

Resistance to whitefly mediated by the Mi gene, acylsugars, and zingiberene in tomato

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Abstract

This study aimed to quantify and compare the effects of Mi gene, high leaf levels of acylsugars (AA), and zingiberene (ZGB) individually and in combination, regarding resistance of tomato lines to *Bemisia argentifolii*. The experiment included 21 genotypes selected simultaneously for high AA and ZGB content, cloned through rooting of axillary shoots with homogeneous sizes, and seven control lines produced from seeds, including 2 genotypes rich in AA (TOM-687 and TOM-688), two genotypes rich in ZGB (TOM-703 and TOM-704), and three lines with low levels of allelochemicals (cv. Santa Clara, TOM-584, and TOM-684), where the TOM-684 line carried the Mi gene. The experiment was conducted in a greenhouse using a randomized complete block design, comprising 28 treatments with 4 replications and 1 plant per plot. The AA and ZGB-rich lines showed higher resistance compared to the other control lines with low levels of allelochemicals, but the TOM-684 control line carrying the Mi gene was more resistant than the non-carriers ('Santa Clara' and TOM-584). The level of resistance conferred by ZGB was similar to that presented by AA. Both ZGB and AA provided higher levels of resistance compared to the Mi gene. The clones selected for both ZGB and AA simultaneously exhibited lower numbers of eggs and nymphs compared to the susceptible controls and were more resistant than the TOM-684 treatment. Overall, the clones even outperformed the lines rich in only one of the allelochemicals, demonstrating a synergistic effect between AA and ZGB for resistance when combined in the same tomato line.

Keywords: allelochemicals, plant breeding, antixenosis, *Solanum lycopersicum*

Introduction

Whitefly, *Bemisia tabaci* (Gennadius, 1889), is a globally significant pest causing economic losses in various important crops, including tomatoes (Zaidi et al., 2017). The most aggressive form of whitefly, known as biotype B, *Bemisia argentifolii* (Bellows & Perring, 1994), is also the most common and abundant (Queiroz et al., 2016). It has a high capacity for transmitting geminiviruses that cause phytotoxic anomalies, also induced by toxins injected during insect feeding (Bragard et al., 2013).

The presence of *B. argentifolii* commonly results in direct and indirect damage to crop production, with chemical control being the primary management method (Shadmany et al., 2015). However, relying solely on pesticides for management can lead to environmental and human health risks and disrupt biological balance (Turchen et al., 2016). One alternative to achieve a better cost-benefit ratio in pest control is the development of

resistant cultivars (Maluf et al., 2010).

Certain wild tomato accessions have shown promising levels of resistance to insect pests due to the presence of allelochemicals such as zingiberene (ZGB), acylsugars (AA), or 2-tridecanone (2-TD) (Maluf et al., 2007). Notable resistant accessions utilized in breeding programs include *Solanum habrochaites* var. *hirsutum* 'PI-127826' and *S. pennellii* LA-716' (Lima et al., 2016; Resende et al., 2006).

Improved lines with high levels of zingiberene (ZGB-703, ZGB-704) and acylsugars (TOM-688, TOM-687) have been obtained and used in tomato breeding programs in Brazil due to their resistance against arthropod pests such as the two-spotted spider mite (*Tetranychus urticae*, Koch 1836), whitefly (*B. tabaci*), and tomato leafminer (*Tuta absoluta*, Meyrick 1917) (Oliveira et al., 2018; Oliveira et al., 2012).

Other pest resistance mechanisms have

been evidenced in tomatoes, such as the Mi gene, which confers resistance to nematodes of the genus *Meloidogyne* spp. and was introduced into cultivated tomatoes from its parent material, *S. peruvianum* (Smith, 1944). Nombela et al. (2000) revealed that this gene, or another intricately linked to it, also confers resistance to whitefly (*B. argentifolii*) in commercial tomato cultivars (*S. lycopersicum*). Marchese (2013) observed that tomato lines carrying the Mi gene reduced whitefly oviposition and nymph survival.

The scarcity of improved lines with high levels of AA or ZGB and unavailability of lines with both high AA and high ZGB contents have resulted in limited studies on the potential synergistic effect of these two allelochemicals on resistance.

