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Development of BC_3F_2 Tomato Genotypes with Arthropod Resistance Introgressed from *Solanum habrochaites* var. *hirsutum* (PI127826)

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Abstract: Arthropod pests are among the biggest problems faced in tomato production worldwide. To overcome the losses caused by these pests, one of the most sustainable and economical strategies is the use of resistance introgressed from wild species. We aimed to develop BC_3F_2 tomato genotypes with high levels of zingiberene (ZGB) and resistance to whitefly (*Bemisia tabaci* biotype B), South American tomato pinworm (*Tuta absoluta*), and the two-spotted spider mite (*Tatranychus urticae*), from the wild accession of *Solanum habrochaites* var. *hirsutum* (accession PI127826). The quantification of ZGB in 520 BC_3F_2 genotypes and in the parentals yielded the selection of five genotypes with high ZGB content and three with low ZGB content, which were then infested with *B. tabaci*, *T. absolute*, and *T. urticae*. In these eight genotypes and in the parents, the types and amounts of trichomes on the leaves were determined. Additionally, molecular markers were used to identify the genotypes with a higher recurrent genome recovery. The results confirmed the transfer of resistance from *S. habrochaites* to the BC_3F_2 genotypes and showed that this resistance seems to be directly related to high concentrations of ZGB and the presence of type IV trichomes.

Keywords: *Solanum lycopersicum*; *Bemisia tabaci*; *Tuta absoluta*; *Tatranychus urticae*; marker-assisted selection; zingiberene

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1. Introduction

The tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetables worldwide, surpassed only by the potato [1–4]. Its wide acceptance and commercialization are due to its rich nutritional value [5] and high versatility—it can be consumed raw, or it can be processed in many ways [6,7]. World tomato production exceeded 180 million tons in the 2019/2020 season [8]. The growing market demand for tomatoes requires a constant effort from breeders. In recent decades, there has been growing consumer interest in tomatoes produced in a sustainable way and with less harmful health effects. For example, the use of insecticides to control diseases and pests needs to be reduced.

Diseases and pests are constant problems in tomato cultivation worldwide [9]. The main pests include the whitefly [*Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) biotype B] [10,11], the South American tomato pinworm [*Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae)] [12,13], and the two-spotted spider mite [*Tatranychus urticae* Koch (Acari: Tetranychidae)] [14,15]. These pests attack tomato crops causing severe damage, which increases production costs and decreases financial profitability for the producer [16,17]. For example, when a tomato plantation is attacked by *T. absoluta* and control is not correctly carried out, economic losses can reach 100%. The main management method used to mitigate the losses caused by arthropod pests in tomatoes is chemical control [11]. However,

the intensive use of chemical products can damage the environment and the health of farmers and consumers [18]. In addition, there may be a reduction in the population of insects that are the natural enemies of pests [19], as well as a reduction in the generation of pest populations resistant to the chemical molecules of insecticides [20]. A healthier and more environmentally friendly strategy is that of genetic resistance [21]. However, domestication has reduced the resistance of cultivated tomato varieties. To increase tomato resistance to pests, commercial cultivars can be crossed with wild accessions that produce secondary metabolites that negatively affect the pests [22–25].

The wild species *Solanum pennellii* Correl and *Solanum habrochaites* S. Knapp & D.M. Spooner var. *hirsutum* confer resistance to a wide range of pests [6,26] due to the presence of chemical compounds called acylsugars and/or zingiberene (referred to as ZGB throughout the text), present in leaflets, which are exuded by glandular trichomes, mainly of type IV and VI [27–32]. These allelochemicals promote resistance because they have deleterious effects on the feeding process and/or life cycle of pests [21]. Promising results are being obtained by crossing commercial cultivars with wild species and by the selection of genotypes with high levels of ZGB or acylsugars and, consequently, high resistance to arthropod pests [33]. Examples of the successful introgression of arthropod pest resistance genes into domesticated tomatoes have generally occurred through the backcrossing of commercial cultivars with wild tomato species *S. pennellii* and *Solanum. peruvianum* (L.) Mill [34–40]. However, despite *S. habrochaites* var. *hirsutum* also presenting high resistance to pests, it has rarely been used in gene introgression programs.

With the advancement of biotechnology, the use of molecular markers has accelerated backcrossing programs for both background and foreground selection [41–43]. Background selection using molecular markers is always advantageous and economically viable as it can accelerate the backcrossing program by allowing the selection of plants with a higher proportion of the recurrent parental genome. Conversely, foreground selection is advantageous when the trait to be incorporated is difficult to assess by phenotype. However, for tomato resistance to arthropod pests, there has been limited research on marker-assisted backcrossing.

