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Tomato Breeding for Sustainable Crop Systems: High Levels of Zingiberene Providing Resistance to Multiple Arthropods

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Abstract: In sustainable cropping systems, the management of herbivorous arthropods is a challenge for the high performance of the tomato crop. One way to reduce the damage caused by these pests is the use of resistant cultivars within a sustainable integrated management system. The host selection of *Tetranychus urticae*, *Bemisia tabaci*, and *Tuta absoluta* was evaluated, characterizing their preference among the tomato genotypes RVTZ2011-79-503-143, RVTZ2011-79-335-164, RVTZ2011-79-185-250 (high zingiberene content—HZC), and RVTZ2011-79-117-273 (low zingiberene content—LZC). Such genotypes were selected in the F₂BC₂ generation (the F₂ generation of the 2th backcross towards *Solanum lycopersicum* after the inicial interspecific cross *S. lycopersicum* × *S. habrochaites* var. *hirsutum*), resulting from crossing *Solanum habrochaites* var. *hirsutum* PI-127826 (HZC and resistant to mites) and the commercial cv. Redenção (*S. lycopersicum*) (LZC and susceptible to mites). In choice and no-choice bioassays by *T. urticae*, and in choice bioassays by *B. tabaci* and *T. absoluta*, arthropods preferred to stay and oviposit in an LZC genotype. In contrast, genotypes with HZC showed repellency to pests and induced a non-preference for oviposition. The F₂BC₂ genotypes selected for HZC are considered sources of resistance genes to these pests for tomato breeding programs, and therefore have excellent potential for sustainable cropping systems. These results represent an advance in obtaining tomato genetic materials which can be used in sustainable production systems with less loss from pests.

Keywords: genetic resistance; natural allelochemicals; organic production; plant defense

1. Introduction

The adoption of genetically resistant plant materials is one of the main strategies for pest management in sustainable tomato crops, in which the use of pesticides should be reduced or mitigated due to their adverse effects on the environment and human health [1–3]. A large number of biochemical and morphological characteristics have been related to tomato resistance to several key and secondary crop pests. Biochemical factors such as allelochemicals, proteins, and several metabolites are often associated with the morphological structures of species like trichomes, mesoderm, and parenchyma, among many other [4–6]. The introgression of genes from wild species has been shown to be the

main alternative for tomato breeding programs, as there are several records of resistance in relation to arthropod pest species in this crop [7–12].

As a way to identify and measure the resistance of tomato genotypes to pest arthropods, biological and behavioral bioassays have been used to characterize cultivars that have resistance mechanisms of the antibiosis or antixenosis type to herbivores in comparison to susceptibility patterns [13]. Thus, it is also possible to identify the mechanisms and degrees of resistance of seedlings to the investigated pest arthropods. In tomato, the resistance mechanism of wild species is associated with the presence and exudation of allelochemicals present mainly in glandular trichomes of stems, leaves, and fruit [14–18]. Zingiberene is an allelochemical with deterrent and repellent actions against pest arthropods, and was found in high concentrations in *Solanum habrochaites* var. *hirsutum* PI-127826 in the glandular trichomes of types IV and VI [19–21]. The inheritance of the content of this allelochemical shows a high heritability, with values above 80%; it is controlled by two genes with incomplete dominance, which allows genetic gains regarding resistance when selecting seedlings with high zingiberene content [22–25].

Recently, the tomato genotype access PI-127826 was been used to increase the degrees of resistance in commercial varieties of *Solanum lycopersicum* through interspecific crosses [20,25–27] in order to obtain isogenic lines for hybrid production in conventional and sustainable production systems. However, the reactions triggered by the allelochemicals on the pests have been varied and complex, resulting in different levels of response in relation to the attack. Therefore, it is necessary to understand the effect of zingiberene and its stereoisomers on the behavior and biology of arthropod pests to determine the level of resistance of the genotypes. In addition, for a tomato breeding program, it is essential to characterize the resistance of generations and confirm the presence of the desirable characteristics of the parents which can be employed in future crossings.

Among the pest arthropods that occur in tomato, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Tetranychus urticae* (Koch) (Acari: Tetranychidae) are cosmopolitan and recurrent in crops grown in diverse production systems. For these arthropods, the deleterious effect of zingiberene has been evidenced in genotypes related to *S. habrochaites* var. *hirsutum*. However, despite all the studies on this subject, obtaining commercial cultivars with acceptable levels of yield and resistance is still an objective yet to be achieved [6,28–32].

In order to characterize resistant tomato genotypes to be used in breeding programs, we investigated the degrees of resistance of F₂BC₂ genotypes with high levels of zingiberene which were obtained from interspecific crossing between *S. habrochaites* var. *hirsutum* and *S. lycopersicum*. The resistance degree of the genotypes to *T. urticae*, *B. tabaci*, and *T. absoluta* were verified upon host selection bioassays.

2. Material and Methods

2.1. Location

The bioassays were carried out at the Midwestern Parana State University, Guarapuava, Paraná, Brazil (25°23′00″ S, 51°29′38.50″ W, elevation 1024 m.a.s.l.). The local climate is classified as humid subtropical mesothermal (Cfb), with moderate summers, winters with frost incidence, and an average annual rainfall of 1711 mm.