This study aimed to quantify and compare the effects of the Mi gene, elevated levels of AA (individually), high levels of ZGB (individually), and high levels of both AA and ZGB (simultaneously) on the resistance of tomato lines to the whitefly *Bemisia argentifolii*.

Material and Methods

Two tomato lines with high foliar levels of acylsugars (TOM-687, TOM-688) and two lines with high foliar levels of zingiberene (ZGB-703, ZGB-704) were previously obtained from crosses with the wild accessions *Solanum pennellii* 'LA-716' and *S. habrochaites* var. *hirsutum* 'PI-127826', respectively. 'TOM-687' and 'TOM-688' were obtained through interspecific crossing of *S. lycopersicum* x *S. pennellii* 'LA-716', followed by three backcrosses with *S. lycopersicum*, and selected based on their high acylsugar content (Gonçalves Neto et al., 2010), following the methodology proposed by Rezende et al. (2002). 'ZGB-703' and 'ZGB-704' were obtained through interspecific crossing of *S. lycopersicum* x *S. habrochaites* var. *hirsutum* 'PI-127826', followed by two backcrosses to *S. lycopersicum*, and selected based on their elevated levels of the sesquiterpene zingiberene (Maluf et al., 2001).

Lines with prominent levels of both acylsugars and zingiberene simultaneously were also obtained: ZGB-703/ZGB-704 were crossed with TOM-688/TOM-689 (the latter also having high acylsugar content), and their segregating F2 to F7 populations were selected, from 2007 to 2013, for high levels of both allelochemicals simultaneously. Two F7 lines resulting from these crosses were considered fixed for elevated levels of both acylsugars and zingiberene and designated as BPX-413E-02-161-207-31-6-20-325 and BPX-413E-02-161-207-31-6-6-367.

In addition to the mentioned genotypes, the low acylsugar and zingiberene lines TOM-584 and Santa Clara

were used, which were susceptible to nematodes (non-carriers of the Mi allele) and previously characterized as susceptible to whitefly by Resende et al. (2006). TOM-684, a line homozygous for the Mi gene that confers resistance to *Meloidogyne* spp. nematodes and has low levels of acylsugars and zingiberene, was also included (Marchese, 2013).

The BPX-413E-02-161-207-31-6-20-325 and BPX-413E-02-161-207-31-6-6-367 lines were sown in polystyrene trays and grown in a plastic greenhouse in Ijaci, MG, Brazil (21°14'16" S latitude and 45°08'00" W longitude, and 918-m altitude). They were grown alongside the control lines TOM-584, TOM-684, TOM-687, TOM-688, ZGB-703, and ZGB-704. Forty plants of each BPX-413E line and twenty plants of each control line were individually evaluated using colorimetric methodologies for zingiberene (Freitas, 1999) and acylsugars (Resende et al., 2002). Two measurements per plant were taken to confirm the presence of high levels of both allelochemicals simultaneously. Five plants from the BPX-413E-02-161-207-31-6-20-325 genotype and sixteen from the BPX-413E-02-161-207-31-6-6-367 genotype (T8 to T28, **Table 1**) were confirmed to have elevated levels and selected for further testing, totaling 21 plants. Averages of AA and ZGB contents were compared by the Duncan's test ($\alpha=0.05$) through SAS statistical package (SAS Institute, 1990). These plants were cloned by stem cutting to obtain 21 clones (named T8 to T28) (Table 1), which were used in the testing of resistance to whitefly.

The experiment consisted of the selected lines from the previous stage (T8 to T28, Table 1), cloned through rooting of axillary shoots with homogeneous sizes, and the same control lines (Santa Clara, TOM-584, TOM-684, TOM-687, TOM-688, ZGB-703, and ZGB-704) grown from seeds, totaling 28 genotypes tested for pest resistance.

For infestation with *B. argentifolii*, an insect rearing was previously established at the Vegetable Sector of UFLA (Federal University of Lavras) in a 12 m² (4.00 m x 3.00 m) screened greenhouse. The greenhouse had a chapel-style structure with a transparent plastic cover of 100 microns thickness and anti-aphid screens on the sides. Adult insects were collected from a Santa Clara tomato cultivar crop and then transferred to the screened greenhouse. Tomato plants of the Santa Clara cultivar grown in 3.5 L pots were used as substrate for egg deposition and subsequent food source for the insects.