In this study, we aimed to explore resistance to arthropod pests in wild tomato. *S. habrochaites* var. *hirsutum*. We describe the development of BC_3F_2 tomato genotypes with resistance to *T. urticae*, *B. tabaci*, and *T. absoluta* derived from *S. habrochaites* var. *hirsutum*. Furthermore, we evaluate which type of trichome was correlated with arthropod resistance in wild species and BC_3F_2 genotypes.

2. Materials and Methods

2.1. Plant Materials and Breeding Strategy

To obtain the 520 genotypes in the BC_3F_2 generation used in this study, three plants of the BC_2F_2 generation, obtained by backcrossing between the commercial cultivar Redenção (*S. lycopersicum*) (referred to as SLR throughout the text) and the wild species *S. habrochaites* var. *hirsutum* (line PI127826) (referred to as SHH throughout the text), were backcrossed with SLR and, the BC_3F_1 genotypes obtained were self-fertilized. All previous steps of the backcrossing breeding program are described in the works of [21,30,44] and summarized in Figure 1. SLR (used as a female parent and recurrent in the backcrossing program) has characteristics suitable for industrial processing, is resistant to geminiviruses and tospoviruses, has low levels of ZGB, and is susceptible to pests. The SHH genotype, used as a male pollen-donor parent, has a high ZGB content and is a known source of pest resistance.

The 520 BC_3F_2 and primary parental genotypes (SLR and SHH) were grown in pots containing sieved soil corrected for acidity and fertilized as recommended for the tomato crop. Thirty days after planting, each plant was cloned using axillary shoots. When the clones reached the four-leaf stage, they were transplanted into 5 L polyethylene pots filled with the soil mentioned above. The plants were trained using a vertical guide and irrigated by a drip system. The plants were kept in a greenhouse with a humidity of 55 $\pm$ 5% and

a temperature of 25 ± 5 °C. The cloning strategy was used to obtain genetically identical plants needed for use in the different experiments carried out in this study (ZGB evaluation, test with three different pests, trichome counts).

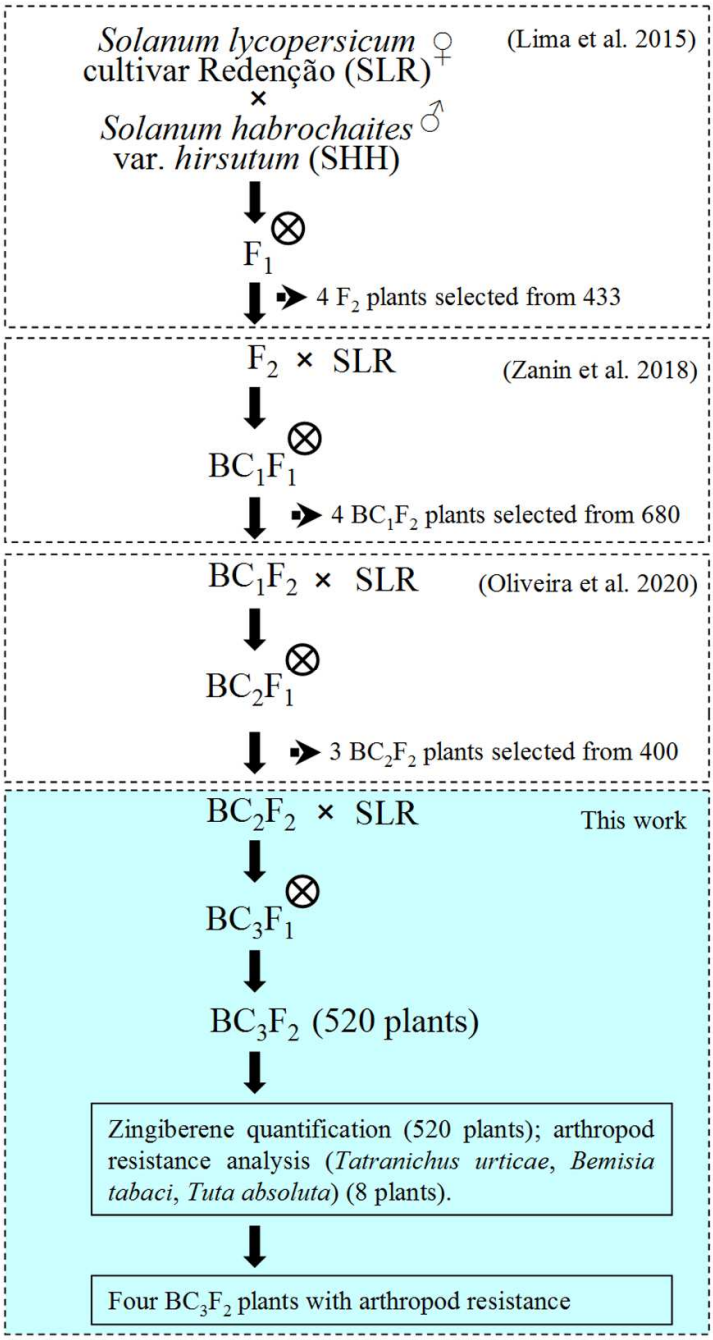

Figure 1. Crossing and backcrossing stages used in the program to select pest tolerant genotypes with high zingiberene content [21,30,44].

