

REPPELENCY TO SPIDER MITE MEDIATED BY THE GENE *Mi* AND BY THE SYNERGISM BETWEEN HIGH FOLIAR CONTENTS OF ACYLSUGAR AND ZINGIBERENE IN TOMATO

REPELÊNCIA AO ÁCARO-RAJADO MEDIADA PELO GENE *Mi* E PELO SINERGISMO ENTRE ALTOS TEORES FOLIARES DE ACILAÇÚCARES E DE ZINGIBERENO EM TOMATEIRO

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ABSTRACT: Tomato fruits (*Solanum Lycopersicum*) are intended for human consumption in its *in natura* or industrially processed form. However, the expansion of its cultivation area has favored the emergence of pests, such as spider mite (*Tetranychus urticae*), which significantly affects the production. The objective of this study was to quantify and evaluate the repellency of tomato lines to spider mite in function of the gene *Mi* and of the individual and synergistic effects of acylsugar and zingiberelene allelochemicals. The experiment consisted of a complete randomized design with four replications. For the bioassay, four fully expanded leaflets with similar size were removed from the upper third of the plants at pre-flowering phenological stage. The bioassay was carried out in a cold chamber, at 16±1°C, and 64 ± 4% RH. The gene *Mi* was not effective in conferring repellency to spider mite. On the other hand, zingiberene and acylsugar were efficient and equivalent regarding repellency to spider mite. When combined in tomato lines, zingiberene and acylsugar had synergistic effect, which increased repellency to spider mite.

KEYWORDS: *Tetranychus urticae*. *Solanum lycopersicum*. Breeding

INTRODUCTION

Tomato (*Solanum lycopersicum*) is grown worldwide. In Brazil, it is cultivated in about 71,000 hectares, with approximately yield of 4.5 million tons (FAO, 2013). The culture is considered as of high risk due to several health problems, especially the high degree of pest infestation (SUINAGA et al., 2003).

Among tomato arthropod-pests, mites of the *Tetranychus* genus stand out for causing drought in leaves, followed by defoliation, reduction in size and number of fruits, and early ripening (FLECHTMANN; BAKER, 1970). The *Tetranychus* genus comprises several species; however, only three are important for tomato culture: the spider mite (*T. urticae* Koch.), and the red spider mites *T. ludeni* Zacher and *T. evansi* Baker Pritchard. Of these three species, spider mite is the most relevant in Brazil (FLECHTMANN, 1989).

Mites in general, including the *Tetranychus* genus, have high capacity of population increase,

reaching 20-25 generations per year (LARA, 1991). Consequently, the effective control of this pest is necessary in order to keep the population below the economic injury level. Currently, chemical control is the most common pest control; however, it is not so effective due to the ability of mites to develop resistance against several groups of acaricides. This resistance probably occurs due to the use of not recommended doses and to the high frequency of spraying (Lara, 1991), which also causes problems to the environment and to human health. Therefore, the development of resistant cultivars is of great importance, since it has permanent effect on the pest population, and does not directly affect their natural enemies, which consequently avoids the use of acaricides (LARA, 1991).

In Brazil, tomato breeding programs aimed at resistance to pests are based on the selection of genotypes with high foliar contents of allelochemicals associated with resistance. Among these allelochemicals, acylsugar and zingiberene stand out for conferring resistance to *Tetranychus urticae*, *Tuta absoluta*, and *Bemisia argentifolii*

(MALUF et al., 2001; GONÇALVES et al., 2006; PEREIRA et al., 2008; RESENDE et al., 2006; RESENDE et al., 2008; SILVA et al., 2009; MACIEL et al., 2011; BLEEKERA et al., 2012; OLIVEIRA et al., 2012; NEIVA et al., 2013;). Thus, the development of lines with high zingiberene or acylsugar content, and which are resistant to arthropods-pests has increased (SILVA et al., 2009; MALUF et al., 2010; OLIVEIRA et al., 2012).

Although high zingiberene and acylsugar contents are important in the obtainment of plants resistant to pests, little is known in relation to the effects on spider mite and to the synergistic effect of these two allelochemicals. According to Marchese (2013), the incorporation of two or more allelochemicals may increase the spectrum of resistance to pests, making it more difficult to be broken. The effect of the gene *Mi* on mite is another key information not found in the literature. The gene *Mi* confers resistance to nematodes of the *Meloidogyne* spp. genus, which was introduced in tomato plants cultivated from *S. peruvianum* species (SMITH, 1944).