2.2. Tomato Genotypes and Growing Seedlings

The tomato genotypes selected by Zanin et al. [25] from a F₂BC₁ population for high levels of zingiberene (RVTZ 2011-079-117, RVTZ 2011-079-185, RVTZ 2011-079-335, and RVTZ 2011-079-503) were used as parents and pollen sources for crosses with *Solanum lycopersicum* cv. Redenção, thus obtaining the F₁RC₂ generation which, after self-fertilization, originated the F₂BC₂ population.

The selection of contrasting genotypes occurred in 400 seedlings of the F₂BC₂ population. The levels of zingiberene were quantified in young and expanded leaflets according to the methodology proposed by de Freitas et al. [33]. Therefore, six leaf disks (1 cm in diameter) were sampled from each genotype,

placed in tubes containing 2 mL of hexane, and then vortexed for 30 s. After that, the leaf disks were removed and the absorbance of the solution was measured using a spectrophotometer (Cary series, UV-Vis Spectrophotometer) at a wavelength of 270 nm. The selection of genotypes was carried out based on the absorbance values, which were above 0.300 nm for the three high-zingiberene genotypes and below 0.150 nm for the low-zingiberene genotype.

As low and high allelochemical standards, 50 tomato seedlings of cv. Redenção and 50 seedlings of the wild genotype *S. habrochaites* var. *hirsutum*, respectively, were used.

The F₂BC₂ genotypes selected indirectly for laboratory resistance based on zingiberene content were RVTZ 2011-79-503-143, RVTZ 2011-79-503-164, RVTZ 2011-79-503-250 (high zingiberene content—HZC) and, to use as a susceptibility standard in bioassays, RVTZ 2011-79-117-273 (low zingiberene content—LZC) (Table 1). The genotypes were cloned from axillary shoots; rooted; transplanted into polyethylene pots (5 L) containing a mixture of commercial substrate and soil (1:1), nitrogen, phosphorus and potassium chemical fertilizer (4:14:8), and acidity correction according to crop demand.

Table 1. Absorbancy at 270 nm (means $\pm$ SD) indicating the zingiberene content of tomato genotypes (*Solanum lycopersicum* cv. Redenção, *Solanum habrochaites* var. *hirsutum*, and genotypes of F₂BC₂).

Genotype	Absorbancy (270 nm)	Classification
<i>S. lycopersicum</i> cv. Redenção	0.032 $\pm$ 0.01	Susceptible standard (SS)
RVTZ 2011-79-117-273	0.100 $\pm$ 0.05	Low zingiberene content (LZC)
RVTZ 2011-79-335-164	0.322 $\pm$ 0.04	High zingiberene content (HZC)
RVTZ 2011-79-185-250	0.330 $\pm$ 0.07	High zingiberene content (HZC)
RVTZ 2011-79-503-143	0.809 $\pm$ 0.18	High zingiberene content (HZC)
<i>S. habrochaites</i> var. <i>hirsutum</i>	0.946 $\pm$ 0.19	Resistant standard (RS)

2.3. Arthropod Rearing

Adults of *T. urticae* were collected and kept on common bean seedlings (*Phaseolus vulgaris*), under controlled conditions (25 $\pm$ 2 °C, 70% $\pm$ 4% relative humidity—RH, 12 h photophase), with daily irrigation. The age-controlled breeding consisted of 10 adult mites (3 males and 7 females) kept on individual bean leaflets, arranged on a sponge moistened with distilled water, and packed in plastic trays (200 $\times$ 300 mm) with the bottom facing up. The mites were kept on the leaflets for 24 h and then the adults were removed, leaving only the eggs.

Individual *B. tabaci* and *T. absoluta* were collected in greenhouses from tomatoes in commercial production. For the rearing of *B. tabaci*, individuals were kept and multiplied on common bean seedlings, grown in pots, and packed in cages in a greenhouse at 25 $\pm$ 3 °C with daily irrigation. Rearing for age control was carried out, keeping bean seedlings with *B. tabaci* nymphs in cages made of voile fabric (100 $\times$ 100 cm) in a protected environment free from infestation. From five to eight days after emergence, the adults were used for bioassays. To rear *T. absoluta*, the individuals were kept on tomato seedlings cv. Santa Cruz, grown in pots, and placed in voile cages (100 $\times$ 100 cm) in a greenhouse at 25 $\pm$ 3 °C. The eggs deposited on the leaves were removed at intervals of three days and transferred to similar cages containing seedlings free from infestation.

2.4. Host Selection Bioassays

2.4.1. *Tetranychus urticae*

Choice and no-choice bioassays were carried out in Petri dish arenas (6 cm in diameter) and covered with a sponge and a cotton layer both moistened in distilled water. In each choice test dish, two leaf discs (3 cm in diameter) were placed under the cotton layer with the abaxial region facing upwards. Two genotypes were placed in each dish, and all of them were tested in combination two by two in order to evaluate the preference of the mites (Figure 1a). The discs were connected to each other

by a transparent coverslip (18 × 18 mm), in the center of which four adult females of *T. urticae* were released, allowing free choice between the discs of the different tomato genotypes. The dishes were kept for 24 h in an air-conditioned chamber (25 ± 1 °C, 70% ± 4% RH, 12 h photophase). Then, the live mites present on each leaf disc were counted, in addition to the eggs deposited on the leaf surface.