2.2. Zingiberene Quantification

The quantification of ZGB in the 520 BC_3F_2 genotypes, and in the parental ZLR (low ZGB content) and SHH (high ZGB content) (used as controls), was performed using a spectrophotometer according to the methodology proposed by the authors of [45]. The sampling of material for ZGB analysis was performed from plants in the pre-flowering period (about 40 d after transplanting) maintained as described above. Six discs of fully expanded young leaflets were collected from the upper third of each plant with the aid of

a paper punching, obtaining a leaf area of 6 cm^2 in total. Discs were placed in test tubes. Subsequently, 2 mL of hexane was added to each tube and stirred for 40 s using a vortex. Immediately afterwards, the absorbance reading was performed at a wavelength of 270 nm in a Cary 60 UV Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The absorbance values obtained were proportional to the amount of ZGB present in the samples [45].

Evaluations were carried out for each genotype in triplicate, and the average of the three evaluations was used to classify the genotypes. After identifying the five plants with the highest concentration of ZGB and the three with the lowest concentration, the plants were cloned again as described above, and the clones obtained were used to evaluate the amount and type of trichomes and their resistance to arthropods.

2.3. Trichome Analysis

To count and identify the types of trichomes present in the eight BC_3F_2 genotypes with high and low ZGB content (selected in the previous step) and the parental genotypes, five young fully expanded leaflets were collected from the middle third of the plants. Sampling was performed 45 d after transplanting. Approximately 12 mm^2 of collected leaflets were cut and prepared on microscope slides. Four blades of each leaf face (abaxial and adaxial) were prepared from each genotype. Ground graphite was added to the material to be analyzed. Slide images were captured (1 mm^2) with four images of each side of the leaf, that is, four repetitions. The images were captured by scanning electron microscope (SEM; Tescan® model Vega3 microscope in XM camera size and HV, Brun, Czech Republic) with an accelerating voltage of 5 kv. After obtaining the images, the identification and counting of the types of trichomes present was carried out using the classification proposed by Luckwill [46].

2.4. Bamesia tabaci Resistance Test

The evaluation of resistance to whitefly (*B. tabaci*) was carried out in the eight BC_3F_2 genotypes selected in the ZGB quantification step and in the parental genotypes. The insects used were obtained from a population maintained on cabbage plants (*Brassica oleraceae* var. *acephala*). A sufficient number of flies for the experiment were obtained by multiplication on the tomato cv. Santa Clara (*S. lycopersicum*), which is susceptible to pests. The infestation of the Santa Clara plant was conducted by placing whitefly-infested cabbage leaves on tomato plants kept in wooden cages (1.0 m wide × 1.0 m deep × 1.20 m high), built with an antifidic screen coating.

The experiment to evaluate resistance to *B. tabaci* in BC_3F_2 tomato genotypes was carried out in a greenhouse with a temperature of 25 ± 3 °C, humidity of 55 ± 5%, and daily irrigation. The experimental design consisted of randomized blocks with four replications. Each pot with one plant was considered an experimental unit. To infest the experimental plants, the infested Santa Clara plants were removed from the cages, and the whiteflies were distributed inside the greenhouse between the lines of the blocks of the BC_3F_2 genotypes and the parental genotypes.

Twenty-one days after the infestation, the number of eggs and nymphs on the leaflets were determined. Three young fully expanded leaflets were collected, one from the upper third, one from the middle third, and one from the lower third of each plant. With the aid of a binocular stereoscopic microscope, eggs and nymphs on the abaxial surface of the leaflet in a leaf area corresponding to 2 cm^2 were counted.

2.5. Tuta absoluta Risistance Test

Resistance to *T. absoluta* was determined using the same genotypes described for *B. tabaci*, with the same experimental design and maintained under the same environmental conditions. The population of *T. absoluta* used in the experiment was also established in the Santa Clara cultivar in a wooden cage with an anti-aphid screen. The infestation of BC_3F_2

and parental genotypes was carried out with the removal of infested Santa Clara cultivar plants from cages between the lines of the blocks of the BC_3F_2 and parental genotypes.

The severity of damage caused by *T. absoluta* was analyzed 21 d after the infestation according to the scale of scores attributed to leaflet lesions proposed by Labory et al. [47]. The scale is as follows: 0, no injury; 1, small and innumerable lesions; 2, small and medium lesions, few in number, and frequently located on the edges of the leaflets; 3, medium and large lesions, numerous and coalescent, and deformed leaflet edges; 4, large coalescent lesions, completely deformed leaflets; and 5, lesions covering the entire leaflet.