Thus, the objective of this study was to evaluate the repellency of tomato lines to spider mite in function of the presence of the gene *Mi*, and the individual and synergistic effects of acylsugar and zingiberene allelochemicals.

MATERIAL AND METHODS

Two tomato lines with high foliar acylsugar content (TOM-687, TOM-688) and two tomato lines with high foliar zingiberene content (ZGB-703, ZGB-704) were obtained from crosses between cultivated tomatoes (*S. lycopersicum*) with the wild accessions *Solanum pennellii* 'LA-716' and *S. habrochaites* var. *hirsutum* 'PI-127826'. 'TOM-687' and 'TOM-688' were obtained from the interspecific cross *S. lycopersicum* x *S. pennellii* 'LA-716', followed by three backcrosses with *S. lycopersicum*, and selected based on their high acylsugar content (PEREIRA et al., 2008; GONÇALVES NETO et al., 2010), according to the methodology proposed by Rezende et al. (2002). 'ZGB-703' and 'ZGB-704' were obtained from the interspecific crosses *S. lycopersicum* x *S. habrochaites* var. *hirsutum* 'PI-127826', followed by two backcrosses with *S. lycopersicum*, and selected based on their high zingiberene sesquiterpene content (GONÇALVES et al., 2006).

Lines with high acylsugar and zingiberene contents were also obtained by crossing the lines

ZGB-703/ZGB-704 and TOM-688/TOM-689 (the latter also presents high acylsugar content). Their segregating populations from F2 to F7 generations were selected for high contents of both allelochemicals. Two F7 populations of these crosses were considered fixed for high contents of both acylsugar and zingiberene, and were designated as BPX-413E-02-161-207-31-6-20-325 and BPX-413E-02-161-207-31-6-6-367. Individual plants of these F7 populations were selected for good agronomic traits (especially size and fruit quality) and cloned via stem cuttings. Clones (T8 to T34, Table 1) were used in repellency tests to spider mite *Tetranychus urticae*. Three lines susceptible to *T. urticae* and with low contents of both acylsugar and zingiberene were also used (RESENDE et al., 2006; MARCHESE, 2013), being two of them ('Santa Clara' and TOM-584) susceptible to nematodes (not carrying the *Mi* allele), and one resistant to nematodes (TOM-684) (carrying the *Mi* allele in homozygosity) (MARCHESE, 2013).

The populations BPX-413E-02-161-207-31-6-20-325 and BPX-413E-02-161-207-31-6-6-367 were sown in polystyrene trays and cultivated in plastic greenhouse, in the municipality of Ijaci, MG (lat. 21°14'16" S, long. 45°08'00" W, at 918 m), together with the lines TOM 584, TOM-684, TOM-687, TOM-688, ZGB-703 and ZGB-704. Forty plants of each BPX-413E populations, and 20 plants of each control lines were evaluated individually, according to the colorimetric methods described by de Freitas (1999) for zingiberene, and by Resende et al. (2002) for acylsugar, in order to confirm the highest contents of the two allelochemicals simultaneously.

Eight plants of the BPX-413E-02-161-207-31-6-20-325 population and 17 of the BPX-413E-02-161-207-31-6-6-367 population (named from T8 to T34 in Table 1) were selected for testing, totaling 25 plants. Plants T8 to T34 (Table 1) were cloned by stem cutting for repellence tests to spider mite (*Tetranychus urticae*). Repellency to spider mite was measured using the bioassay proposed by Weston and Snyder (1990).

The experiment consisted of a complete randomized design with four replications. Four expanded leaflets of similar size were removed from the upper third of the plants at pre-flowering phenological stage. Mites were collected from tomato and green bean plants, obtained by means of the rearing and maintenance of the Acarology Laboratory of the Regional EPAMIG of South of Minas Gerais, located in Lavras, Brazil.

Table 1. Description of zingiberene content (ZGB), acylsugar content (AA) and presence of the gene *Mi* in 34 tomato genotypes. UFLA: Lavras, 2014.

