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Article

New insights into the use of dwarf tomato plants for pest resistance

Gabriel Mascarenhas Maciel[1,*], Camila Soares de Oliveira[1], Ana Carolina Silva Siquieroli[2], Lucas Medeiros Pereira[1], Ana Luisa Alves Ribeiro[1], Frederico Garcia Pinto[3], Brena Rodrigues Mota Ikehara[3], Nilo Cesar Queiroga Silva[1], Ana Karoline Silva Rocha de Farias[1]

1. Universidade Federal de Uberlândia – Instituto de Ciências Agrárias – Monte Carmelo (MG), Brazil.
2. Universidade Federal de Uberlândia – Instituto de Biotecnologia – Monte Carmelo (MG), Brazil.
3. Universidade Federal de Viçosa – Rio Paranaíba (MG), Brazil.

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***Corresponding author:** gabrielmaciel@ufu.br

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ABSTRACT: Tomato (*Solanum lycopersicum* L.), a globally significant species, faces substantial economic losses due to pest insects. However, exploiting dwarf germplasm for pest resistance, particularly the UFU MC TOM1 lineage, remains underexplored. The objectives of this study were to select backcross populations from dwarf tomato plants through direct and indirect selection and to elucidate whether there are consequences in resistance level as backcross generations progress aiming at a genetic background of interest. Backcross populations 1, 2, and 3 of dwarf tomato plants were obtained. In the conducted experiment, featuring 20 treatments and four repetitions in a randomized block design, we assessed foliar injuries and herbivory through image analysis. Additionally, we quantified the content of acylsugars, conducted chromatographic analysis, and analyzed the metabolomic profile in the leaflets. Promisingly, the UFU 13_1 dwarf plant population showed potential for enhancing genetic improvement programs targeting pest insect resistance. Moreover, the progression of backcross generations yielded varied resistance levels, likely influenced by inheritance patterns and acylsugar content. The UFU MC TOM1 line, distinguished by its high acylsugar content, exhibited robust resistance against both the tomato leafminer (*Tuta absoluta* Meyrick 1917) and the leafminer (*Liriomyza huidobrensis* Blanchard). Furthermore, our analysis revealed the expression of metabolites in the UFU MC TOM1 lineage, potentially linked to various biotic and abiotic stress responses. These findings offer promising prospects for leveraging the dwarf tomato variety, UFU MC TOM1, in genetic improvement programs aimed at combating pest insects in tomatoes.

Key words: *Solanum lycopersicum* L., *Liriomyza huidobrensis*, *Tuta absoluta*, pest resistance, backcross populations.

INTRODUCTION

Among the diverse array of cultivated botanical species, *Solanum lycopersicum* L., known as the tomato plant and belonging to the Solanaceae family, stands out as the second most widely consumed and cultivated non-starchy vegetable globally (Caballero et al. 2016). Despite its economic prominence, the tomato plant remains susceptible to biotic stresses, with pest infestations posing challenges (Li et al. 2023). Notably, the tomato leafminer (*Tuta absoluta* Meyrick 1917) and the leafminer (*Liriomyza huidobrensis* Blanchard) have emerged as prominent concern in tomato cultivation landscapes. These pest species, recognized for their global presence, inflict substantial agricultural losses worldwide (Biondi et al. 2018, Verheggen and Fontus 2019).

In response to these challenges, researchers have advanced on the development of insect-resistant tomato cultivars, employing a strategic approach that involves enhancement of acylsugars (Resende et al. 2020, Oliveira et al. 2022, Gomes et al. 2023, Mutschler et al. 2023). The presence of acylsugars in the leaflets confers a broad spectrum of resistance to pest insects (Maluf et al. 2010), due to their deleterious effects on insect development and their capacity to reduce oviposition and feeding (Maciel et al. 2018, Ben-Mahmoud et al. 2019). Notably, resistance mediated by acylsugars represents a complex and polygenic quantitative trait (Mutschler et al. 2023).

The incorporation of resistance to pest insects into tomato plants has primarily been achieved through introgression from the wild accession of *Solanum pennellii* (Peixoto et al. 2020, Mutschler et al. 2023). However, *S. pennellii* is characterized by its non-commercial nature and small fruit size (Dariva et al. 2020). An additional challenge associated with utilizing wild species of the *Solanum* genus pertains to intra and interspecific reproductive barriers (Chakraborty et al. 2023). When cross-compatibility is established, the introgression of resistance occurs through successive backcrosses, resulting in extended timeframes for breeding program execution (Gonçalves Neto et al. 2010).

Another strategy for incorporating resistance to pest insects in tomatoes involves the use of a dwarf parental line. Evidence suggests that dwarf tomato genotypes exhibit high acylsugar content in their leaflets, allowing for an indirect method of selection (Kortbeek et al. 2021, Gomes et al. 2021, Finzi et al. 2022, Oliveira et al. 2022, Gomes et al. 2023).

The inheritance pattern of dwarf stature, as investigated within the UFU MC TOM 1 lineage, follows a monogenic and recessive model, enabling the production of hybrids exhibiting normal stature (Maciel et al. 2015). Despite the promising prospects of utilizing dwarf tomato varieties, significant knowledge gaps persist regarding their application in conferring resistance to pest insects, particularly concerning the potential of the UFU MC TOM1 lineage and the presence of other bioactive compounds within the leaflets. Furthermore, the implications of advancing three successive backcrosses to establish a genetic background for pest insect resistance using a dwarf parental line remain elusive.

In line with this context, the present study aimed to select backcross populations originating from dwarf tomato plants for resistance against *T. absoluta* and *L. huidobrensis*, employing both direct (foliar injuries) and indirect (acylsugar levels) selection methods. The overarching goal was to cultivate a genetic background of particular interest, underpinning a nuanced approach to pest resistance in tomato breeding.