2.6. Tatranichus Urticae Resistance Test

The population of *T. urticae* used in the experiment was maintained on bean plants (*Phaseolus vulgaris* L.) in a controlled environment (temperature, 20 ± 3 °C; humidity, 55 ± 5%). The experiment was carried out with insects of the same age. These age-uniform insects were obtained by the microscopic selection of adult females for oviposition. After selection, these females were transferred to bean leaflets kept in Petri dishes and placed in a temperature- and humidity-controlled growth chamber, as previously described. After 72 h, the females were removed, and the leaflets were kept in a controlled environment for the eggs to hatch. The population obtained was used in the bioassays as described below.

A test known as a 'mite race' was carried out following the methodology proposed by Weston and Snyder [48]. Young, fully expanded, uniformly sized leaflets were removed from the upper third of each genotype to be tested in the pre-flowering phase of the plants (45 d after transplanting). Subsequently, these leaflets were placed on a sheet of A4 bond paper, and this was fixed on a Styrofoam plate. In the center of each leaflet, with the adaxial side facing upwards, a 9 mm diameter thumbtack was fixed. Then, ten mites were released in the center of the thumbtack with the aid of a fine brush. After 60 min, the distances traveled by the mites on the leaflets from the center of the thumbtack were measured in millimeters. According to Weston and Snyder [48], the shorter the distance traveled by the mite on the leaflet surface, the higher the resistance level. Therefore, for the mites that remained on the tack, the distance covered was considered to be zero. The maximum potential distance was from the center of the thumbtack to the leaf tip.

2.7. Statistical Analyses

The identification of statistical differences between the amount of trichomes and the level of resistance to whitefly *B. tabaci*, *T. absoluta*, and *T. urticae* found in each genotype and in the parental genotypes was performed by an analysis of variance, considering the assumptions of normality of errors (Shapiro–Wilk test), homogeneity of variances (Levene's test), and Tukey's multiple-comparison test. All analyses were performed using the agricolae package available in R software (https://www.r-project.org/, accessed on 11 November 2022).

2.8. Evaluation of Recurrent Genome Recovery

The identification of BC_3F_2 genotypes with a higher recurrent genome recovery was performed using inter-simple sequence-repeat (ISSR) molecular markers developed by the University of British Columbia, Canada. The DNA of the five genotypes with high ZGB content and the recurrent parent was extracted following the protocol proposed in [49]. After extraction, the DNA was amplified by PCR using 10 ISSR primers (Table 1). The amplification reaction for each genotype was composed of 20 ng genomic DNA, 0.5 µM primer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 1 U Taq DNA polymerase, 1.5 µL 1× PCR buffer, and water to make the final volume 12.5 µL. PCR amplification was performed in a Veriti thermocycler (Applied Biosystems, Foster City, CA, USA) with the following program: initial DNA denaturation at 94 °C for 5 min; 35 cycles at 94 °C for 45 s, primer annealing temperature for 45 s (according to Table 1), and 72 °C for 90 s to extend the fragments; and final extension at 72 °C for 5 min. All reagents used were from Invitrogen (Carlsbad, CA, USA).

Table 1. Inter-simple sequence-repeat (ISSR) primers used to identify BC_3F_2 genotypes with higher recurrent genome recovery. AT °C, annealing temperature.

Primer	Sequence * (5′–3′)	AT °C
UBC-807	(AG)8T	52
UBC-808	(AG)8C	50
UBC-809	(AG)8G	55
UBC-810	(GA)8T	52
UBC-811	(GA)8C	52
UBC-815	(CT)8G	52
UBC-827	(AC)8G	53
UBC-835	(AG)8YC	52
UBC-836	(AG)8YA	53
UBC-848	(CA)8AGG	55

* Y = (C, T).

The amplification products were resolved by electrophoresis on a 1.8% agarose gel, stained with ethidium bromide (0.5 μg mL^{-1}), subjected to a constant current of 110 V for 4 h, visualized under UV light, and photo-documented with a L-PIX-HE digital system (Loccus, Cotia, SP, Brazil. A DNA ladder (100 bp) was used as standard to determine the size of the amplified fragments in base pairs. A binary matrix, obtained by genotyping individuals according to the presence (1) or absence of bands (0), was used to calculate the genetic similarity between genotypes based on Jaccard's coefficient using NTSYS-pc 2.2 software [50].