Id.	Treatments	AA content	ZGB content	Presence of the <i>Mi</i> allele
T1	Santa Clara	-	-	-
T2	TOM 584	-	-	-
T3	TOM 684	-	-	+
T4	TOM 687	+	-	-
T5	TOM 688	+	-	-
T6	ZGB 703	-	+	-
T7	ZGB 704	-	+	-
T8	BPX-413E-02-161-207-31-6-20-325-107	+	+	-
T9	BPX-413E-02-161-207-31-6-20-325-116	+	+	-
T10	BPX-413E-02-161-207-31-6-20-325-125	+	+	-
T11	BPX-413E-02-161-207-31-6-20-325-128	+	+	-
T12	BPX-413E-02-161-207-31-6-20-325-129	+	+	-
T13	BPX-413E-02-161-207-31-6-6-367-102	+	+	-
T14	BPX-413E-02-161-207-31-6-6-367-107	+	+	-
T15	BPX-413E-02-161-207-31-6-6-367-111	+	+	-
T16	BPX-413E-02-161-207-31-6-6-367-112	+	+	-
T17	BPX-413E-02-161-207-31-6-6-367-113	+	+	-
T18	BPX-413E-02-161-207-31-6-6-367-114	+	+	-
T19	BPX-413E-02-161-207-31-6-6-367-115	+	+	-
T20	BPX-413E-02-161-207-31-6-6-367-126	+	+	-
T22	BPX-413E-02-161-207-31-6-6-367-128	+	+	-
T23	BPX-413E-02-161-207-31-6-6-367-129	+	+	-
T24	BPX-413E-02-161-207-31-6-6-367-132	+	+	-
T25	BPX-413E-02-161-207-31-6-6-367-133	+	+	-
T26	BPX-413E-02-161-207-31-6-6-367-134	+	+	-
T27	BPX-413E-02-161-207-31-6-6-367-135	+	+	-
T28	BPX-413E-02-161-207-31-6-6-367-137	+	+	-
T29	BPX-413E-02-161-207-31-6-20-325-104	+	+	-
T30	BPX-413E-02-161-207-31-6-20-325-110	+	+	-
T31	BPX-413E-02-161-207-31-6-20-325-123	+	+	-
T32	BPX-413E-02-161-207-31-6-20-325-124	+	+	-
T33	BPX-413E-02-161-207-31-6-6-367-101	+	+	-
T34	BPX-413E-02-161-207-31-6-6-367-104	+	+	-

- Low content; + high content

The bioassay was carried out in a cold chamber, at $16 \pm 1^\circ\text{C}$ and $64 \pm 4\%$ RH. Leaflets of each genotype were fixed with a metallic thumbtack (9 mm diameter) in the central region of the abaxial leaf surface, in a sulphite paper sheet, on a styrofoam tray, on which they were randomly distributed. Five female mites were taken from the rearing and transferred to the center of each thumbtack with the aid of a thin brush. The mean distance traveled by mites (in mm) on the surface of each leaflet were measured from the center of the

thumbtack after 20, 40 and 60 minutes. Smaller distances travelled by mites were considered indicative of higher levels of repellency. The distance traveled by the mites which remained on the thumbtack was considered zero.

Data were analyzed using the SAS statistical package (SAS Institute, 1990), and treatment means were compared by the Duncan test. Non-orthogonal contrasts were used for contrasts of interest among treatment groups (Table 2) and tested by the t test, at 5% nominal significance.

Table 2. Description of contrasts of interest used for contrasts between genotypes and/or groups of genotypes with different zingiberene contents (ZGB) and acylsugar contents (AA). UFLA: Lavras, 2014.

Id.	Estimated contrasts	Description
C1	T1-T2	Controls susceptible to nematodes vs low AA and ZGB contents
C2	T1- T3	Control susceptible to nematodes (low AA and low ZGB) vs control resistant to nematodes (low AA and low ZGB), carrying the gene <i>Mi</i>
C3	T2-T3	Control susceptible to nematodes (low AA and low ZGB) vs control resistant to nematodes (low AA and low ZGB), carrying the gene <i>Mi</i>
C4	T4-T5	Between lines with high AA content (both susceptible to nematodes)
C5	T6-T7	Between lines with high ZGB content (both susceptible to nematodes)

C6	$(T1+T2)/2 - (T4+T5)/2$	Effect of high AA content (alone)
C7	$(T1+T2)/2 - (T6+T7)/2$	Effect of high ZGB content (alone)
C8	$(T1+T2)/2 - (T8+T9+....+T34)/27$	Controls susceptible to nematodes (low AA and low ZGB) vs BPX-413 genotype = effect of high AA content and high ZGB content, simultaneously, compared with controls with low AA + low ZGB
C9	$(T4+T5)/2 - (T8+T9+....+T34)/27$	Controls with high AA and low ZGB vs BPX-413 lines = effect of high AA content and high ZGB content, simultaneously, compared with the controls with high AA
C10	$(T6+T7)/2 - (T8+T9+....+T34)/27$	Control with high AA and high ZGB vs BPX-413 lines = effect of high AA content+ high ZGB content, simultaneously, compared with the controls with high ZGB