MATERIAL AND METHODS

Obtaining backcross populations in dwarf tomato plants

The stages for obtaining genetic material and conducting the experiment on pest insect resistance were carried out between 2018 and 2021 (18°42’43.19” S, 47°29’55.8” W, at an altitude of 873 m). All stages were conducted in a greenhouse of the arched type (7 × 21 m), with a ceiling of 4-m height, covered with 150-micron transparent polyethylene film, ultraviolet-resistant additive, and equipped with white anti-aphid side curtains.

The dwarf tomato populations investigated in this study derive from the UFU MC TOM 1 (Maciel et al. 2015) and UFU TOM5 lineages, sourced from the UFU germplasm bank. Serving as the female parent (♀), the UFU TOM5 lineage a recurrent parent is a homozygous, pre-commercial line with normal stature (dwarf gene, DD) and an indeterminate growth habit (SPSP), produce saladette-type fruits and knows for its favorable agronomic attributes. Meanwhile, the UFU MC TOM 1 lineage, chosen as the male parent (♂), embodies a homozygous dwarf line (dd), featuring an indeterminate growth pattern (SPSP), but bearing very small mini-tomato fruits (Maciel et al. 2015, Finzi et al. 2017).

Following the acquisition of F1 generation (UFU-TOM5 ♀ versus UFU MC TOM 1 ♂), the first backcross (F1BC1) was conducted, succeeded by self-pollination, resulting in the F2BC1 generation (BC1). From the F2BC1 cohort, dwarf specimens were selected, initiating the subsequent backcross (F1BC2), followed by self-pollination, resulting in the F2BC2 generation (BC2). Subsequently, from the F2BC2 progeny, another round of backcrossing (F1BC3) was conducted, followed by self-pollination, culminating in the F2BC3 generation (BC3). In the F2BC1, F2BC2, and F2BC3 generations, only dwarf plants (Fig. 1) with a genetic background of saladette-type fruits were selected, resulting in 17 populations from backcrosses BC1, BC2, and BC3, as follows: four populations of dwarf saladette-type tomato plants from the first backcross BC1 (UFU_13_1, UFU_4_3, UFU_17_6, and UFU_10_4), four populations of dwarf saladette-type tomato plants from the second backcross BC2 (UFU_13_1_2, UFU_17_6_1, UFU_4_6_1, and UFU_10_4_5), and nine populations of dwarf saladette-type tomato plants from the third backcross BC3 (UFU_10_4_5_6, UFU_10_4_5_4, UFU_17_6_1_3, UFU_17_6_1_2, UFU_13_1_2_2, UFU_13_1_2_1, UFU_4_6_1_1, UFU_17_4_1_1, and UFU_10_4_5_1).

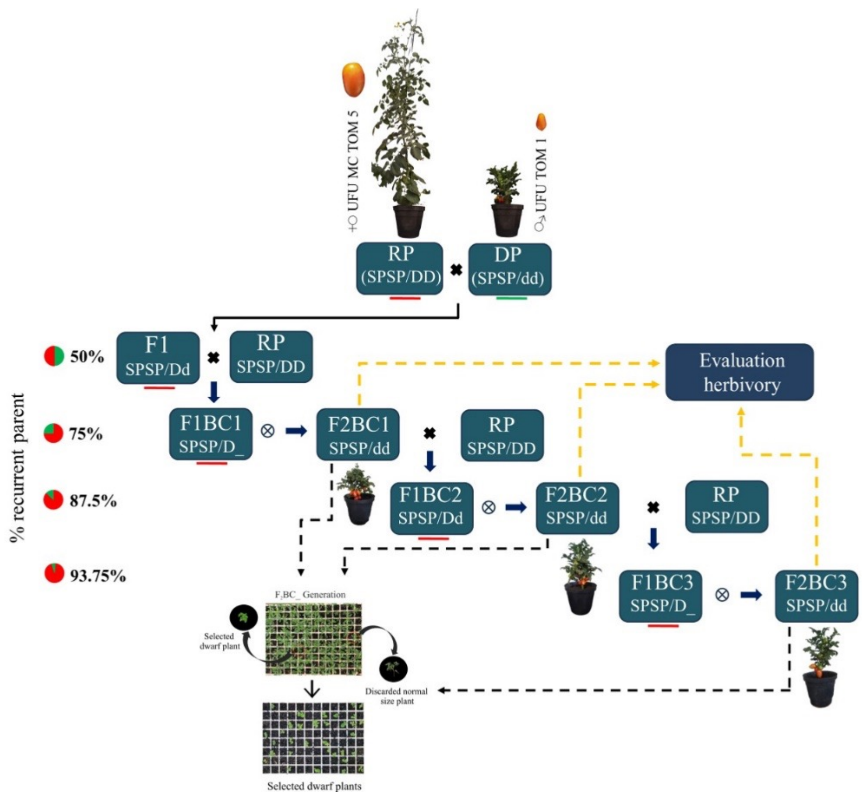


Figure 1. Scheme for obtaining the F2BC1, F2BC2, and F2BC3 dwarf tomato populations evaluated in the experiment.

For comparative purposes, the donor parent UFU MC TOM 1, the recurrent parent UFU TOM 5, and the commercial hybrid cv. Vivacy were incorporated into the experiment. Thus, the experiment was conducted in a randomized complete block design, totaling 20 treatments distributed across four blocks, with a total of 80 plots. Each plot consisted of six plants, resulting in a grand total of 480 plants involved in the experiment.

The sowing was carried out on July 1, 2021. The seedlings were produced in polyethylene trays containing 200 cells filled with a coconut fiber-based substrate. At 35 days after sowing, the seedlings were transplanted into 5-L pots containing the same substrate used for sowing. Cultural management adhered to established for tomato cultivation (Alvarenga 2013). Notably, no insecticide application was carried out during the experiment.