At 20 days after transplanting the clones (previously obtained through rooting of axillary shoots) and the control lines, the genotypes were transferred to the screened greenhouse that had been previously infested with an established population of *B. argentifolii*.

Table 1. Contents of zingiberene (ZGB) and acylsugar (AA) in leaflets of tomato lines and clones, expressed as units of absorbance, coefficient of variation, and genotypic coefficient of determination. UFLA: Lavras, MG, 2013

| Treatment | ZGB | AA |
|---|----------------|----------------|
| T1=Santa Clara | Not determined | Not determined |
| T2=TOM 584 | 0.071 E | 0.204 D |
| T3=TOM 684 | 0.070 E | 0.199 D |
| T4=TOM 687 | 0.080 DE | 0.307 BC |
| T5=TOM 688 | 0.080 DE | 0.299 C |
| T6=ZGB 703 | 0.183 AB | 0.137 E |
| T7=ZGB 704 | 0.186 AB | 0.137 E |
| T8=BPX-413E-02-161-207-31-6-20-325-107 | 0.123 CD | 0.299 C |
| T9=BPX-413E-02-161-207-31-6-20-325-116 | 0.189 AB | 0.271 C |
| T10=BPX-413E-02-161-207-31-6-20-325-125 | 0.173 ABC | 0.276 C |
| T11=BPX-413E-02-161-207-31-6-20-325-128 | 0.174ABC | 0.280 C |
| T12=BPX-413E-02-161-207-31-6-20-325-129 | 0.145 BC | 0.282 C |
| T13=BPX-413E-02-161-207-31-6-6-367-102 | 0.154 BC | 0.273 C |
| T14=BPX-413E-02-161-207-31-6-6-367-107 | 0.125 BC | 0.281 C |
| T15=BPX-413E-02-161-207-31-6-6-367-111 | 0.211 A | 0.398 A |
| T16=BPX-413E-02-161-207-31-6-6-367-112 | 0.153 BC | 0.328 BC |
| T17=BPX-413E-02-161-207-31-6-6-367-113 | 0.129 CD | 0.277 C |
| T18=BPX-413E-02-161-207-31-6-6-367-114 | 0.139 BC | 0.290 C |
| T19=BPX-413E-02-161-207-31-6-6-367-115 | 0.135 BC | 0.300 C |
| T20=BPX-413E-02-161-207-31-6-6-367-126 | 0.135 BC | 0.288 C |
| T21=BPX-413E-02-161-207-31-6-6-367-127 | 0.175 ABC | 0.281 C |
| T22=BPX-413E-02-161-207-31-6-6-367-128 | 0.128 CD | 0.328 BC |
| T23=BPX-413E-02-161-207-31-6-6-367-129 | 0.127 CD | 0.365 AB |
| T24=BPX-413E-02-161-207-31-6-6-367-132 | 0.150 BC | 0.309 BC |
| T25=BPX-413E-02-161-207-31-6-6-367-133 | 0.173 ABC | 0.332 BC |
| T26=BPX-413E-02-161-207-31-6-6-367-134 | 0.153 BC | 0.324 BC |
| T27=BPX-413E-02-161-207-31-6-6-367-135 | 0.149 BC | 0.310 BC |
| T28=BPX-413E-02-161-207-31-6-6-367-137 | 0.148 BC | 0.331 BC |
| Contrasts of interest | | |
| C1= (T2-T3) | 0.00NS | 0.00NS |
| C2=(T2+T3)/2-(T4+T5)/2 | -0.002NS | -0.10* |
| C3=(T2+T3)/2 - (T6+T7)/2 | -0.11* | 0.06* |
| C4=(T4+T5)/2-(T6+T7)/2 | -0.10* | 0.16* |
| C5=(T2+T3)/2-(T8.....T28)/21 | -0.08* | -0.10G* |
| C6=(T4+T5)/2-(T8.....T28)/21 | -0.07* | -0.00NS |
| C7=(T6+T7)/2 - (T8.....T28)/21 | 0.03NS | -0.16* |

Means followed by the same letter in the column do not differ from each other, according to the Duncan's test at a 5% probability level.

The plants used as breeding grounds remained in the greenhouse; however, their irrigation was discontinued, resulting in their death.

The experiment was set up and conducted in a greenhouse at the Federal University of Lavras in 2013. A randomized complete block design (RCBD) was used, consisting of 28 treatments with four replications, and one plant was used per plot, totaling 112 plants.