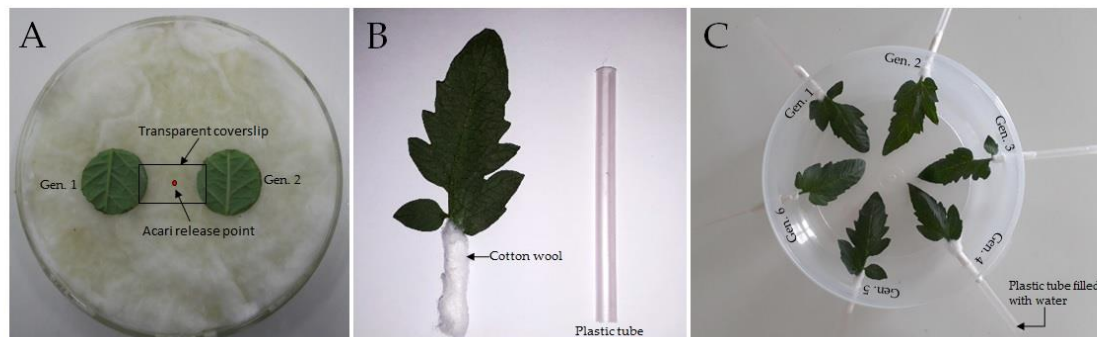


Figure 1. Material used in the host selection bioassays. (A) Arena for *Tetranychus urticae* with two tomato genotypes connected by a transparent coverslip where the mites were released. (B) Tomato leaflet wrapped in cotton wool and plastic tube. (C) Tomato genotypes (6 treatments) arranged equidistant presenting the design of the *Bemisia tabaci* and *Tuta absoluta* bioassays. Each leaflet wrapped in cotton wool was introduced into a plastic tube containing water in order to maintain the humidity.

The experiment was designed with six treatments (genotypes) that were combined in pairs (total of 14 combinations) with ten repetitions per pair, and the arenas were distributed in a completely randomized design.

For the no-choice test, a leaf disc (3 cm in diameter) of each genotype was inserted in each arena, on which six adult females were transferred with the aid of a stereoscopic microscope (Nikon SMZ745T, Japan). The females were kept on the leaf discs for 24 h, and then the live mites and eggs deposited on the abaxial face of each disc were counted. The experiment was carried out in a completely randomized design, with six treatments (genotypes) and ten repetitions. The dishes were kept in an air-conditioned chamber (25 ± 1 °C, 70% ± 4% RH, 12 h photophase).

2.4.2. *Bemisia tabaci*

To evaluate the host selection behavior for the oviposition of *B. tabaci*, a choice test was adopted using circular plastic arenas (29.0 cm in diameter × 10.0 cm in height) with the cover lined with voile fabric to allow air circulation. From six equidistant holes in the side walls of the arena, tomato leaflets were inserted in a horizontal position, with the petiole kept externally and the leaf area internally within the arena. In order to keep the leaflet moist, the petiole was wrapped in cotton wool before being inserted into the plastic tube (0.3 cm in diameter × 10.0 cm in length) filled with water, whose function was to keep the cotton and petiole moist (Figure 1B,C). Thus, each arena contained a leaflet of each tomato genotype, comprising six leaflets, which were available for the choice of the insects.

In each arena, 30 individuals of *B. tabaci* were inserted and released with the aid of a suction device (transparent plastic tube 0.3 cm in diameter) from a hole made in the bottom of the container. After 24 h, the individuals and eggs present on the abaxial face of each leaflet were counted in order to calculate the attractive and preference index.

This bioassay was carried out in a completely randomized design with 6 treatments (genotypes) and 20 repetitions, and the arenas were kept in a greenhouse with temperature control (25 ± 3 °C).

2.4.3. *Tuta absoluta*

The behavior and deposition of the *T. absoluta* were evaluated in choice bioassays, using arenas similar to those described above for *B. tabaci*, allowing the free choice of insects in relation to the genotypes. However, for *T. absoluta* the arenas were made with a rectangular EVA

(ethylene vinyl acetate) plate arranged in a conical shape forming a tube (25.0 cm in diameter $\times$ 40.0 cm in height), with side holes arranged in an equidistant way, which allowed the coupling of the leaflets in the horizontal position. The leaflets were inserted in the arena with the leaf area on the inside and the petiole wrapped in cotton and a plastic tube on the outside (Figure 1B,C). In turn, the upper and lower openings of the arena were lined with voile fabric to prevent the escape of insects and to allow air to circulate.

In the center of each arena, 12 females were released (10 to 12 days old), which remained for 72 h until the number of individuals and the number of eggs deposited on the adaxial and abaxial surfaces of the leaflets of each genotype were evaluated.

The bioassay was carried out in a completely randomized design, with 6 treatments (genotypes) and 10 repetitions. The insects were kept in air-conditioned rooms (25 ± 1 °C, $70\% \pm 4\%$ RH, photophase 12 h).

2.5. Data Analysis

The number of adults and eggs of *T. urticae*, *B. tabaci*, and *T. absoluta* present in each treatment in the choice and no-choice tests were submitted to a normality analysis (Bartlett, $p \leq 0.05$) and analysis of variance (ANOVA, $p \leq 0.05$), and the means were compared by the Tukey test ($\alpha \leq 0.05$). The non-normal data were transformed by $(x + 0.5)^{1/2}$. These analyses were performed using the Statistica 7.0 software [34].

The data referring to the choice bioassays for *T. urticae* were analyzed using the Chi-square test ($p \leq 0.05$) and Kruskal–Wallis ($p \leq 0.05$), respectively, to test the hypothesis of equality between the observed and expected frequencies from individuals in each treatment.