3. Results

3.1. Zingiberene Quantification

The quantification of ZGB yielded the identification of the BC_3F_2 genotypes with higher allelochemical contents (RVTZpl#348, RVTZpl#361, RVTZpl#344, RVTZpl#448, and RVTZpl#346) and those with lower contents (RVTZpl#128, RVTZpl# 125, and RVTZpl#126). The genotypes with high ZGB contents showed values close to those observed in SHH (control for high ZGB contents). Additionally, those selected with low contents had values close to SLR, which is recognized for having low ZGB contents (Table 3).

Table 2. Correlation between zingiberene (ZGB) concentration and type and amount of trichomes with intensity of infestation by *Bemisia tabaci*, *Tuta absoluta*, and *Tetranychus urticae* in BC_3F_2 tomato genotypes obtained from a cross between *Solanum lycopersicum* cv. Redeção (used as the default susceptibility genotype) and *Solanum habrochaites* var. *hirsutum* (PI127826) (used as the default resistance genotype). SGB Abs—Zingiberene absorbance at 270 nm; GT IV—type IV glandular trichomes; GT VI—type VI glandular trichomes; NGT—non-glandular trichomes; DT—Distance traveled in millimeters; AB—abaxial face of the leaflet; AD—adaxial face of the leaflet; DAI—days after infestation.

		Trichomes						*B. tabaci*		*T. absoluta*	*T. urticae*
	ZGB Abs &	GT IV &		GT VI &		NGT &		Number of Eggs &	Number of Nymphs &	Lesions &	DT &
Genotype		AB	AD	AB	AD	AB	AD	21 DAI	21 DAI	21 DAI	60 min
S. habrochaites var. *hirsutum*	0.31 a	5.00 a	2.00 a	0.00 e	47.00 a	0.00 c	21.50 a	4.75 h	2.00 e	1.60 d	2.21 e
RVTZ pl#344 (high ZGB)	0.19 abc	1.00 c	0.50 bc	4.50 a	1.50 ab	30.00 bc	20.50 a	30.25 e	6.00 cd	2.26 bcd	7.49 de

Table 3. *Cont.*

		Trichomes						*B. tabaci*		*T. absoluta*	*T. urticae*
	ZGB Abs [&]	GT IV [&]		GT VI [&]		NGT [&]		Number of Eggs [&]	Number of Nymphs [&]	Lesions [&]	DT [&]
Genotype		**AB**	**AD**	**AB**	**AD**	**AB**	**AD**	**21 DAI**	**21 DAI**	**21 DAI**	**60 min**
RVTZ pl#346 (high ZGB)	0.17 abc	1.25 bc	0.75 abc	1.00 cde	0.75 abc	20.00 cdefg	12.00 cde	20.75 f	4.00 de	1.70 d	10.52 cd
RVTZ pl#348 (high ZGB)	0.26 ab	3.75 ab	1.50 ab	3.00 ab	2.00 a	12.25 g	12.50 cde	12.75 g	4.00 de	2.20 bcd	8.01 d
RVTZ pl#361 (high ZGB)	0.22 abc	0.75 c	0.25 bc	2.75 abc	1.25 ab	24.00 cdef	14.75 bc	16.25 fg	5.25 cd	2.23 cd	12.24 bcd
RVTZ pl#448 (high ZGB)	0.18 abc	250 abc	1.00 abc	2.25 bcd	1.25 ab	22.25 cdefg	12.50 cde	33.75 e	5.75 cd	2.18 cd	11.58 abcd
S. lycopersicum cv. Redenção	0.06 bc	1.00 c	0.50 bc	0.75 de	0.50 abc	26.25 bcde	14.75 bc	153.50 a	25.00 b	4.15 a	19.52 ab
RVTZ pl#125 (low ZGB)	0.05 c	0.50 a	0.25 bc	0.25 e	0.50 abc	14.25 fg	10.25 cde	128.75 b	47.50 a	3.90 ab	16.19 abc
RVTZ pl#126 (low ZGB)	0.04 c	0.25 c	0.00 c	0.50 de	0.25 bc	37.00 ab	17.75 ab	141.75 ab	29.00 b	3.65 ab	21.26 a
RVTZ pl#128 (low ZGB)	0.05 c	0.50 c	0.00 c	0.75 de	0.25 bc	22.25 cdefg	8.00 e	106.50 c	25.50 b	4.40 a	20.21 ab
Pearson correlation (r)											
ZGB	-	0.72 *	0.73 *	0.32 [ns]	0.33 [ns]	0.06 [ns]	0.31 [ns]	−0.91 *	−0.87 *	−0.91 *	−0.94 *
GT IV AB leaflet side	0.72 *	-	-	-	-	-	-	−0.58 *	−0.51 [ns]	−0.54 *	−0.78 *
GT IV AD leaflet side	0.73 *	-	-	-	-	-	-	−0.60 *	−0.53*	−0.59 *	−0.83 *
GT VI AB leaflet side	0.32 [ns]	-	-	-	-	-	-	−0.46 [ns]	−0.42 [ns]	−0.32 [ns]	−0.37 [ns]
GT VI AD leaflet side	0.33 [ns]	-	-	-	-	-	-	−0.50 [ns]	−0.39 [ns]	−0.34 [ns]	−0.37 [ns]
NGT AB leaflet side	0.06 [ns]	-	-	-	-	-	-	0.05 [ns]	−0.05 [ns]	−0.03 [ns]	−0.22 [ns]
NGT AD leaflet side	0.31 [ns]	-	-	-	-	-	-	−0.21 [ns]	−0.25 [ns]	−0.30 [ns]	−0.50 [ns]