After six days of infestation, while still in the vegetative stage, the genotypes were evaluated for egg deposition by counting the number of eggs. Four leaflets from the upper third of each tested plant were sampled, and a binocular stereomicroscope with a magnification of 20 to 80 times was used to assist in the counting of eggs in a 2 cm² leaf area. Twenty-three days after the infestation date, the same previously marked leaflets, identified with white adhesive tape, were evaluated

for nymph development (number of nymphs in the final instar) using the binocular stereomicroscope (Walker et al., 2010) (Maluf et al., 2010).

The data obtained were analyzed using the SAS statistical package (SAS Institute, 1990). Treatment means were compared using the Duncan test at a 5% significance level. Non-orthogonal contrasts were used to compare specific groups of treatments based on their relevance.

Results and Discussion

Significant differences at a 5% level were observed among the genotypes for zingiberene (ZGB) and acylsugar (AA) contents, as determined by the F-test (Table 1).

The genotypes TOM-584 and TOM-684, used as control lines with low levels of ZGB and AA, exhibited

similar and low concentrations of these allelochemicals and did not differ statistically from each other (Table 1, contrast C1). Any potential level of resistance to whitefly observed in TOM-684 compared to TOM-584 should therefore be attributed to the presence of the Mi gene and not to higher levels of ZGB or AA.

No detectable difference was found by the mean test in ZGB concentrations between the homozygous genotypes with high levels of AA (TOM-687 and TOM-688) and the control lines TOM-584 and TOM-684 (low levels of both ZGB and AA). However, regarding AA concentrations, the genotypes TOM-687 and TOM-688 (high AA content) showed significantly higher levels of AA compared to the control lines TOM-584 and TOM-684 (Table 1, contrast C2).

The homozygous genotypes with elevated levels of ZGB (ZGB-703 and ZGB-704) exhibited significantly higher levels of ZGB compared to the control lines TOM-584 and TOM-684. However, in terms of AA concentrations, the control lines showed higher levels. The positive value in contrast C3 (0.0645) for AA concentration indicates the average higher content of this allelochemical in the control lines compared to the ZGB-703 and ZGB-704 genotypes (Table 1, contrast C3).

Both the mean test and contrast C4 demonstrate that the ZGB content is higher in the ZGB-703 and ZGB-704 treatments compared to the TOM-687 and TOM-688 treatments. On the other hand, in terms of AA concentrations, the TOM-687 and TOM-688 treatments are significantly higher (Table 1, contrast C4).

The results presented confirm that the treatments used as high ZGB content controls (ZGB-703 and ZGB-704) indeed exhibited a high level of ZGB and a low level of AA, while the treatments used as high AA content controls (TOM-687 and TOM-688) contained a high level of AA and a low level of ZGB.

The clones T8 to T28, which were selected for high levels of both ZGB and AA, showed significantly higher levels of these allelochemicals compared to the low-level controls, TOM-584 and TOM-684 (Table 1, contrast C5).

In general, the levels of ZGB in these clones were as high as in the ZGB-703 and ZGB-704 treatments, except for the following treatments: T8, T17, T22, and T23, which had intermediate levels of ZGB but were still significantly higher than the controls TOM-584 and TOM-684. All the clones exhibited higher AA levels compared to ZGB-703 and ZGB-704 (Table 1, contrast C7). Regarding AA concentration, the clones did not differ from the controls TOM-687 and TOM-688 but showed higher ZGB levels (Table 1, contrast C6).

Response of tomato lines and clones to whitefly infestation

The presence of the allelochemicals zingiberene (ZGB) and acylsugars (AA) in the tested tomato genotypes significantly affected both the oviposition and nymph survival of *Bemisia argentifolii* (Table 2).

The homozygous genotypes with elevated levels of acylsugars, TOM-687 and TOM-688, showed lower preference (antixenosis) in terms of both oviposition and the number of nymphs when compared to the susceptible controls 'Santa Clara' and TOM-584 (Table 2, contrast C5'). This effect was also observed in the homozygous genotypes with prominent levels of ZGB, as the ZGB-703 and ZGB-704 treatments exhibited lower numbers of both eggs and nymphs (Table 2, contrast C6'). This demonstrates the clear non-preference of the pest for the lines rich in these allelochemicals, ZGB or AA, which provides greater resistance by reducing both whitefly oviposition and nymph survival (Table 2, contrasts C5' and C6').

