinworms in the plant (−0.81), plant damage index (−0.83), types of leaflet lesions (−0.81), percentage of attacked leaflets (−0.81), attractiveness index (−0.68), and oviposition preference index (−0.69). The density of type IV glandular and total glandular trichomes correlated directly with acylsugar levels (0.78 and 0.75, respectively), and inversely with all the evaluated resistance characteristics: number of eggs (−0.68), attractiveness index (−0.85), number of pinworms (−0.85), percentage of attacked leaflet (−0.83), plant damage index (−0.78), number of lesions per leaflet (−0.81), attractiveness index (−0.85), and oviposition preference index (−0.75).

Considering all the variables observed in the experiment, acylsugar content (AC), density of glandular trichomes (TGT), density of glandular trichomes type IV (IVGT), non-glandular trichomes (NGT), number of eggs (NE), number of pinworms (NP), plant damage index (PDI), types of leaflet lesions (TLL), percentage of attacked leaflets (PAL), attractiveness index (AI), and oviposition preference index (OPI), a heatmap grouping analysis was performed (Fig. 5). It is possible to observe the formation of two groups, separated in

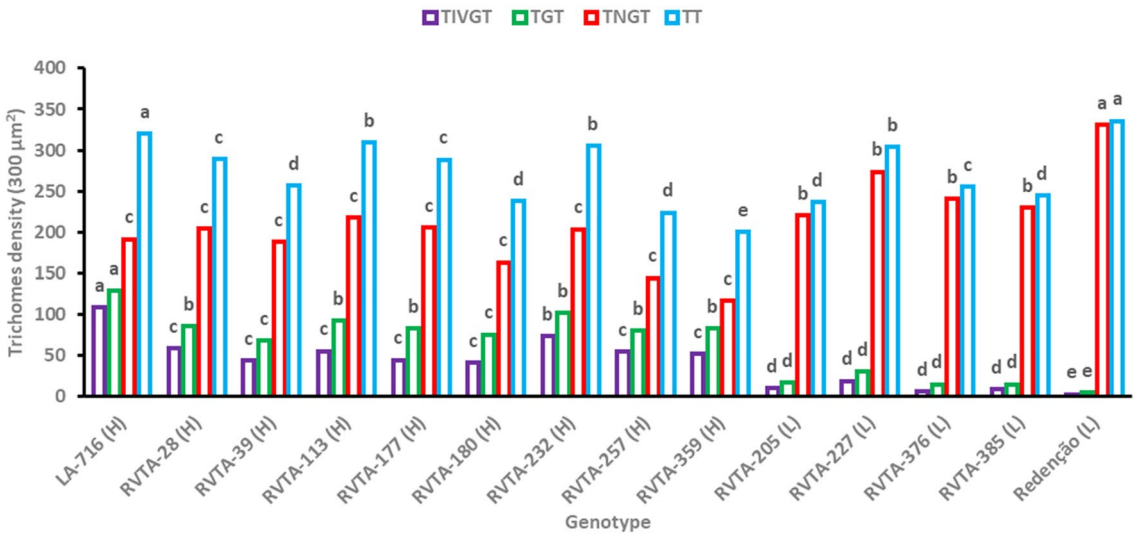

Fig. 2 Type IV glandular trichomes (TIVGT), total glandular trichomes (TGT), total non-glandular trichomes (TNGT), and total trichomes (TT) in F_2BC_2 tomato genotypes with different levels of acylsugars, *S. pennellii* accession LA-716, and *S. lycopersicum* cv. Redenção. Means followed by the same letter in the column with de same color do not differ by the Scott–Knott test ($P < 0.05$) of probability