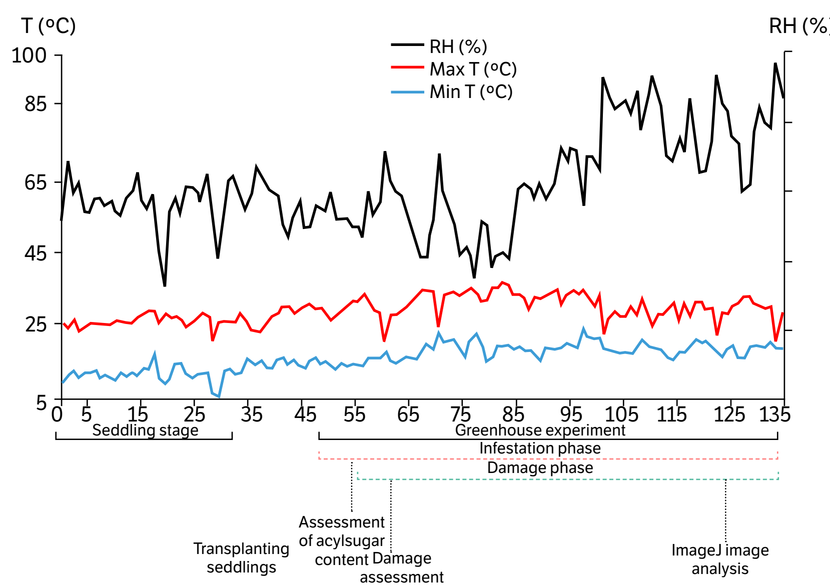
Acylsugar quantification

The extraction of acylsugars was carried out by removing, with a 3/8" diameter disc drill, six samples of leaflet discs, equivalent to 4.21 cm^2 of leaf area. Samples were collected from the upper third of plants 20 days after transplanting. Sample collection took place between 5 and 6 a.m. in winter. The leaf discs were placed in test tubes, and 1 mL of dichloromethane (CH2Cl2) was added to extract the acylsugars. The tubes were then shaken in a vortex device for 30 seconds, removing the leaflets, evaporating the solvent and adding 0.5 mL of 0.1 N sodium hydroxide (NaOH), dissolved in methanol, evaporating right away. The residue was kept at high temperature (100°C), with 0.5 mL of methanol added three times, at intervals of 2 minutes. After complete evaporation of methanol, the residue was dissolved in 0.4 mL of water.

Subsequently, 0.1 mL of 0.04 N hydrochloric acid (HCl) was added, heating for 5 minutes until boiling. The solution obtained was cooled and then 0.5 mL of Sommogy and Nelson's reagent was added (reagent A + reagent B; ratio of 25:1) (Nelson 1960). The solution was heated to boiling for 10 minutes and cooled under running water. Sequentially, 0.5 mL of arsenic molybdate was added and stirred for 15 seconds in a vortex apparatus (Resende et al. 2002). Then, the samples were read on a Thermo Scientific spectrophotometer (MultiskanFC, Skan IT 2.5.1 software) in 96-well plates (Maciel and Silva 2014) to obtain absorbance. For the absorbance values obtained to be expressed in nanomoles/cm^2 of leaf area, the glucose standard curve was performed at 80 mg/L (Resende et al. 2002).

Reaction of plants to infestation of leafminer (*Tuta absoluta* Meyrick, 1917) and (*Liriomyza huidobrensis* Blanchard)

The same plants employed for acylsugar quantification were utilized for assessing herbivory by *T. absoluta* and *L. huidobrensis*. Throughout the infestation period until the final evaluation day, maximum and minimum temperatures, along with relative air humidity, were monitored (Fig. 2).



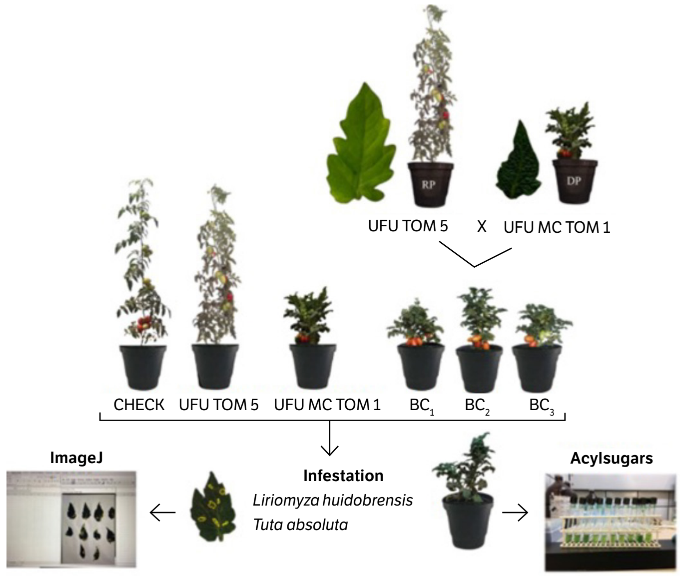
Max T: maximum temperature; Min T: mininum temperature; RH: relative humidity.

Figure 2. Climate conditions during the execution of the experiment.

The artificial infestation was established 14 days after transplantation. Adults of *T. absoluta* and *L. huidobrensis* sourced from colonies were released within the greenhouse. Release was conducted with pots of Santa Clara cv. (host plants) placed side by side every 50 cm, saturating the area with insects.