The susceptible controls, 'Santa Clara' and TOM-584, showed similar resistance reactions in terms of antixenosis to the insect, with no significant difference between them. They had the highest oviposition and nymph numbers among the tested genotypes (Table 2, contrast C1'). There was no difference between the ZGB-rich treatments (ZGB-703 and ZGB-704) and the AA-rich treatments (TOM-687, TOM-688), indicating that both ZGB and AA provide similar levels of antixenotic resistance to whiteflies in tomatoes. Comparable results were reported by Neiva et al. (2013), who noted that the allelochemicals ZGB, AA, and 2-tridecanone, at the levels found in the tested lines, were similarly efficient in reducing oviposition of *B. argentifolii*. Likewise, Oliveira et al. (2012) reported that Zingiberene, acylsugar, and 2-tridecanone were also equally effective in reducing oviposition in tomato moth, promoting repellency against the mite of the genus *Tetranychus urticae*, as discussed by Oliveira et al. (2018). Moreover, there are many studies in the literature that demonstrate the resistance conferred by ZGB or AA alone (Oliveira et al., 2018; Neiva et al., 2013; Oliveira et al., 2012; Maciel et al., 2011; Gonçalves Neto et al., 2010; Maluf et al., 2007).

The TOM-684 treatment, carrying the Mi gene that confers resistance to nematodes, showed less preference by the whitefly compared to the susceptible controls 'Santa Clara' and TOM-584. Although these three treatments had low levels of AA and ZGB, the one carrying the Mi gene (TOM-684) was less susceptible to whitefly infestation (Table 2, Contrast C3). However, when comparing the means of the homozygous treatments for

Table 2. Average number of eggs and nymphs/2 cm² of leaf area, measured at 6 and 23 days, respectively, after infestation with *Bemisia argentifolii* in the upper third of the plant. Lavras, 2013

| Treatment | Number of eggs /2 cm ² of leaflet | Number of nymphs /2 cm ² of leaflet |
|--|---|---|
| T1=Santa Clara | 42.43 H | 31.00 I |
| T2=TOM 584 | 41.18 H | 30.50 I |
| T3=TOM 684 | 34.06 G | 25.18H |
| T4=TOM 687 | 20.56 EF | 15.12G |
| T5=TOM 688 | 20.81 F | 15.31G |
| T6=ZGB 703 | 18.18 BCDEF | 14.18FG |
| T7=ZGB 704 | 16.68 BCDEF | 13.56EFG |
| T8=BPX-413E-02-161-207-31-6-20-325-107 | 17.56 BCDEF | 13.50 EFG |
| T9=BPX-413E-02-161-207-31-6-20-325-116 | 16.37 BCDEF | 10.43ABCDEF |
| T10=BPX-413E-02-161-207-31-6-20-325-125 | 15.25 ABCDE | 10.31 ABCDEF |
| T11=BPX-413E-02-161-207-31-6-20-325-128 | 14.93 ABCD | 10.00 ABCDE |
| T12=BPX-413E-02-161-207-31-6-20-325-129 | 15.75 BCDEF | 11.50 ABCDEFG |
| T13=BPX-413E-02-161-207-31-6-6-367-102 | 15.06 ABCD | 10.75 ABCDEF |
| T14=BPX-413E-02-161-207-31-6-6-367-107 | 19.18 DEF | 12.56 DEFG |
| T15=BPX-413E-02-161-207-31-6-6-367-111 | 10.17 A | 7.56 A |
| T16=BPX-413E-02-161-207-31-6-6-367-112 | 15.18 ABCDE | 8.93 ABCD |
| T17=BPX-413E-02-161-207-31-6-6-367-113 | 18.31 CDEF | 12.93 DEFG |
| T18=BPX-413E-02-161-207-31-6-6-367-114 | 15.37 ABCDE | 12.62 DEFG |
| T19=BPX-413E-02-161-207-31-6-6-367-115 | 16.37 BCDEF | 12.3CDEFG |
| T20=BPX-413E-02-161-207-31-6-6-367-126 | 15.87 BCDEF | 11.75 BCDEFG |
| T21=BPX-413E-02-161-207-31-6-6-367-127 | 13.50 ABC | 11.50 ABCDEFG |
| T22=BPX-413E-02-161-207-31-6-6-367-128 | 13.56 ABC | 11.91 BCDEFG |
| T23=BPX-413E-02-161-207-31-6-6-367-129 | 13.37 ABC | 10.37 ABCDEF |
| T24=BPX-413E-02-161-207-31-6-6-367-132 | 12.75 AB | 10.00 ABCDE |
| T25=BPX-413E-02-161-207-31-6-6-367-133 | 12.81 ABC | 8.18AB |
| T26=BPX-413E-02-161-207-31-6-6-367-134 | 15.00 ABCD | 8.43ABC |
| T27=BPX-413E-02-161-207-31-6-6-367-135 | 13.68 ABC | 11.00 ABCDEF |
| T28=BPX-413E-02-161-207-31-6-6-367-137 | 12.93 ABC | 10.81 ABCDEF |
| Contrasts of interest | | |
| C1'=T1-T2 | 1.25 ns | 0.50ns |
| C2'=(T1+T2)/2 - T3 | 7.75 * | 5.56* |
| C3'=T4-T5 | -0.25 ns | -0.18ns |
| C4'=T6-T7 | 1.50 ns | 0.62ns |
| C5'=(T1+T2)/2 - (T4+T5)/2 | 21.12 * | 15.53* |
| C6'=(T1+T2)/2 - (T6+T7)/2 | 24.37 * | 16.87* |
| C7'=(T1+T2)/2 - (T8+T9+T10+.... +T28)/21 | 26.90 * | 19.91* |
| C8'=(T4+T5)/2 - (T8+T9+T10+.... +T28)/21 | 5.78 * | 4.38* |
| C9'=(T6+T7)/2 - (T8+T9+T10+.... +T28)/21 | 2.53 * | 3.042* |
| CV% | 17.65 | 18.10 |