In the choice bioassays for *B. tabaci* and *T. absoluta*, the attractiveness index was estimated as $AI = 2G/(G + S)$, where AI = attractiveness index; G = number of insects attracted to the evaluated genotype; and S = number of insects attracted to the susceptible standard (*S. lycopersicum* cv. Redenção). The AI values varied between zero and two, in which AI = 1 indicates a similar attraction between the evaluated genotypes (repellent test plant) and the standard (attractive test plant); AI < 1 corresponds to less attraction (greater repellency) to the genotype; and AI > 1 indicates a greater attraction to the evaluated genotype in relation to the standard. This index is an adaptation of the formula mentioned by Lin et al. [35] and used by Baldin et al. [36].

The oviposition preference index (OPI) was also calculated as $OPI = [(T - S)/(T + S)] \times 100$, where T = number of eggs counted in the evaluated treatment and S = number of eggs counted in the standard genotype (*S. lycopersicum* cv. Redenção). This index ranges from +100 (high preference) to −100 (non-preference or oviposition inhibition). The classification of genotypes was performed by comparing the average of eggs from the treatments with the average of the *S. lycopersicum* cv. Redenção [37].

3. Results

3.1. Tetranychus Urticae Host Selection

The combinations of genotypes offered to *T. urticae* influenced the choice of females, which showed a rejection of the F₂BC₂ genotypes with HZC (RVTZ 2011-79-503-143, RVTZ 2011-79-503-164, and RVTZ 2011-79-503-250) when combined with the standard susceptibility tomato cv. Redenção (Chi-square, GL = 1, $p < 0.05$) (Figure 2). Although it was with less intensity, this response pattern was repeated when this cultivar was compared to the low-zingiberene genotype (RVTZ 2011-79-503-273) (Chi-square, GL = 1, $p = 0.016$).

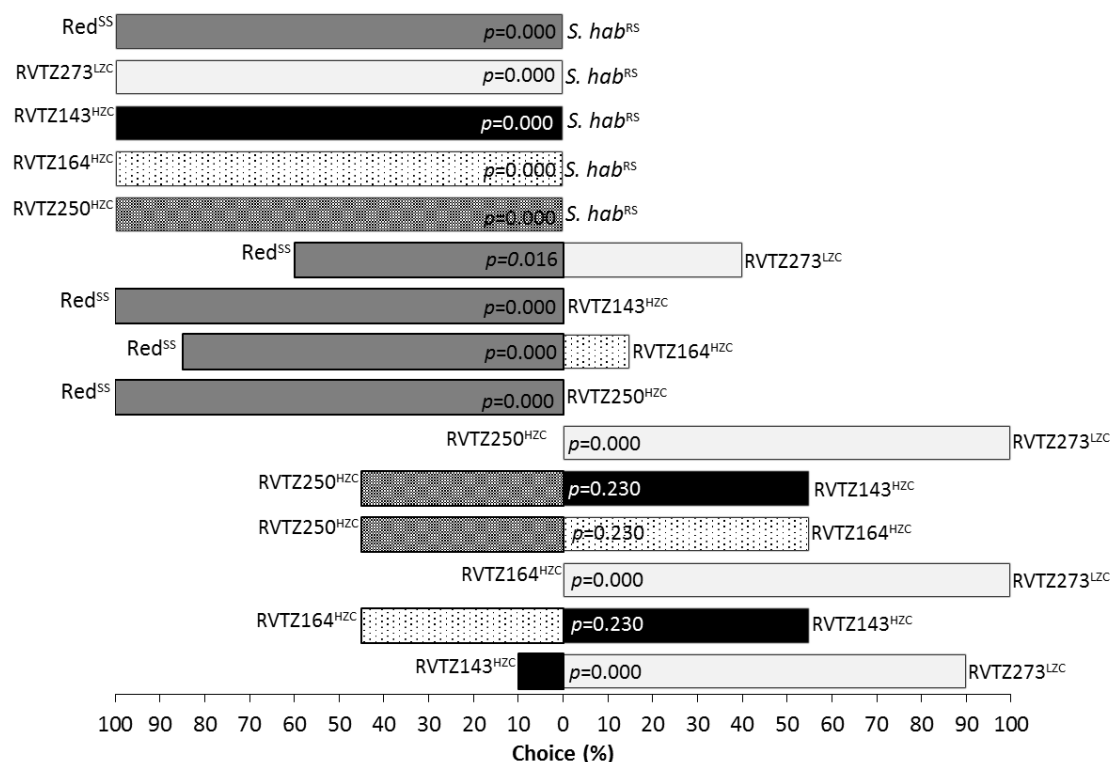


Figure 2. Frequency of choice of *Tetranychus urticae* in relation to tomato F₂BC₂ genotypes ($n = 10$). Red = *Solanum lycopersicum* cv. Redenção; PI-127826 = *Solanum habrochaites* var. *hirsutum* PI-127826; 273 = RVTZ 2011-79-117-273 (with low zingiberene content); 143 = RVTZ 2011-79-503-143; 164 = RVTZ 2011-79-503-164; and 250 = RVTZ 2011-79-503-250 (with high zingiberene content). p -value indicates the statistical significance according to the Chi-square test. SS = Susceptible Standard genotype; RS = Resistant standard genotype; HZC = high zingiberene content; LZC = low zingiberene content.