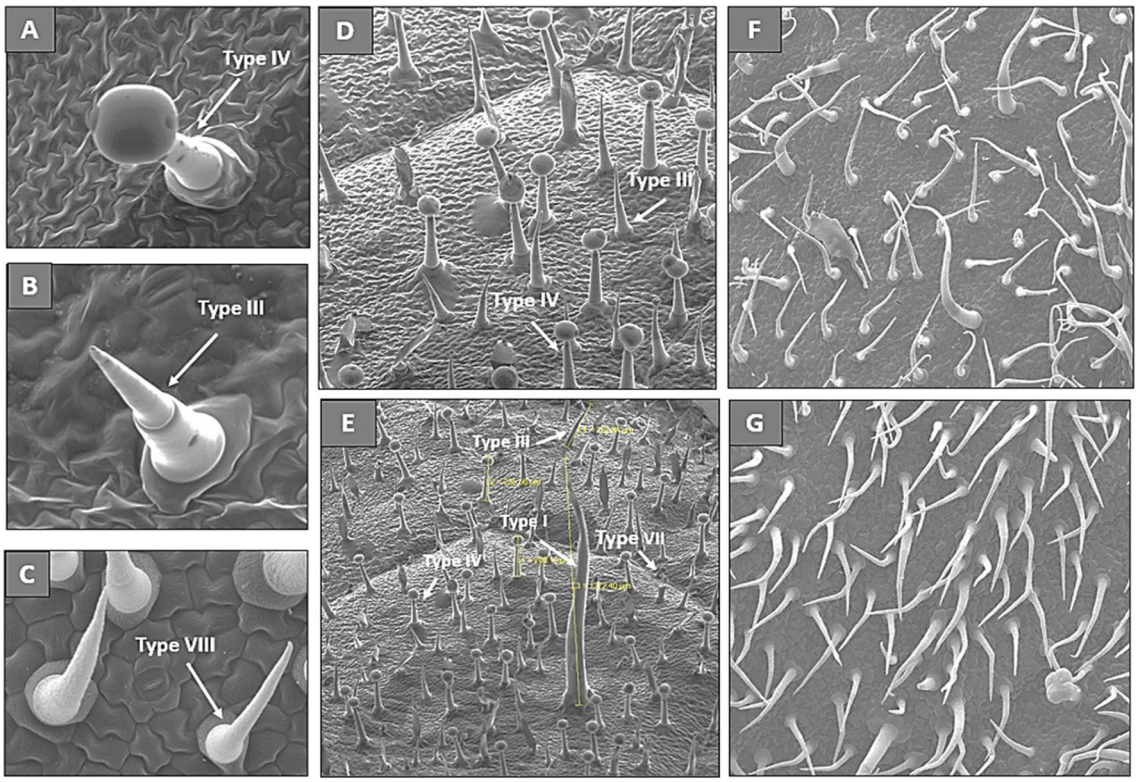

Fig. 3 Glandular trichome type IV present in genotypes with a high content of acylsugars (**A**). Type III non-glandular trichome in low-acylsugar genotypes (**B**). Non-glandular trichome type VIII (**C**). Abaxial surface of the genotype RVTA-232H (**D**) and the wild accession S. pennelli. (**E**) covered with glandular trichomes of types IV, VI, VII, and non-glandular types I and III. Abaxial surface of the RVTA-385L (**F**) genotype and abaxial surface of *the commercial cv. Redenção* (**G**)

low and high levels of acylsugars. The low-acylsugar genotypes were grouped with the susceptible genotype 'Redenção'. The other group was formed by *S. pennellii* LA-716 and the acylsugar-rich genotypes. In the second group, *S. pennellii* LA-716 diverges from the other high-acylsugar genotypes since it has even higher levels of this allelochemical in the leaflets and, consequently, has greater resistance. Among the genotypes with high levels of acylsugars, it is possible to identify two groups, from which the genotypes RVTA-39H, RVTA-232H, RVTA-257H, and RVTA-359H stood out with the greatest resistance to the South American tomato pinworm.

Discussion

The introgression of genetic resistance is the main strategy for integrated pest management in sustainable production systems, especially for pests with high damage potential and that are difficult to control (Trapero et al. 2016; Alphey and Bonsall 2018). Thus, the search for resistance genes in wild species is crucial, especially when the trait has a high heritability in populations from interspecific crosses, such as in the present study. The lower susceptibility of wild species to pests is related to the presence of allelochemicals known as acylsugars secreted by glandular trichomes.

The evaluation of the similarity between the genotypes with a high content of acylsugars and the parents showed genetic proximity to the cv. Redenção (Table 2). Of the 13 genotypes subjected to the similarity analysis, eight were selected with an index equal to or greater than 0.50 (Table 2). In order to attain better efficiency in the selection of plants with greater resistance to the South American tomato pinworm combined with greater similarity to the recurrent parent, the use of molecular markers is essential. According to the literature, the use of molecular markers for assisted selection has been one of the most efficient applications of biotechnology in agriculture (Collard and Mackill 2008; Foolad and Panthee 2012; Rai et al. 2013). The markers allow differentiating genotypes by using similarity coefficients, resulting in greater selection gains, especially in recurrent crosses (Kiani and Siahchehreh 2018). In tomato, marker-assisted selection has been an important tool to develop superior materials (Osei et al. 2018). In this study, the use of assisted selection allowed the identification of genotypes with greater recovery of the recurrent genome and