* Means followed by the same letter in the column do not differ from each other, according to the Duncan's test ($P < 0.05\%$).

ZGB or AA (TOM-687, TOM-688, ZGB-703, and ZGB-704) with the TOM-684 treatment, it becomes evident that the level of antixenotic resistance mediated by acylsugars or zingiberene is higher than the resistance level conferred by the Mi gene. These findings are consistent with those of Marchese (2013), who evaluated resistance to whiteflies mediated by acylsugars and the Mi gene in tomato plants. These authors also observed a reduction of 18.78% and 21.39% in the number of nymphs per leaflet in the Mi gene-carrying lines (TOM-684 and TOM-598), compared to the commercial control 'Santa Clara', indicating some level of resistance. However, the level of whitefly resistance conferred by high levels of acylsugars was higher than that conferred by the presence of the

Mi gene. Nombela et al. (2003) also reported resistance mediated by the presence of the Mi gene in tomato plants, with a reduction of 50% in the average number of nymphs per leaflet compared to plants without the resistance allele. In this study, the reduction in the number of nymphs in the TOM-684 treatment, carrying the Mi gene, was approximately 20% compared to 'Santa Clara' and TOM-584, which do not have the Mi gene, similar to the findings of Marchese (2013).

The BPX-413E clones, selected for elevated levels of both ZGB and AA, showed a lower number of eggs and nymphs compared to the susceptible controls 'Santa Clara' and TOM-584 (Table 2, Contrast C7'). They exhibited even greater resistance to whiteflies than the TOM-684

treatment, carrying the Mi gene, indicating that the resistance conferred by the presence of the Mi gene is of lower magnitude than that conferred by prominent levels of allelochemicals, either individually or in combination.

Comparisons of the clones T8 to T28 with the TOM-687 and TOM-688 treatments, homozygous for AA, showed superior resistance in the clones (Table 2). This is also true when comparing these clones to the treatments rich only in ZGB, ZGB-703, and ZGB-704. Among the 21 selected clones, 11 (T11, T13, T15, T21, T22, T23, T24, T25, T26, T27, T27) had significantly lower oviposition than TOM-687 (the standard resistant line), while the others did not differ from it. In terms of nymph count, it is also observed that all genotypes from T8 to T28 had lower or at least equal numbers to the resistant controls TOM-687, TOM-688, ZGB 703, ZGB 704. Differences detected through contrasts (Table 2, Contrast C8' and C9') confirm the higher average level of resistance of the clones compared to these controls (TOM-687, TOM-688, ZGB 703, ZGB 704), both in oviposition and nymph count.