Less attractiveness compared to the standard resistance genotype *S. habrochaites* var. *hirsutum* PI-127826 was observed in all the evaluated combinations, even when compared to the genotypes selected for HZC in the F₂BC₂ generation (Chi-square, GL = 1, $p < 0.05$). *T. urticae* females did not differentiate between the genotypes with HZC, presenting a similar frequency of choice regardless of the combined genotypes (Chi-square, GL = 1, $p > 0.05$).

In no-choice bioassays, the number of live mites was influenced by the presence of zingiberene. *S. lycopersicum* cv. Redenção leaflets provided more than 90% survival after 24 h of exposure of mites, with an average of 5.75 living individuals. The LZC genotype RVTZ 2011-79-117-273 did not differ from the standard susceptible genotype (Tukey, GL = 5, $\alpha = 0.912$). In turn, HZC triggered acari mortality, as occurred with the resistant standard genotype (Tukey, GL = 5, $\alpha < 0.0001$). The average number of eggs deposited in the leaflets was higher in the susceptible cultivar and in the low allelochemical genotype, differing from the high-content genotypes (Tukey, GL = 5, $\alpha < 0.0001$). These genotypes did not differ among themselves (Tukey, GL = 5, $\alpha > 0.1$) (Table 2).

3.2. Bemisia tabaci Host Selection

S. lycopersicum cv. Redenção was the most attractive for permanence and oviposition, followed by RVTZ 2011-79-117-273 (LZC) (Tukey, GL = 5, $p = 0.01$). *S. habrochaites* var. *hirsutum* PI-127826 and other genotypes selected for their high levels of zingiberene triggered the same insect responses (RVTZ 2011-79-503-143, RVTZ 2011-79-335-164, RVTZ 2011-79-185-250) (Tukey, GL = 5, $\alpha > 0.9$) (Table 3).

Table 2. Number ($\pm$ standard error) of live adults and eggs of *Tetranychus urticae* mites on the abaxial face of leaf discs of tomato genotypes in no-choice oviposition bioassays ($n = 10$).

Genotypes	Number of Individuals	Number of Eggs
<i>Solanum lycopersicum</i> cv. Redenção	5.75 $\pm$ 0.12 a	46.6 $\pm$ 1.80 a
RVTZ 2011-79-117-273 ^{LZC}	4.25 $\pm$ 0.14 ab	27.2 $\pm$ 1.20 b
RVTZ 2011-79-503-143 ^{HZC}	3.25 $\pm$ 0.15 b	3.1 $\pm$ 0.60 c
RVTZ 2011-79-335-164 ^{HZC}	3.50 $\pm$ 0.14 b	3.9 $\pm$ 0.53 c
RVTZ 2011-79-185-250 ^{HZC}	3.25 $\pm$ 0.30 b	0.7 $\pm$ 0.14 c
<i>Solanum habrochaites</i> var. <i>hirsutum</i>	3.75 $\pm$ 0.12 b	1.8 $\pm$ 0.18 c
F-value	81.9	328
ANOVA <i>p</i> -value	0.03	0.0001

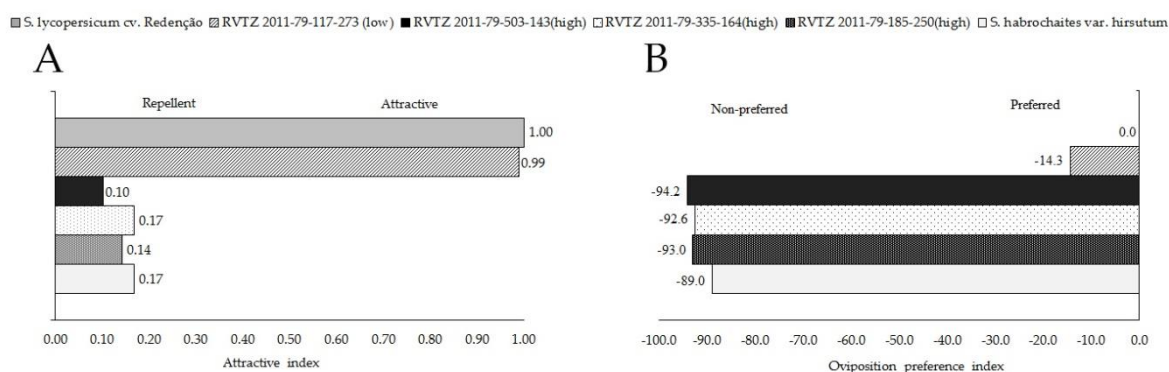
^{LZC} low zingiberene content; ^{HZC} high zingiberene content. Means followed by the same letter in a column do not differ statistically from each other by the Tukey test (GL = 5, $\alpha \leq 0.05$). For analysis, the average number of eggs was transformed by $(x + 0.5)^{1/2}$.

Table 3. Number ($\pm$ standard error) of *Bemisia tabaci* live adults and eggs on the abaxial face of leaflets from tomato genotypes in the choice bioassay ($n = 10$).