Eight days after infestation, seven assessments were conducted at two-day intervals, during which ratings were assigned for injuries on the plants and leaflets (Oliveira et al. 2012). At the end of the seven assessments, the ratings were summed, obtaining the total value for each plot. Plants were individually assessed for injury levels on the plant and leaflets according to rating scales ranging from 1 to 5, where 1 indicates the lowest level of injury and 5 the highest, as proposed by Maluf et al. (1997) and Labory et al. (1999).

Following the conclusion of the rating assessments, the calculation of the herbivory area caused by T. absoluta and L. huidobrensis was performed using the ImageJ software for image processing and analysis (Schneider et al. 2012). To obtain the mean value for each plot in the experiment, 10 leaflets from the upper third of the plant were scanned. A comprehensive overview of all experimental stages is illustrated in the flowchart (Fig. 3).



RP: recurrent parental; DP: donor parental.
Figure 3. Flowchart depicting the experiment's stages.

Chromatographic analysis and metabolomic profile

Leaflet samples (n = 6) were collected from the middle portion of the plant and crushed with liquid nitrogen using a mortar and pestle until a fine powder was obtained. Metabolite analysis via gas chromatography-mass spectrometry (GC-MS) was conducted using 100 mg of freeze-dried material. The extraction, derivatization and GC-MS analysis were carried out as described by Lisec et al. (2006).

Statistical analysis

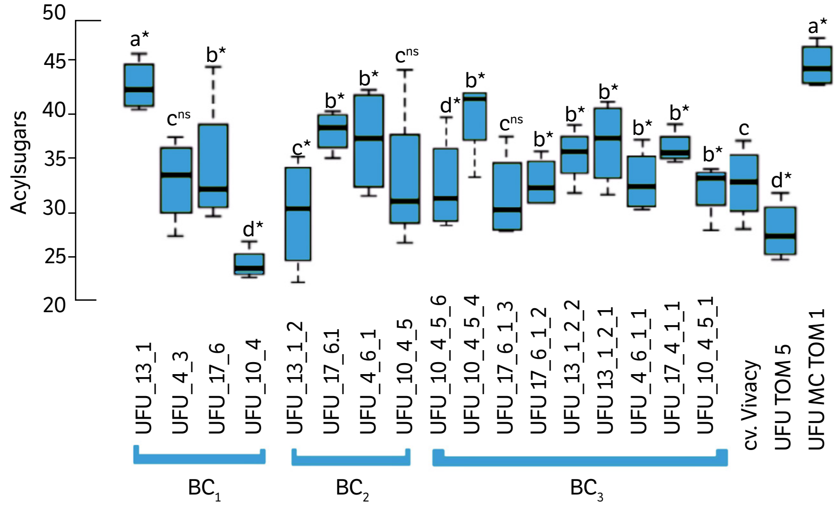
Statistical assumptions were assessed through tests for normality (Lilliefors' test), homogeneity (Oneill-Matheus' test), and additivity (Tukey's test). Except for the acylsugars variable, all others were transformed using $\sqrt{x} + 1$ to meet the assumptions and tabulate the actual values. Data were subjected to analysis of variance using the F test ($p < 0.05$). Means were compared using the Scott-Knott's test ($p < 0.05$) and Dunnett's test ($p < 0.05$), with the commercial cultivar Vivacy serving as the control for comparison purposes.

Genetic diversity was represented by a dendrogram, and a heat map was obtained from maximum and minimum distances. Statistical analyses were conducted using the GENES software (Cruz 2013) and R (R Core Team 2021).

RESULTS AND DISCUSSION

Throughout the experiment, minimum temperatures of 9°C and maximum temperatures of 38°C were observed. The relative humidity ranged from 36 to 98% (Fig. 2). Climatic factors are recognized as crucial regulators of insect populations. In this study, as demonstrated, it can be affirmed that the conditions were favorable for the development of *T. absoluta* (Mohamed et al. 2022) and *L. huidobrensis* (Mujica et al. 2017), with the average temperature and relative humidity during the assessments recorded at 30.61°C and 63.75%, respectively.

For decades, quantifying the content of acylsugars in tomato leaflets has been the prime strategy for the indirect selection of plants resistant to pest insects (Resende et al. 2002, Maciel and Silva 2014). Following the quantification of acylsugar levels in tomato leaflets, a significant difference among treatments was observed ($p < 0.05$). It became evident that the UFU MC TOM1 lineage is rich in acylsugars as it significantly differed from the commercial control (cv. Vivacy) and recurrent parent (UFU TOM5) ($p < 0.05$) (Fig. 4).



*Significant by the Dunnett's test, p < 0.05 compared to cv. Vivacy; nsnon-significant by the Dunnett's test, $p < 0.05$ compared to cv. Vivacy.

Figure 4. Levels of the allelochemical acylsugars (nmol·cm^{-2} of leaf area) in tomato leaflets. Means followed by the same letters do not differ significantly according to the Scott-Knott test, $p < 0.05$.

The potential utilization of the UFU MC TOM 1 dwarf lineage for gene introgression, specifically targeting resistance against pest insects, notably those mediated by acylsugars, has been previously elucidated in various studies (Gomes et al. 2021, Oliveira et al. 2022, Gomes et al. 2023). The findings obtained in this current investigation further affirm the efficacy of the UFU MC TOM1 lineage for such purposes. Additionally, it is noteworthy to emphasize that UFU MC TOM1 exhibited comparable traits to the wild-type *S. pennellii* accession concerning acylsugar levels in leaflets, as detailed in previous research by Finzi et al. (2022).