The data obtained here differs from the conclusions of Silva et al. (2009), who reported no synergistic effect between zingiberene and acyl sugars in the double heterozygous genotypes regarding the levels of resistance to whitefly. In the present assay, the reductions in the number of eggs and nymphs of the clones, compared to the average of the TOM-687/TOM-688 lines, were 27.97% and 28.80%, respectively; compared to the average of ZGB-703/ZGB-704, the reductions were 14.51% and 20.69%, respectively, indicating a synergistic effect of the presence of both allelochemicals in the same lineage. Silva et al. (2009) did not detect synergistic effects of the simultaneous presence of ZGB and AA in the leaflets compared to the presence of ZGB or AA alone. However, the effects measured in the present study represent levels of AA and ZGB for genotypes with loci in homozygosity for the presence of both allelochemicals, while those of Silva et al. (2009) represent AA and ZGB for loci in heterozygosity.

Maluf et al. (2010) demonstrated the existence of a synergistic effect of AA and ZGB in resistance to the tomato leafminer. Greater resistance to the tomato leafminer was found in genotypes that were double heterozygous for both AA and ZGB, compared to genotypes that were heterozygous for AA only or heterozygous for ZGB only.

The results obtained indicate that there is an advantage in selecting genotypes with high levels of both ZGB and AA simultaneously, as their resistance to *B. argentifolii* is of a higher degree compared to

genotypes that are homozygous for only one of these allelochemicals.

The presence of either one or both allelochemicals was sufficient to reduce oviposition compared to susceptible controls, indicating their effectiveness (Table 2, Contrasts C5', C6', C7', C8', and C9'). However, genotypes rich in both allelochemicals simultaneously induce a higher degree of resistance and, as suggested by Silva et al. (2009), possibly function as a more effective barrier against pest arthropod biotypes that may overcome resistance mediated by only one of the allelochemicals alone.

Conclusions

Zingiberene (ZGB) and acylsugars (AA) are equally efficient in promoting resistance against whiteflies.

The resistance conferred by the allelochemicals, ZGB and AA, is of a higher level than that conferred by the Mi gene against whiteflies.

Zingiberene (ZGB) and acylsugars (AA), when combined in tomato lines, show a synergistic effect in enhancing resistance against whiteflies compared to using genotypes with elevated levels of ZGB and AA individually.

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References

- Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J.J., MacFarlane, S., Peters, D., Susi, P., Torrance, L. 2013. Status and prospects of plant virus control through interference with vector transmission. *Annual Review of Phytopathology* 51: 177-201.
- Freitas, J.A. 1999. *Resistência genética de tomateiro *Lycopersicon* spp. à mosca-branca *Bemisia* spp. mediada por zingibereno contido em tricomas glandulares*. 93 f. (Tese de Doutorado) - Universidade Federal de Lavras, Lavras, Brasil.
- Gonçalves Neto, A.C., Silva, V.F., Maluf, W.R., Maciel, G.M., Nizio, D.A.C., Gomes, L.A.A., Azevedo, S.M. 2010. Resistência à traça-do-tomateiro em plantas com altos teores de acilaçúcares nas folhas. *Horticultura Brasileira* 28: 203-208.
- Lima, I.P., Resende, J.T.V., Oliveira, J.R.F., Faria, M.V., Dias, D.M., Resende, N.C.V. 2016. Selection of tomato genotypes for processing with high zingiberene content,

resistant to pests. *Horticultura Brasileira* 34: 387-391.

Maciel, G.M., Maluf, W.R., Silva, V.F., Gonçalves Neto, A.C., Gomes, L.A.A. 2011. Híbridos pré-comerciais resistentes a *Tuta absoluta* obtidos de linhagem de tomateiro rica em acilacúcares. *Horticultura Brasileira* 29: 151-156.

Maluf, W.R., Campos, G.A., Cardoso, M.G. 2001. Relationships between trichome types and spider mite (*Tetranychus evansi*) repellence in tomatoes with respect to foliar zingiberene contents. *Euphytica* 121: 73-80.