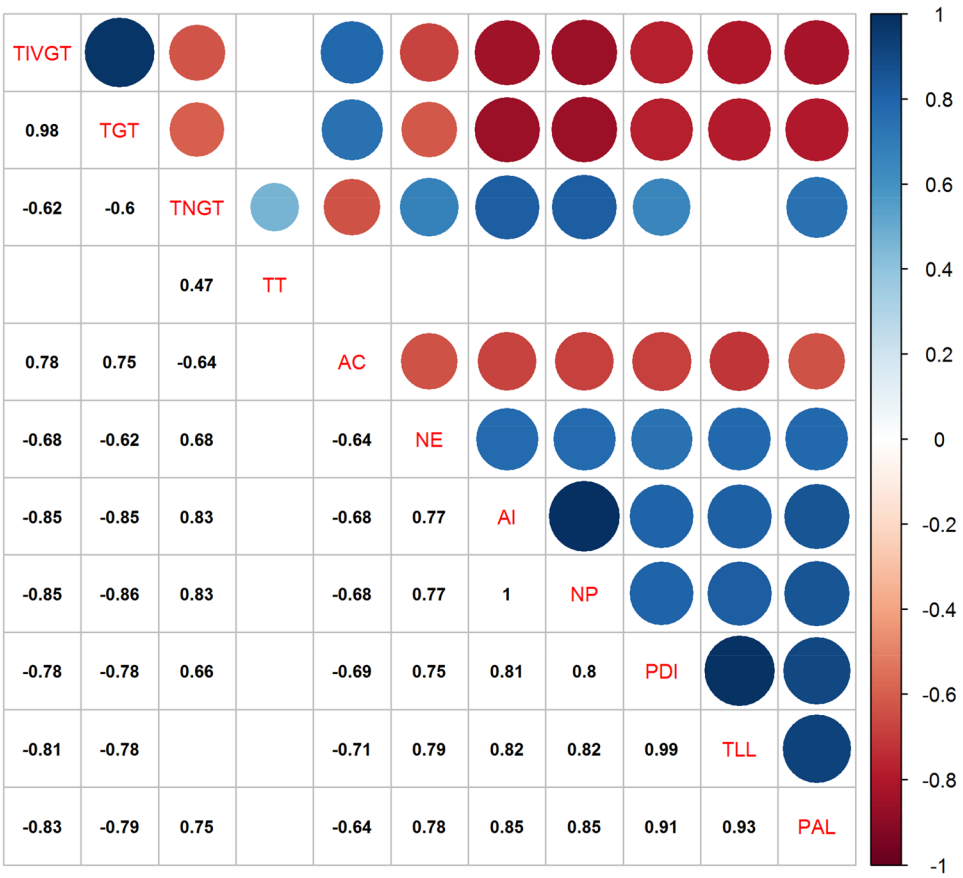

Fig. 4 Spearman's rank correlation coefficient between the *evaluated characteristics.* AC *A*cylsugar content, PDI *Plant damage index,* NLL *N*umber *of lesions per leaflet,* PAL *Percentage of attacked leaflets,* NP *N*umber of pinworms, NE *N*umber of eggs, AI *Attractiveness* index, OPI oviposition preference index, TIVGT *Type* IV glandular trichomes, TGT *T*otal glandular trichomes, TNGT *T*otal non-glandular *trichomes;* TT *Total trichomes*

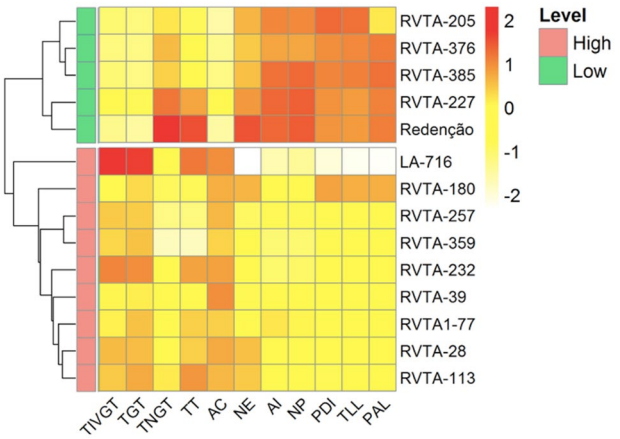

Fig. 5 Heatmap with the normalized means of the variables and hierarchical grouping of the separate genotypes based on the level (high and low) of acylsugars. TIVGT *T*ype IV glandular trichomes, TGT *T*otal glandular trichomes, TNGT *Total* non-glandular trichomes, TT *Total* trichomes, NE *Number* of eggs, AI *Attractiveness* index, NP *N*umber of pinworms, PDI *Plant* damage index, NLL *N*umber of lesions per leaflet, PAL *P*ercentage of attacked leaflets, in F_2BC_2 *tomato genotypes*

also demonstrated that the backcrossing process efficiently recovers the recurrent genome without losing the genetic resistance to the South American tomato pinworm.