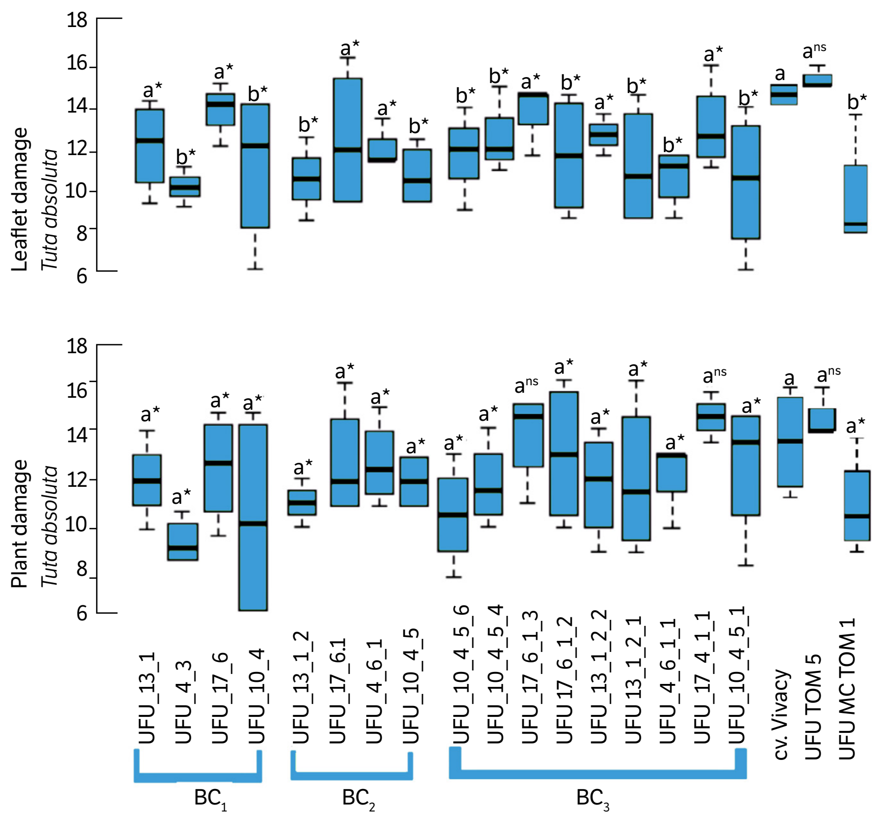
Regarding the performance of the BC1, BC2, and BC3 populations resulting from the cross between UFU-TOM5♀ and UFU MC TOM 1♂, promising populations were identified. In the initial backcross, among the four evaluated populations, two exhibited noteworthy acylsugar levels (UFU_13_1 and UFU_17_6 44.07 and 34.31 nmol·cm^{-2} of leaf area, respectively), representing increases of 60.07 and 24.62% compared to the recurrent parent (UFU TOM5) and 32.09 and 6.89% compared to the commercial cultivar (cv. Vivacy), respectively (Fig. 4). The population that distinguished itself in BC1 was UFU_13_1, boasting a notable acylsugar content (44.07 nmol·cm^{-2} of leaf area), similar to the resilient donor/parent (UFU MC TOM1) (Fig. 4).

Among the four populations of BC2, UFU_17_6_1 (1.16 time higher than the commercial control) and UFU_4_6_1 (1.13 time higher compared to the commercial control) excelled. Among the nine populations evaluated in BC3, seven surpassed the commercial control and the recurrent parent. Notably, the UFU_17_4_1_1 population exhibited a heightened acylsugar content (39.46 nmol·cm^{-2} of leaf area), marking a 22.94% increase over the commercial control ($p < 0.05$) (Fig. 4).

The resistance conferred by acylsugars in tomato plants presents a complex scenario. Mutschler et al. (2023) propose that acylsugar-mediated resistance represents a sophisticated, polygenic quantitative trait. It has been reported that intermediate levels of acylsugars within tomato leaflets are sufficient to confer a broad-spectrum resistance against pest insects (Maluf et al. 2010). These observations were based on investigations conducted by Gonçalves et al. (2007), which advocate that acylsugar content might be governed by a single gene, exhibiting partial dominance for lower concentrations and incomplete dominance for higher concentrations.

Similarly, Maluf et al. (2010) advocate for the utilization of the TOM-687 lineage (abundant in acylsugars, derived from wild parentage) to yield hybrids with intermediate levels. A novel strategy unearthed in the current study entails the exploitation of dwarf plants from the UFU_10_4_5_4, UFU_17_6_1_2, UFU_13_1_2_2, UFU_13_1_2_1, UFU_4_6_1_1, UFU_17_4_1_1, and UFU_10_4_5_1 populations. It is suggested that self-pollination of plants from these populations would yield homozygous lines, facilitating the development of insect-resistant tomato hybrids utilizing a dwarf parental line. Beyond pest resistance, these novel lines may confer additional agronomic benefits, as highlighted by Finzi et al. (2017).

The selection of plants based on their resistance to pest insects through acylsugar levels represents an indirect method (Resende et al. 2002, Maciel and Silva 2014). Primarily, the heritability of pest insect resistance tends to be low. This phenomenon can be attributed to the challenges associated with maintaining a consistent environment, which impacts both the plant and the pest arthropod (Resende et al. 2002, Gonçalves et al. 2007), thus reducing the precision of selection (Santos et al. 2018). While direct selection may yield superior gains, simultaneous selection (both direct and indirect) could potentially enhance maximization and accuracy (Santos et al. 2018). Consequently, in addition to indirect selection based on foliar acylsugar content, the dwarf tomato populations were subjected to direct evaluation for leaflet and plant injuries. Significant differences among treatments were observed ($p < 0.05$). It became apparent that the UFU MC TOM1 lineage exhibits resistance to pest insects *T. absoluta*. There was a notable difference compared to the commercial control (cv. Vivacy) and the recurrent parent (UFU TOM5) when evaluating leaflet damage (Fig. 5).