Genotypes	Number of Individuals	Number of Eggs
<i>Solanum lycopersicum</i> cv. Redenção	13.00 $\pm$ 0.90 a	47.10 $\pm$ 3.70 a
RVTZ 2011-79-117-273 ^{LZC}	12.90 $\pm$ 1.0 b	35.30 $\pm$ 3.50 b
RVTZ 2011-79-503-143 ^{HZC}	0.70 $\pm$ 0.20 c	1.45 $\pm$ 0.50 c
RVTZ 2011-79-335-164 ^{HZC}	1.20 $\pm$ 0.30 c	1.80 $\pm$ 0.40 c
RVTZ 2011-79-185-250 ^{HZC}	1.00 $\pm$ 0.15 c	1.75 $\pm$ 0.35 c
<i>Solanum habrochaites</i> var. <i>hirsutum</i>	1.20 $\pm$ 0.30 c	2.50 $\pm$ 0.40 c
F-value	683.8	177.8
ANOVA <i>p</i> -value	0.0001	0.0001

^{LZC} low zingiberene content; ^{HZC} high zingiberene content. Means followed by the same letter in each column do not differ statistically from each other by the Tukey test ($\alpha \leq 0.05$). For analysis, the average number of eggs was transformed by $(x + 0.5)^{1/2}$.

According to the values of the attractiveness index calculated after 24 h of infestation, all the genotypes with a HZC were classified as repellent to *B. tabaci* when compared to the susceptible standard genotype *S. lycopersicum* cv. Redenção (Figure 3a). In addition, the preference index for oviposition calculated in the choice bioassay classified all the materials as non-preferred to *B. tabaci* oviposition when compared to the susceptible *S. lycopersicum* cv. Redenção, even for the low zingiberene genotype (Figure 3b).

**Figure 3.** *Bemisia tabaci* responses to tomato genotypes in choice bioassays ($n = 10$). (A) Attractiveness index of genotypes for adults of *B. tabaci*. (B) Oviposition preference index for *B. tabaci* in relation to genotypes. The data regarding the number of individuals were transformed by $(x + 0.5)^{1/2}$.

3.3. *Tuta Absoluta* Host Selection

T. absoluta females preferred to remain in the LZC genotypes. Nevertheless, they did not differ from RVTZ 2011-79-335-164, which was selected for the high content of this compound (Tukey, GL = 5, $\alpha > 0.1$) (Table 4). However, even in the genotype with a HZC, *T. absoluta* females oviposited compared to the susceptible standard and that with a LZC (Tukey, GL = 5, $\alpha < 0.05$) (Table 4). Therefore, the number of eggs deposited was significantly higher in the standard susceptibility cultivar than in the other genotypes (Tukey, GL = 5, $\alpha < 0.05$) (Table 4).

Table 4. Number ($\pm$ standard error) of *Tuta absoluta* adults and eggs on the adaxial and abaxial sides of leaflets of tomato genotypes in a choice bioassay ($n = 10$).

Genotypes	Number of Adults	Number of Eggs
<i>Solanum lycopersicum</i> cv. Redenção	1.4 $\pm$ 0.15 a	47.0 $\pm$ 1.40 a
RVTZ 2011-79-117-273 ^{LZC}	1.4 $\pm$ 0.15 a	37.0 $\pm$ 1.30 b
RVTZ 2011-79-503-143 ^{HZC}	0.5 $\pm$ 0.16 b	4.7 $\pm$ 0.10 c
RVTZ 2011-79-335-164 ^{HZC}	1.0 $\pm$ 0.2 ab	8.2 $\pm$ 0.30 c
RVTZ 2011-79-185-250 ^{HZC}	0.6 $\pm$ 0.15 b	9.1 $\pm$ 0.20 c
<i>Solanum habrochaites</i> var. <i>hirsutum</i>	0.5 $\pm$ 0.16 b	3.6 $\pm$ 0.09 c
F-value	6.1	111.6
ANOVA <i>p</i> -value	0.0001	0.0001

^{LZC} low zingiberene content; ^{HZC} high zingiberene content. Means followed by the same letter in each column do not differ statistically from each other by the Tukey test ($\alpha \leq 0.05$). For analysis, the average number of eggs was transformed by $(x + 0.5)^{1/2}$.

The calculated indices demonstrated that the LZC and susceptible genotypes were more attractive to *T. absoluta* than the genotypes with a HZC (Figure 4a). In contrast, the HZC genotypes presented less attractiveness (RVTZ 2011-79-335-164 and RVTZ 2011-79-185-250) or repellency (RVTZ 2011-79-503-143) to *T. absoluta* (Figure 4a). Furthermore, the selected HZC genotypes suppressed *T. absoluta* oviposition, triggering more than 60% of the oviposition index when compared with the standard genotype (*S. lycopersicum* cv. Redenção) (Figure 4b).

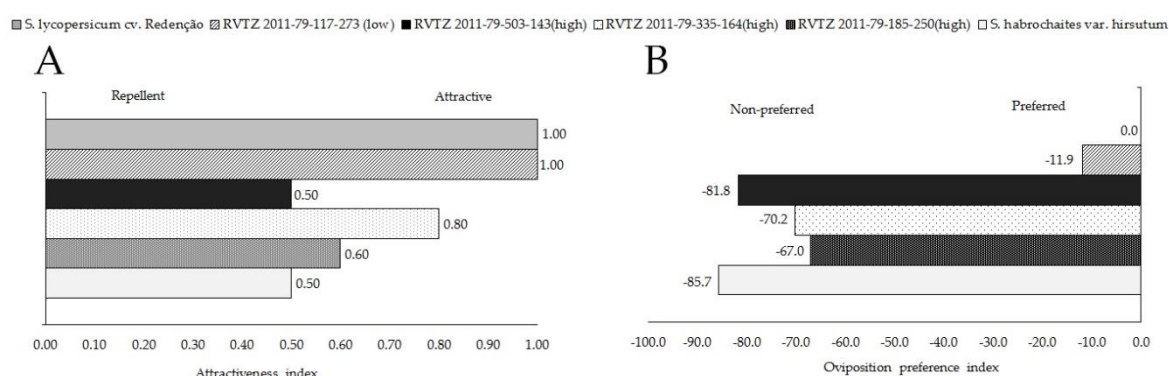


Figure 4. *Tuta absoluta* responses to tomato genotypes in choice bioassays. (A) Attractiveness index of genotypes for adults of *T. absoluta*. (B) Oviposition preference index of *T. absoluta* in relation to genotypes. The data regarding the number of individuals were transformed by $(x + 0.5)^{1/2}$.