Among the F_2BC_2 genotypes selected for high levels of acylsugars, some plants presented greater resistance to the South American tomato pinworm than the cv. Redenção. The presence of acylsugars in tomato leaflets and their influence on pest behavior has been previously investigated (Dias et al. 2016; Maluf et al. 2010; Resende et al. 2009). The obtained data assure that the selection of plants with high content of acylsugars, in laboratory, represents a promising tool to find genotypes with resistance to pests in a faster and cheaper process. Maluf et al. (2010) reported that the presence of high levels of acylsugars in the leaflets provides resistance to the South American tomato pinworm. Furthermore, they affirm that the presence of the allelochemical in the leaflets negatively affects the behavior of other arthropod pests, when subjected to biological tests with plants containing high content of acylsugars. In addition to being present in *S. pennellii* LA-716 (Rakha et al. 2017; Silva et al. 2016), the acylsugars showed a high correlation with glandular trichomes, especially the type IV, as previously reported by Bitew (2018) and Lucini et al. (2015), demonstrating that these structures can also be a strong indicator for the selection of pest-resistant genotypes. The acylsugar-rich

genotypes, besides showing higher resistance to the South American tomato pinworm, presented the highest densities of glandular trichomes, indicating the positive relationship between these traits, working together for greater pest control efficiency.

Low-acylsugar genotypes had a higher concentration of non-glandular trichomes, demonstrating that in larger arthropods, such as the South American tomato pinworm, only physical barriers are insufficient to reduce the attack. The RVTA-332H genotype presented a diversity of trichomes on the leaf surface, with several types of glandular and non-glandular trichomes, which is an interesting condition for pest control, as they constitute associated chemical and physical barriers (Fig. 3C) (Lucini et al. 2015; Oliveira et al. 2018; Silva et al. 2019; Zhang et al. 2020).

In this study, most genotypes with high content of acylsugars showed lower values for number of eggs, number of pinworms, plant damage index, number of lesions per leaflet, and percentage of attacked leaflets when compared to low-acylsugar plants and Redenção (Table 3). The genotypes RVTA-180H, RVTA-113H, and RVTA-28H were inconsistent for resistance to the South American tomato pinworm, especially for oviposition preference, even though they have high acylsugar content and high density of glandular trichomes. Moreover, the South American tomato pinworm caused similar damage to the RVTA-180H genotype and the low-acylsugar genotypes. This can be explained by the types and concentration of the hexose molecules that constitute the acylsugars of the plant. It is known that acyl esters may consist of acyglucoses and acylsucroses (Weinhold and Baldwin 2011), and the higher concentrations of acylsugars glucose-based better improve the plant endogenous defense against pests. Besides the type, the proportion of acylsugars present in the leaflets interferes with the level of the plant resistance to arthropods. Thus, although this characteristic has not been evaluated in the present study, it can be assumed that there are differences in the chemical constitution of acylsugar compounds among the genotypes with high levels of the allellochemical, leading to different responses regarding resistance to the South American tomato pinworm.

Results from the literature support this hypothesis. For instance, Leckie et al. (2016) found that when the acylsugars extracted from *S. pennellii* LA-716 were isolated as acylglucose and acylsucrose and applied individually to thrips (*Frankliniella* spp. Thysanoptera: Thripidae), they did not control the arthropod efficiently. However, when applying the combined compounds, the results were similar to that observed in *S. pennellii* LA-716, which proves the role played by different types of acylsugars in pest resistance and explains the difference in resistance levels among the selected genotypes with high levels of acylsugars. Thus, even though the acylsugar content represents an important indirect marker of selection for the South American tomato pinworm resistance, it does not eliminate the need to expose the genotypes to the arthropod pest. These results are related to the erosion of genes responsible for these factors during backcross generations to obtain segregating populations.

Considering the hierarchical grouping (Fig. 4), among the F_2BC_2 plants, the genotypes RVTA-39H, RVTA-232H, RVTA-257H, and RVTA-359H stood out for containing high concentrations of acylsugars in leaves, density of glandular and glandular trichomes type IV, and higher resistance to the South American tomato pinworm, since they showed less preference for oviposition, fewer pinworms, low attractiveness, and reduced severity of damage caused by the South American tomato pinworm, behaving similarly to *S. pennellii*, the resistant control (Table 3).

Considering the results obtained in this work, plants with the best results and the highest proportions of the recurrent parent genome were maintained for advances in the breeding program. Thus, the plants RVTA-232H and RVTA-257H, which had a performance similar to the resistant control, *S. pennellii* LA-716, and had greater similarity with the cv. Redenção (0.60 and 0.64, respectively), are the ones that will result in greater genetic gains in future crosses, representing great options to be included in sustainable farming systems.

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