*Significant by the Dunnett's test, $p < 0.05$ compared to cv. Vivacy; ^{ns}non-significant by the Dunnett's test, $p < 0.05$ compared to cv. Vivacy.

Figure 5. Injuries progress curve over time for leaflet and plant injuries (sum of the scores from the seven evaluations). Means followed by the same letters do not differ significantly according to the Scott-Knott test, $p < 0.05$.

Regarding herbivory caused by *T. absoluta*, it was observed that all populations (BC1, BC2, and BC3) exhibited reduced consumption of leaf mesophyll in the leaflets, except for the UFU_17_6_1_3 population. Notably, the UFU_17_6_1_3 population, which displayed the highest leaf mesophyll consumption (plant injury = 13.75 and leaflet injury = 15.25) (Fig. 5), demonstrated a low level of acylsugars in the leaflets (Fig. 4). While genotypes with elevated acylsugar levels have shown greater resistance to *T. absoluta* compared to others, their resistance levels may be influenced by genetic background. Moreover, it is suggested that, as backcross generations progress, other characteristics related to plant morphology or secondary compounds may be compromised (Oliveira et al. 2022, Gomes et al. 2023).

As reported, it is known that obtaining insect-resistant tomato plants primarily involves the use of wild accessions of the *Solanum* genus, particularly *S. pennellii* (Maciel et al. 2018, Peixoto et al. 2020, Mutschler et al. 2023). The results obtained in this study demonstrate the potential of the UFUMC TOM 1 lineage in obtaining introgression lines rich in acylsugars. As research in this area is still in its nascent stages regarding the potential use of dwarf tomato plants for insect pest resistance, it is suggested that other compounds may exist in the leaflets of the UFU MC TOM 1 lineage. In this context, chromatographic analysis and metabolomic profiling have contributed to advancing knowledge. It was possible to observe the presence of other compounds of interest for further investigations (Fig. 6).

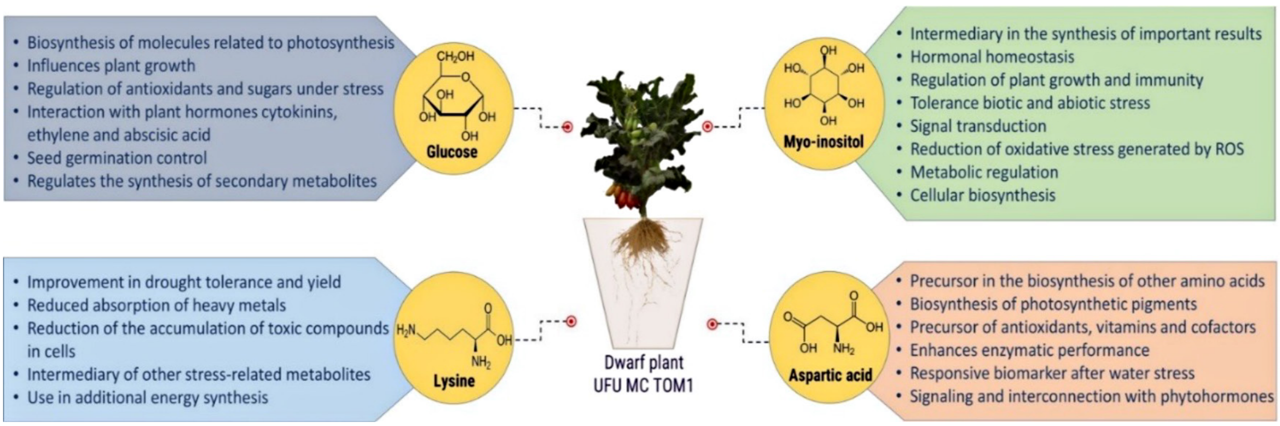


Figure 6. Compounds identified by total leaf gas chromatography-mass spectrometry of dwarf tomato plants UFU MC TOM 1 and their actions on plant metabolism.

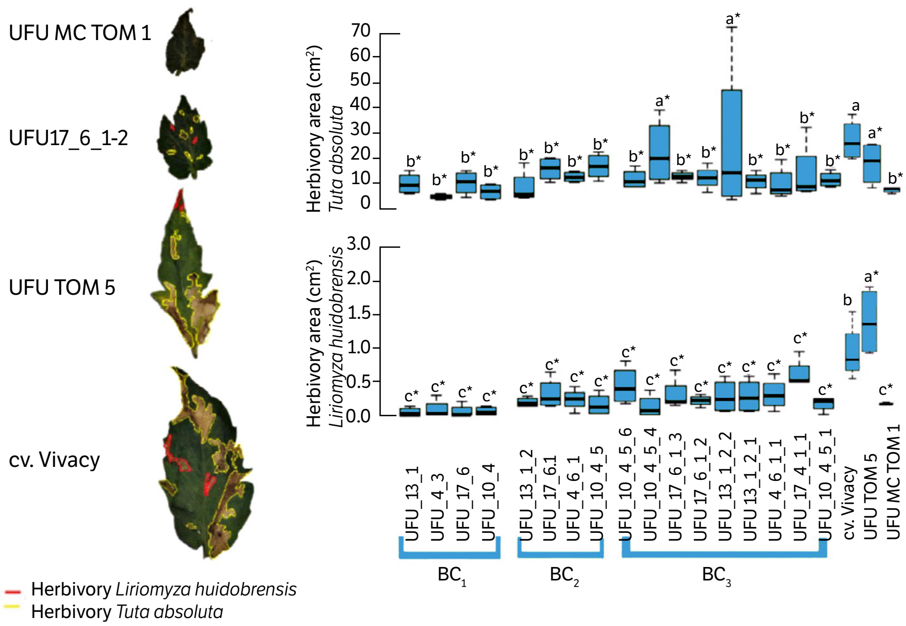
There are reports suggesting that compounds such as glucose, lysine, myo-inositol, and aspartic acid may be also associated with promoting resistance to various types of biotic and abiotic stress in plants (Riemer et al. 2022, Xu and Fu 2022). These findings could potentially unveil a complex array of compounds capable of providing broad-spectrum resistance. Additionally, each compound could be investigated individually or in combination to pursue tomatoes with heightened resistance levels and decreased dependency on insecticide applications.