Maluf, W.R., Inoue, I.F., Ferreira, R.P.D., Gomes, L.A.A., Castro, E.M., Cardoso, M.G. 2007. Higher glandular trichome density in tomato leaflets and repellence to spider mites. *Pesquisa agropecuária brasileira* 42: 1227-1235.

Maluf, W.R., Maciel, G.M., Gomes, L.A.A., Cardoso, M.G., Gonçalves, L.D., Silva, E.C., Knapp, M. 2010. Broad-Spectrum Arthropod Resistance in Hybrids between High- and Low-Acylsugar Tomato Lines. *Crop Science* 50: 439-450.

Marchese, A. 2013. *Resistência à Mosca-Branca e ao Ácaro-Rajado Mediada por Acilacúcares e pelo Gene Mi em Tomateiro*. 63 f. (Tese de Doutorado) - Universidade Federal de Lavras, Lavras, Brasil.

Neiva, I.P., Andrade Júnior, V.C., Maluf, W.R., Oliveira, C.M., Maciel, G.M. 2013. Role of allelochemicals and trichome density in the resistance of tomato to whitefly. *Ciência e Agrotecnologia* 37: 61-67.

Nombela, G., Beitia, F., Muñoz, M. 2000. Variation in tomato host response to *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to acyl sugar content and presence of the nematode and potato aphid resistance gene *Mi*. *Bulletin of Entomological Research* 90: 161-167.

Nombela, G., Williamson, V.M., Muniz, M. 2003. The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. *Molecular Plant-Microbe Interactions* 16: 645-649.

Oliveira, C.M., Andrade Júnior, V.C., Maluf, W.R., Neiva, I.P., Maciel, G.M. 2012. Resistance of tomato trains to the moth *tuta absoluta* imparted by allelochemicals and trichome density. *Ciência e Agrotecnologia* 36: 45-52.

Oliveira, C.M., Maluf, W.R., Marchese, A., Lasmar, A., Andrade, T.M., Menino, G.C.O., Azevedo, A.M. 2018. Reppelency to spider mite mediated by the gene *mi* and by the synergism between high foliar contents of acylsugar and zingiberene in tomato. *Bioscience Journal* 34: 1532-1539.

Queiroz, P.R., Martins, E.S., Klautau, N., Lima, L., Praça, L., Monnerat, R.G. 2016. Identification of the B, Q, and native Brazilian biotypes of the *Bemisia tabaci* species complex using Scar markers. *Pesquisa Agropecuária Brasileira* 51: 555-562.

Resende, J.T.V., Maluf, W.R., Cardoso, M.G., Nelson, D.L., Faria, M.V. 2002. Inheritance of acylsugar contents in tomatoes derived from an interspecific cross with the wild tomato *Lycopersicon pennellii* and their effect on spider

mite repellence. *Genetics and Molecular Research* 1: 106-116.

Resende, J.T.V., Maluf, W.R., Faria, M.V., Pfann, A.Z., Nascimento, I.R. 2006. Acylsugars in tomato leaflets confer resistance to the south american tomato pinworm, *Tuta absoluta* Meyr. *Scientia Agricola* 63: 20-25.

SAS Institute. 1990. SAS/STAT User's Guide. *SAS Institute* 6: 1-2.

Shadmany, M., Omar, D., Muhamad, R. 2015. Biotype and insecticide resistance. *Applied Entomology* 139: 67-75.

Silva, V.F., Maluf, W.R., Cardoso, M.G., Neto, A.C.G., Maciel, G.M., Nízio, D.A.C., Silva, V.A. 2009. Resistência mediada por aleloquímicos de genótipos de tomateiro à mosca-branca e ao ácaro-rajado. *Pesquisa agropecuária brasileira* 44: 1262-1269.

Smith, P.G. 1944. Embryo culture of a tomato species hybrid. *Proceedings of the American Society of Horticultural Science* 44: 413-416.

Turchen, L.M., Golin, V., Butnariu, A.R., Guedes, R.N.C., Pereira, M.J.B. 2016. Lethal and sublethal effects of insecticides on the egg parasitoid *Telenomus podisi* (Hymenoptera: Platygasteridae). *Journal of Economic Entomology* 109: 82-94.

Zaidi, S.S.E.A., Briddon, R.W., Mansoor, S. 2017. Engineering dual begomovirus *bemisia tabaci* resistance in plants. *Trends Plant Sci.* 22: 6-8.

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