[&] Means followed by the same letter in the column do not differ by Tukey's test ($p > 0.05$); * Significant at 5% level; [ns]: Not significant; -: Not evaluated.

3.2. Trichome Analysis

Among the types of trichomes evaluated, there were more type IV glandular trichomes (Figure 2a,b) in the SHH parental genotypes and the genotypes with higher concentrations of ZGB. Additionally, this type of trichome was the only one that showed a positive and significant correlation with leaflet ZGB concentration (Table 3). Conversely, type VI glandular trichomes (Figure 2c,d) were present in low amounts in the both SHH and SLR parental genotypes and in the genotypes derived from these parentals. Non-glandular trichomes were absent on the abaxial surface, but they were present in high numbers on the adaxial surface of SHH leaflets and in high numbers on both sides of the SLR leaflets (Table 3).

3.3. Bamesia tabaci Resistance Test

The genotypes with high and low ZGB content showed differences in whitefly resistance (Figure 3, Table 3). The smallest number of whitefly eggs and nymphs deposited on leaflets was observed in genotypes with a high ZGB content (Table 3). The results of these genotypes were similar to those observed for the wild parental SHH genotype, which had the lowest ovoposition rates and number of nymphs among all evaluated genotypes.

The RVTZpl#348 and RVTZpl#361 genotypes with a high ZGB content showed a low incidence of eggs (Table 3). The RVTZpl#346 and RVTZpl#348 genotypes, also with a high ZGB content, had the lowest number of nymphs on leaflets (Table 3). There was a significant negative correlation between mean ZGB level and both mean number of eggs and mean number of nymphs (Table 3). Significant negative correlations were also observed between

the mean number of type IV glandular trichomes on the abaxial face and the whitefly mean number of eggs, as well as between the mean number of type IV glandular trichomes on the adaxial surface and the mean number of eggs and nymphs (Table 3).

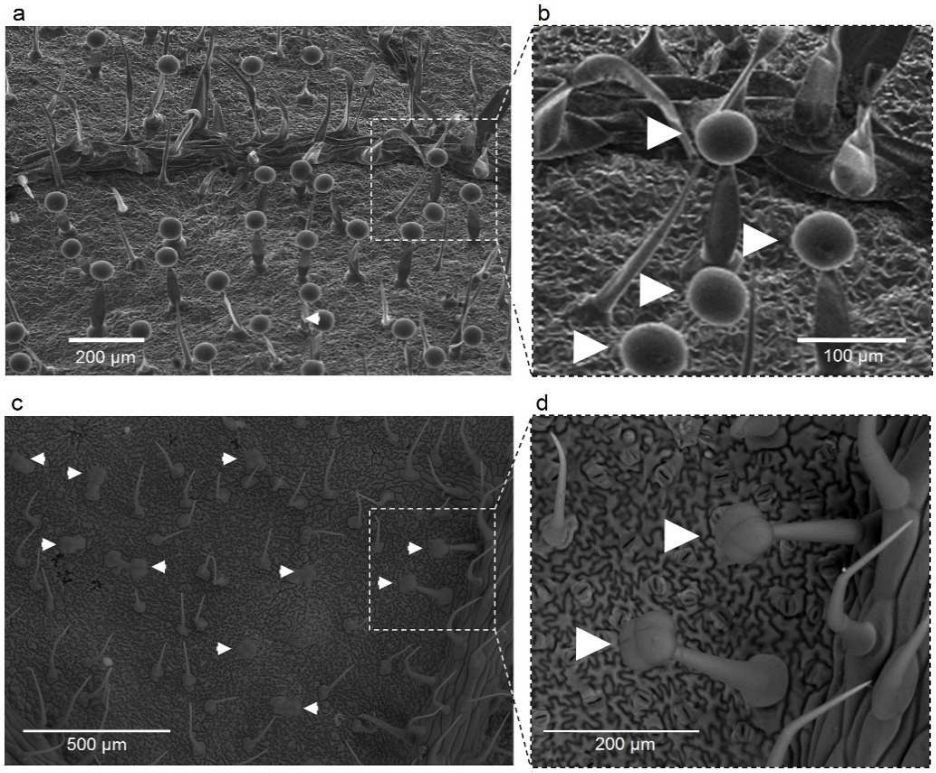

Figure 2. Type IV (**a**,**b**) and type VI (**c**,**d**) glandular trichomes present in leaflets of tomato genotypes with high zingiberene contents.