With the aim of refining the technique proposed by Maluf et al. (1997) and Labory et al. (1999), the calculation of herbivory area through image analysis was conducted. Coherence was observed between the results obtained from image analysis and those obtained for plant injuries, foliar injuries, and acylsugar levels. Consistent with the other assessments, it became evident that the UFU MC TOM1 lineage exhibits resistance to the pest insects *T. absoluta* and *L. huidobrensis*. A significant difference ($p < 0.05$) was observed compared to the commercial control (cv. Vivacy) and recurrent parent (UFU TOM5) (Fig. 7).

Similarly, to what was observed for leaf and plant injuries (Fig. 5), all backcross populations (BC1, BC2, and BC3) exhibited a smaller herbivory area calculated from images. The utilization of imaging for direct selection in tomato genotypes is suggested as a valuable tactic for evaluation. The fact that the dwarf lineage UFU MC TOM1, rich in acylsugars (Fig. 4), manifested smaller lesions on both plants and leaves (Fig. 5), and a reduced consumed area (Fig. 7) in comparison to the commercial cultivar and the recurrent parent (both featuring low levels of acylsugars) may be linked to non-preference, thereby underscoring antixenosis-type resistance. Various studies have already unraveled similar resistance effects, albeit with a background originating from the wild accession *S. pennellii* (Gonçalves Neto et al. 2010).

Indeed, the dwarf lineage UFU MC TOM1 exhibits considerable potential to stimulate forthcoming breeding programs aimed at combating insect pests. It is noteworthy that, besides conferring insect pest resistance, the dwarf lineage (UFU MC TOM1) may impart diverse advantages to hybrids concerning plant morphology, yield, and fruit quality (Finzi et al. 2017, Rajendran et al. 2022). The acquisition of hybrids with normal size remains feasible even when employing a dwarf parent. This phenomenon arises from the monogenic and recessive nature of the dwarfism trait (Maciel et al. 2015). The recommendation to utilize the dwarf lineage UFU MC TOM1 is extended for the procurement of alternative genetic backgrounds across diverse tomato lineages worldwide, with the aim of resistance against *T. absoluta* and *L. huidobrensis*.

After the evaluations using both indirect (Fig. 4) and direct (Figs. 5 and 7) selection methods, responses for heat map and hierarchical clustering (double dendrogram) for traits related to insect pest resistance in tomato were obtained (Fig. 8).



*Significant by the Dunnett's test, $p < 0.05$ compared to cv. Vivacy; ns non-significant by the Dunnett's test, $p < 0.05$ compared to cv. Vivacy.

Figure 7. Herbivory area consumed by *Tuta absoluta* and *Liriomyza huidobrensis* obtained with the assistance of ImageJ software. Means followed by the same letters do not differ significantly according to the Scott-Knott test, $p < 0.05$.

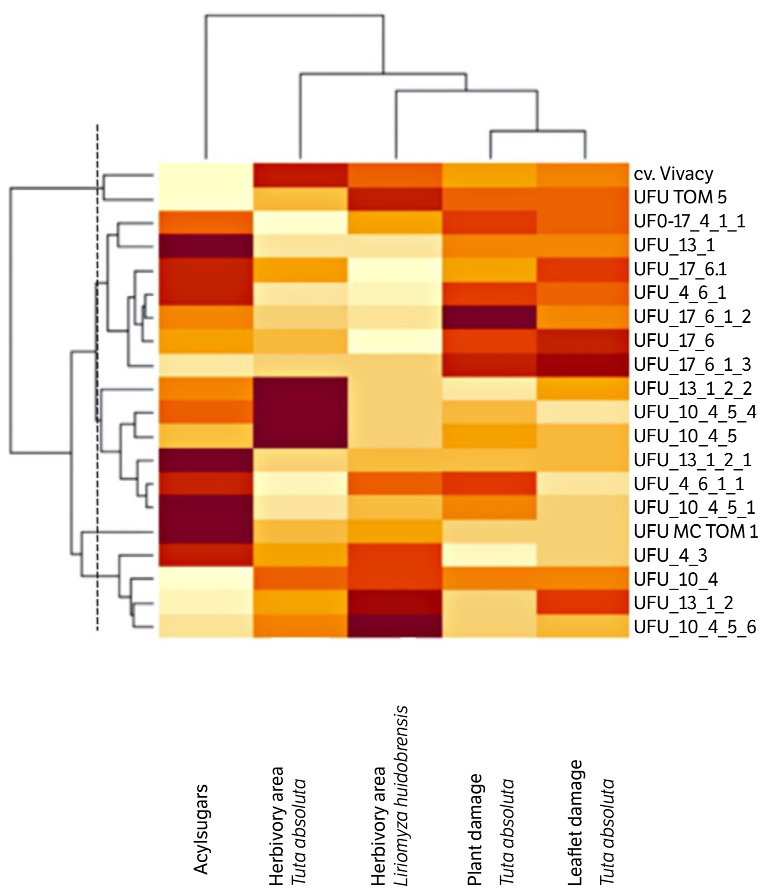


Figure 8. Heat map responses and hierarchical clustering (double dendrogram) to traits related to resistance to *Tuta absoluta* and *Liriomyza huidobrensis* in tomato plants.