4. Discussion

Genetics and plant breeding are two main sciences in the service of man that contribute in various fields of activities, especially food production. Genetic improvement considers the relationship of physical, chemical, biological, economic, and social components to the environment, aiming at the best possible adjustment and resulting in the sustainability of a productive system. Thus, genetic improvement is essential for plants to adapt sustainably to the growing environment, providing greater productivity and less possible damage to the system balance.

Tomato production is characterized by low sustainability, largely due to the high number of applications for pest and disease control. In this context, breeding aimed at the greater sustainability of the systems has sought to develop genotypes more tolerant to abiotic and biotic stresses. The use of wild tomato genotypes as a source of resistance to pests has been used on a recurring basis, aiming to seek adjustment to the sustainable production system.

The resistance in these cases is related to the presence of allelochemicals that are exuded by the tomato leaf trichomes, including zingiberene, a volatile sesquiterpene with a proven effect in pest control. The best way to introduce resistance in commercial cultivars is through interspecific crossings. In the present work, resistance was introduced through backcrosses and, as shown, the results were promising.

Glandular trichomes are specialized structures found on the surface of about 30% of all vascular plants and are responsible for a significant portion of a plant's secondary chemistry. Glandular trichomes are an important source of allelochemicals, which provide the plant with protection against herbivores and pathogens. The storage compartment of glandular trichomes is usually located at the tip of the morphological structure and is part of the glandular cell, or cells that are metabolically active. This knowledge now helps classic breeding programs, as well as targeted genetic engineering, with the aim of optimizing the density and physiology of trichomes for the purpose of biocides to improve crop protection [38,39].

Glandular trichomes and zingiberene contribute to increased tomato resistance against herbivore attack [39]. The ability to accumulate zingiberene in glandular trichomes is being transferred to commercial cultivars through crossbreeding, contributing to the increased resistance of plants to mites and whiteflies [40]. The presence of zingiberene in tomato genotypes induced a non-preference for the oviposition of arthropod pests. Such evidence demonstrates that F₂BC₂ seedlings selected for high zingiberene content have desirable degrees of resistance to arthropods, and that selection for this characteristic has been successful in terms of the biological response of the target organisms. These genotypes, in some cases, showed similar degrees of resistance to the standard *S. habrochaites* var. *hirsutum* PI-127826, which was expected due to the high heritability (81.9%) of zingiberene content character in plants [22,25].

Normally, the genetic inheritance of a concentration of allelochemicals associated with the presence of glandular trichomes on tomato species is mediated by at most two larger genes, and is associated with smaller genes with an additive effect. The heritability in a broad sense was estimated at above 50%, considered as moderate to high [41–43]. Heterozygous genotypes for zingiberene showed levels of allelochemicals intermediate to those of their parents of high and low content, indicating incomplete dominant gene action for the content of allelochemicals [7].

The concentration of this allelochemical is controlled by the two larger gene loci, with incomplete dominance in the sense of the lowest content in populations of interspecific crosses of *S. lycopersicum* with *S. habrochaites* var. *hirsutum* [22]. Other studies related to the presence of zingiberene reported that genetic inheritance was controlled by a single dominant gene of *S. habrochaites* var. *hirsutum* in the generation F₂ and in the BC progeny of *S. lycopersicum* with *S. habrochaites* var. *hirsutum*, and also as a single recessive gene in the F₂ generation and in the BC progeny of *S. habrochaites* var. *hirsutum* × *S. habrochaites* var. *glabratum* [33,44].

This fact, together with the high heritability of the character, may reflect satisfactory genetic gains in backcrossing over generations, using indirect selection for resistance to arthropod pests based on the zingiberene content [25]. The most important aspect of the genetic breeding is hereditary variability and, particularly, the genetic component, which has a strong influence on the response to selection. The information generated from the character association studies appeared as an important indicator for a selection program. The present study also contributed to the suitability of several characters for indirect selection, because the selection for one or more characteristics resulted in a correlated response in several other studies [45].

The two larger gene loci are located very close to each other on chromosome 8 (ShZIS and ShSBS) [40]. However, it is assumed that only one of the gene loci is primarily responsible for the synthesis of zingiberene [44]. Evidence suggested that this locus must contain ShZIS synthase, which produces 7-epizingiberene, a zingiberene sesquiterpene stereomer, in glandular trichomes from Z-Z-farnesyl-diphosphate (zFPP) in plastids, which promotes resistance to various tomato pests.

It is also assumed that ShZIS is allelic to ShSBS and linked to zFPS. Gonzales-Vigil et al. [46] suggested that ShZIS originated before ShSBS, with the mutagenesis of only three amino acids at the active site of a synthesizing enzyme, predominantly zingiberene, elucidating the probable pathway for the synthesis of this sesquiterpene. Thus, indirect selection for resistance facilitated the process of genetic improvement, as it allows the use of tools that minimize the difficulties of selecting in large populations of plants, as in the case of segregating populations [7].