Figure 3. Level of adult whitefly infestation in genotypes selected for contrasting levels of zingiberene. (**a**) refers to the genotype RVTZpl#125 with low content of the allelochemical, and (**b**) refers the genotype RVTZpl#348 with high content of the allelochemical.

3.4. Tuta absoluta Risistance Test

There was no significant difference in leaflet damage caused by *T. absoluta* between genotypes with a high ZGB content and the SHH genotype. There was also no significant different between genotypes with a low ZGB content and the SLR genotype (Table 3). More severe leaflet lesions were observed in genotypes with low ZGB contents (Figure 4). There was a significant negative correlation between the mean ZGB content and mean area of leaflet lesions (Table 3), as well as between the mean area of leaflets lesions and the mean number of type IV glandular trichomes on the abaxial and adaxial face (Table 3).

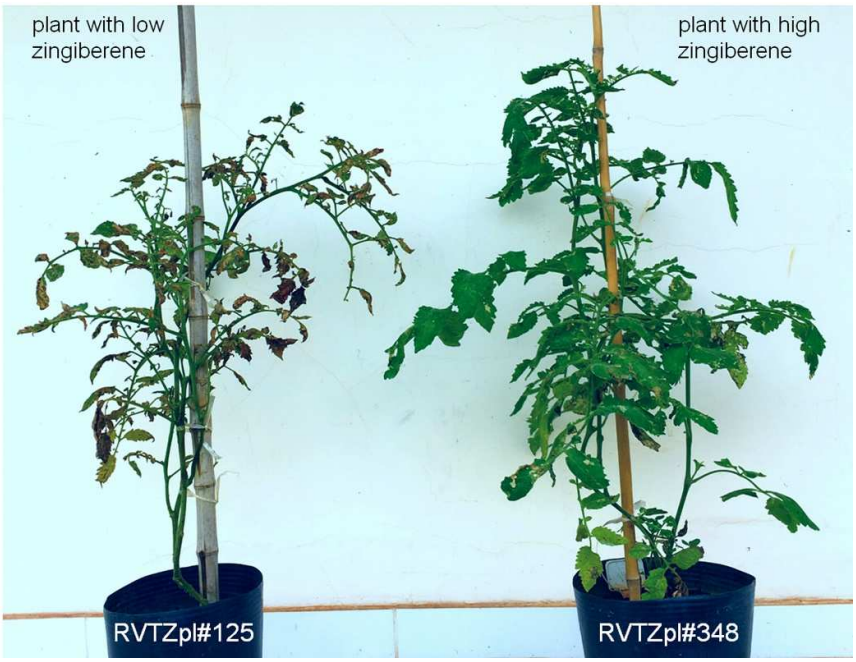

Figure 4. Level of damage caused by tomato moth on low zingiberene (RVTZpl#125) and high (RVTZpl#348) tomato genotypes selected from BC_3F_2 backcross.

3.5. Tatranichus urticae Resistance Test

In the mite race study on the leaflets, there was a lower average distance traveled in genotypes with a a high ZGB content compared to in genotypes with a low ZGB content. There was a negative significant correlation between the mean distance traveled by *T. urticae* and the average content of ZGB in the genotypes (Table 3), as well as between the mean distance traveled and the mean number of type IV glandular trichomes on both sides of the leaflets. The shortest distance traveled by the mites occurred on the wild parent SHH, followed by the genotype RVTZpl#344 (Table 3).

3.6. Evaluation of Recurrent Genome Recovery

The 10 ISSR primers used amplified 104 loci and, of these, 49 were polymorphic. The genetic similarity between the evaluated genotypes and the recurrent parent SLR was 0.48 with RVTZpl#348, 0.29 with RVTZpl#361, 0.43 with RVTZpl#344, 0.35 with RVTZpl#448, and 0.25 with RVTZpl#346.

4. Discussion

The BC_3F_2 tomato genotypes with high levels of ZGB introgressed from *S. habrochaites* var. *hirsutum* (PI127826) were found to be promising genotypes for the development of tomato lineages with broad resistance to arthropods. This conclusion is based on the resistance behavior of these genotypes in response to infestation with *B. tabaci* biotype B, *T. absoluta*, and *T. urticae*. The level of resistance of the BC_3F_2 tomato genotypes was equal to that of the wild tomato species SHH, recognized for its resistance to arthropods [34,51,52].