The heat map graph delineates the relative abundance of each tomato genotype (rows) within each assessed trait (columns). Meanwhile, the dendrogram illustrates the hierarchical clustering of tomato genotypes based on the Euclidean distance measure and the unweighted pair group method using arithmetic averages (UPGMA) clustering method.

The determination of the number of groups in the dendrogram was predicated on the sudden changes in branches, as described by Cruz et al. (2014). This analysis revealed the formation of four distinct groups. Group I comprised populations UFU_4_3, UFU_10_4, UFU-13_1_2, and UFU_10_4_5_6. Group II consisted solely of the donor parent (UFU MC TOM1). Group III encompassed a broader range of populations, including UFU_10_4_5_1, UFU_4_6_1_1, UFU_13_1_2_1, UFU_10_4_5, UFU_10_4_5_4, UFU_13_1_2_2, UFU_17_6_1_3, UFU_17_6, UFU_17_6_1_2, UFU_4_6_1, UFU_17_6.1, UFU_13_1, and UFU_17_4_1_1. Lastly, Group IV was composed of the commercial cultivar Vivacy and the recurrent parent UFU TOM5. The dendrogram exhibited a cophenetic correlation coefficient of 88% with a distortion of 4.73%. The outcome of the multivariate analysis reinforces the potential of the dwarf tomato lineage (UFU MC TOM1) to foster genetic improvement programs aimed at pest resistance. Notably, this lineage stood isolated in Group II. Conversely, the commercial control (cv. Vivacy) and the recurrent parent (UFU TOM5) clustered together in the same group. These findings align with those obtained in the univariate analysis (Figs 4 and 5).

The inner section of the dendrogram was depicted as a heat map, with more intense colors (tending towards red) indicating stronger responses to the analyzed variable. Notably, the resistant lineage UFU MC TOM1 appeared in lighter shades for plant and leaf injuries, as well as leaf area, while displaying a darker hue (deep red) for acylsugar content in the leaves. Conversely, both the cv. Vivacy and the recurrent parent UFU TOM5 exhibited lighter tones for acylsugar levels in the leaves, juxtaposed with darker shades for plant and leaf injuries, along with leaf area. Among the analyzed variables, the highest relative contributions of traits were presented by acylsugar content and the area of herbivory caused by *T. absoluta*, accounting for 34.69 and 56.15%, respectively. This observation underscores resistant populations with high levels of acylsugars and highlights the importance of employing image-based techniques for herbivory assessment.

Regarding the resistance level among the backcross populations (BC1, BC2, and BC3) evaluated, there was no prevalence of populations originating from BC3. This phenomenon could be attributed to the complex manner in which acylsugar-mediated resistance operates. Several populations demonstrated potential for lineage acquisition. Population UFU 13_1 did not differ significantly ($p < 0.05$) from the resistant benchmark UFU MC TOM1, rich in acylsugars. This population holds promise for lineage acquisition and exploration of combinatorial capabilities aimed at hybrid attainment. There are reports indicating that intermediate levels of acylsugars are sufficient to confer broad-spectrum resistance to insect pests. Some populations exhibited intermediate levels (populations UFU_10_4_5_4, UFU_17_6_1_2, UFU_13_1_2_2, UFU_13_1_2_1, UFU_4_6_1_1, UFU_17_4_1_1, UFU_10_4_5_1, UFU_17_6.1, UFU_4_6_1, UFU_17_6), with the same potential to confer resistance to insect pests.

These findings confirm the potential of dwarf tomato populations for the development of introgression lines targeting resistance to *T. absoluta* and *L. huidobrensis*, potentially leading to a range of compounds capable of providing broad-spectrum resistance to insect pests.

CONCLUSION

The population of dwarf tomato plants UFU 13_1 emerges as a promising candidate for driving genetic enhancement programs targeting insect pest resistance.

The progression through successive generations of backcrossing resulted in different levels of resistance, likely influenced by the mode of inheritance and the genetic mechanisms governing acylsugar production.

The UFU MC TOM1 lines, characterized by elevated levels of acylsugars, have demonstrated consistent resistance against the tomato leafminer (*T. absoluta* Meyrick 1917) and the serpentine leafminer (*L. huidobrensis* Blanchard) across all evaluations conducted.

In addition to acylsugars, the leaflets of UFU MC TOM1 lineage exhibit heightened content of metabolites such as glucose, lysine, myo-inositol, and aspartic acid, thereby presenting promising avenues for further investigations into resistance against diverse biotic and abiotic stresses.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Maciel, G. M.; **Methodology:** Pereira, L. M., Ribeiro, A. L. A., Oliveira, C. S. and Farias, A. K. S. R.; **Software:** Pereira, L. M., Ribeiro, A. L. A., Oliveira, C. S. and Farias, A. K. S. R.; **Validation:** Pereira, L. M., Pinto, F. G. and Ikehara, B. R. M.; **Formal analysis:** Maciel, G. M., Siquieroli, A. C. S. and Pinto, F. G.; **Investigation:** Pereira, L. M. and Maciel, G. M.; **Resources:** Maciel, G. M. and Siquieroli, A. C. S.; **Data curation:** Maciel, G. M. and Siquieroli, A. C. S.; **Writing—original draft:** Pereira, L. M.; **Writing—review and editing:** Maciel, G. M., Siquieroli, A. C. S., Pinto, F. G. and Silva, N. C. Q.; **Visualization:** Pereira, L. M., Maciel, G. M., Siquieroli, A. C. S., Ikehara, B. R. M. and Silva, N. C. Q.; **Supervision:** Maciel, G. M.; **Project administration:** Maciel, G. M.; **Funding acquisition:** Maciel, G. M. and Siquieroli, A. C. S.

DATA AVAILABILITY STATEMENT

All dataset were generated or analyzed in the current study.

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