The environment can interfere in expression of zingiberene content, and thus low heritability can be great challenge for breeding programs. In-depth knowledge of quantitative genetic parameters and, in particular, genetic variation and heritability, help to overcome these challenges. Although the expression of the characters is influenced by the environment, the high heritability described in the literature implies success in transfer to progeny through crosses [47].

The high heritability of the traits is the fundamental basis for the genetic improvement of this particular trait. In addition, the nature and magnitude of genetic variation that governs inheritance of yield and yield components are essential for breeding. In general, heritability is the reason for variation due to differences between genotypes and the total phenotypic variation of a trait in a population [48]. Heritability estimates, along with genetic advancement, are more accurate in predicting genetic gain under selection than heritability alone. In addition, the relationship between the yield and the characteristics attributable to yield is of high importance for the direct and indirect selection of characteristics [49].

Resistance depends on the relationship of the target insect to the levels of zingiberene. The bioassays provided important results, inferring that the adults of *T. urticae* (Figure 2) and *B. tabaci* (Table 3) rejected the genotypes with high zingiberene content. For these two arthropods, the number of eggs deposited on leaflets of the susceptible standard treatment (*S. lycopersicum* cv. Redenção) and the low zingiberene genotype was higher compared to the high content genotypes and *S. habrochaites* var. *hirsutum*.

The results of this work corroborated the research of others who evaluated the selection of genotypes from different generations resulting from interspecific crosses between *S. habrochaites* var. *hirsutum* and *S. lycopersicum*. Interspecific hybrids with a high content of zingiberene, derived from a diallel and demonstrated by means of GCA (General Combining Ability) and SCA (Specific Combining Ability), have a high potential for use in improving tomato pest resistance [50]. Studies reported that genes responsible for high levels of zingiberene were passed to segregating generations through crosses, maintaining the resistance of plants in advanced populations with commercial backgrounds [20,40,51].

The high number of eggs deposited by *T. urticae* (Table 2), *B. tabaci* (Table 3), and *T. absoluta* (Table 4) on the leaflets of the RVTZ genotype 2011-79-117-273 demonstrated that, although it has a low zingiberene content, it is highly susceptible to the evaluated pests. Studies with other tomato lines have also shown a positive correlation between zingiberene content and resistance to these pest arthropods [7,52,53]. Genotypes with a high zingiberene content (ZGB-703 and ZGB-704) had a lower oviposition and a lower number of nymphs of whitefly when compared with genotypes with a low acyl-sugar (AS) and zingiberene content (Santa Clara, TOM-695, TOM-556, and TOM-584) [54].

Bleeker et al. [46] observed a severe reduction in the fertility of two of the main tomato pests, *B. tabaci* and *T. urticae*, mediated by a zingiberene stereoisomer, 7-epizingiberene, also present in the PI-127826 genotype. Bleeker et al. [40] found that the survival rate and reproductive success of *B. tabaci* adults in F₂ genotypes from the cross between PI-127826 and *S. lycopersicum* were severely compromised, although the levels of 7-epizingiberene on the F₂-7 and F₂-71 leaf surface were lower than that observed in wild tomato PI-127826.

For *T. absoluta*, although adults remained on the leaflets of genotypes with high levels of zingiberene, their rejection was verified by the reduced number of eggs deposited compared to the susceptibility standard and the low allelochemical content genotype (Table 4). Homozygous lines with a high zingiberene content, simple heterozygous genotypes for zingiberene, and controls with a low zingiberene content ('Débora Max' and 'TOM-684') and a high zingiberene content (PI-127826) were subjected to infestation with adult pinworm from South America. High zingiberene genotypes showed significantly lower egg counts than the low ones [7]. The damage by *T. absoluta* was greater in the low zingiberene controls, as in the present study.

Silva et al. [55] found that volatile compounds such as sesquiterpenes negatively affected the preference (antixenosis) or survival (antibiosis) of herbivorous insects in tomatoes by modulating the flight behavior and oviposition of females of *T. absoluta* during mating. In addition, they observed that females landed more frequently and oviposited in the most susceptible genotype. However, this proportions of preference for feeding and oviposition were not substantially higher in the resistant genotypes when compared to susceptible ones.

Al-Bayati [56], when evaluating nine tomato hybrids (BC₃F₃ and BC₃F₄) selected based on the variation of the type IV trichome density and zingiberene concentration of an interspecific cross with *S. habrochaites*, concluded that the resistance to the spider mite had been successfully transferred to hybrids through backcrossing and indirect selection. There was a significant negative correlation between almost all the behavioral and biological responses of the mites with the density of type IV trichomes and the zingiberene content. Therefore, these results are in agreement with those obtained in the present study for the relationship between zingiberene and pests.

The results of this work showed that the genotypes with a high zingiberene content affected the behavior and development of arthropods. Thus, it confirms that the use of *S. habrochaites* var. *hirsutum* PI-127826 enabled the introgression of genes to increase the degrees of resistance in the F₂BC₂ genotypes. Finally, the results suggested that the genotypes RVTZ 2011-79-503-143, RVTZ 2011-79-335-164, and RVTZ 2011-79-185-250 can be used as potential sources of genes for resistance to *T. urticae*, *B. tabaci*, and *T. absoluta* in tomato breeding programs. The results also showed that the genetic improvement of this species, mediated by allelochemicals present in wild species, is an excellent alternative for obtaining cultivars with better adaptation to sustainable production systems.

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