Our study shows that the transfer of resistance from SHH to cultivated tomatoes is an effective and sustainable strategy to obtain tomato lineages with a broad spectrum of resistance to the main arthropods that cause damage to the crop.

The high concentrations of ZGB in BC_3F_2 genotypes show that the genes responsible for this characteristic in the SHH line PI127826 were effectively transferred in our backcrossing program. The presence of high levels of ZGB is widely known as a resistance factor to several pest arthropods in tomatoes, such as *B. tabaci* [27,44,53–56], *T. absoluta* [28,44,54,57–59], and *T. urticae* [30,33,52,60,61]. The inheritance of ZGB production in SHH is possibly controlled by a major gene [53], which facilitates introgression in commercial cultivars. Despite this, SHH has rarely been explored in breeding for resistance to arthropods. Other than [54], only our research group has documented the development of arthropod-resistant genotypes using SHH as a gene source [21,30,44,59,61].

In BC_3F_2 genotypes, type IV glandular trichomes seem to be fundamental in resistance to the three pest species studied (Table 1). Furthermore, the positive and significant correlation between the amount of trichomes in the BC_3F_2 genotypes and high levels of ZGB confirms the role of these trichomes in resistance. In wild tomatoes, type I, IV, and VI trichomes have been the most important in pest resistance [29,58]. In *S. habrochaites* line LA2329, resistance to *T. urticae* was related to a high density of type IV and VI trichomes [52]. The same was true for whitefly resistance in line PI127826 [53]. An analysis of trichomes in three different SHH lines (G1.1561, LA1718, LA1777) showed that the amount of type IV trichomes was different in each lineage studied [58]; thus, they may present different degrees of resistance to arthropods. The fact that we did not find a significant correlation between type VI trichomes and zingiberene content (Table 3) indicates that there are genetic/morphological differences in resistance between accessions PI127826 and LA2329 of SHH. Additionally, considering that the authors of [53] used the same SSH lineage as in our study (PI127826), it is possible to conclude that the inheritance of the two types of trichomes are independent. It is possible that the selection performed in our study yielded the maintenance of plants with a higher prevalence of type IV trichomes. Interestingly, type IV trichomes were the only ones to confer resistance to the whitefly in all life stages of the pest [27]. Therefore, our data indicate that the high presence of glandular type IV trichomes, derived from the SHH line PI127826 and associated with a high concentration of ZGB, may be sufficient to guarantee resistance to pest arthropods in tomatoes.

The bioassays confirmed that the presence of type IV trichomes in BC_3F_2 genotypes, which were associated with high concentrations of ZGB, seems to promote mostly antixenosis resistance to *B. tabaci*. This conclusion is based on the oviposition results and the consequent reduction in the number of nymphs. Genotypes with a high ZGB content decrease *B. tabaci* oviposition per unit of leaf area, leading to a reduction in pest populations to levels that may not be harmful to the plant [27,54–56]. Furthermore, it is noteworthy that, in our study, the proportion of the number of eggs and the number of nymphs was similar in resistant and susceptible genotypes, which indicates weak, or an absence of, antibiosis resistance in this phase of the pest's life cycle. Additionally, antixenosis resistance seems to be acting in *T. urticae* as they avoided moving over the leaflet, and type IV trichomes, associated with high ZGB content, may be acting as a repellent. As for *T. absoluta*, it was not possible to confirm which type of resistance was active in the BC_3F_2 genotypes as the number of eggs was not counted. Therefore, the smaller area of lesions may be related both to the repellence of winged adults to oviposition and to the toxicity to caterpillars after the eggs hatch and they feed on plant tissue.

The use of ISSR markers allowed us to identify different levels of similarity between the BC_3F_2 genotypes resistant to arthropods and the recurrent cultivar SLR, which showed different rates of recovery of the recurrent genome. The application of the marker-assisted selection of resistance to arthropods in tomatoes is not an approach that is being used and/or documented, as only one study was found [62]. However, the incorporation of resistance to fungal, bacterial, viral, and nematode diseases using markers for foreground and/or background selection has been widely documented in tomatoes [63]. In this sense,

considering that we found genetic differences in SLR between the BC_3F_2 genotypes, the method was efficient. Therefore, the genotypes RVTZpl#348, RVTZpl#344, and RVTZpl#448 obtained in our breeding program are the most promising for further use in the backcrossing program and for a quick recovery of market characteristics in the obtained genotypes.

In summary, our data confirms the successful introgression of resistance to *B. tabaci*, *T. absoluta*, and *T. urticae* from *S. habrochaites* var. *hirtsutum* (line PI127826) for BC_3F_2 genotypes. Furthermore, the combination of resistance data and recurrent genome recovery identified the BC_3F_2 genotypes RVTZpl#348, RVTZpl#344, and RVTZpl#448 as the most promising for continuation of the breeding program.

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