RESULTS AND DISCUSSION

The genotypes tested presented significant differences by the F test at 5% probability in relation to repellency to spider mite in the measured times (20, 40 and 60 minutes) (Table 3). Homozygous genotypes with high AA content (TOM-687 and TOM-688), and homozygous genotypes with high ZGB content (ZGB-703 and ZGB-70) (Table 1) presented relatively short distance travelled by mites on the leaflets (Table 3), at all evaluation times, when compared with

controls with low ZGB and AA contents ('Santa Clara' and TOM 584). This indicates the efficiency of both allelochemicals acting alone in repellency to mite (Table 3 and Table 4, C6 and C7 contrasts). Controls susceptible to nematodes and with low allelochemicals content ('Santa Clara' and TOM-584) did not differ in relation to the distance travelled by mites in the first 20 minutes; subsequently, after 40 and 60 minutes, Santa Clara genotype presented travelled distances slightly greater than that of TOM-584 (Table 3 and Table 4, contrast C1).

Table 3. Mean distance travelled by mites on the leaflets surface, UFLA, Lavras, MG, 2014.

Id.	Treatments	Distance traveled by the mite on the leaflet surface (mm)		
		20 min*	40 min*	60 min*
T1	Santa Clara	21,00g	29,60 j	35,600k
T2	TOM 584	20,00g	25,20 i	29,250j
T3	TOM 684	18,200g	24,75i	27,750j
T4	TOM 687	9,75f	14,20h	18,900i
T5	TOM 688	8,40def	14,15h	18,950i
T6	ZGB 703	9,05ef	13,25fgh	18,550i
T7	ZGB 704	8,25def	12,90efgh	18,400hi
T8	BPX-413E-02-161-207-31-6-20-325-107	8,60def	11,75defgh	15,00defghi
T9	BPX-413E-02-161-207-31-6-20-325-116	6,70bcdef	12,70efgh	15,400efghi
T10	BPX-413E-02-161-207-31-6-20-325-125	4,65abcd	8,55abcdef	13,00bcdefg
T11	BPX-413E-02-161-207-31-6-20-325-128	6,60bcdef	11,65cdefgh	14,60defghi
T12	BPX-413E-02-161-207-31-6-20-325-129	7,85cdef	14,35h	13,30bcdefgh
T13	BPX-413E-02-161-207-31-6-6-367-102	4,80abcd	10,75bcdefgh	10,80abcde
T14	BPX-413E-02-161-207-31-6-6-367-107	8,00def	13,75gh	12,95bcdefg
T15	BPX-413E-02-161-207-31-6-6-367-111	1,90 a	4,40 a	6,90a
T16	BPX-413E-02-161-207-31-6-6-367-112	5,25bcdef	6,90abcde	8,40ab
T17	BPX-413E-02-161-207-31-6-6-367-113	7,15cdef	13,50gh	13,25bcdefgh
T18	BPX-413E-02-161-207-31-6-6-367-114	6,85cdef	10,95cdefgh	11,85bcdef
T19	BPX-413E-02-161-207-31-6-6-367-115	6,05bcdef	10,60bcdefgh	13,35bcdefgh
T20	BPX-413E-02-161-207-31-6-6-367-126	8,15def	10,60bcdefgh	11,20bcdef
T22	BPX-413E-02-161-207-31-6-6-367-128	2,75ab	5,75 ab	8,00ab
T23	BPX-413E-02-161-207-31-6-6-367-129	8,50def	12,00defgh	16,25fghi
T24	BPX-413E-02-161-207-31-6-6-367-132	8,35def	13,40fgh	14,45cdefghi
T25	BPX-413E-02-161-207-31-6-6-367-133	7,45cdef	10,35bcdefgh	14,35cdefghi
T26	BPX-413E-02-161-207-31-6-6-367-134	7,20cdef	12,55efgh	12,95bcdefg
T27	BPX-413E-02-161-207-31-6-6-367-135	7,60cdef	12,25efgh	12,50bcdefg

T28	BPX-413E-02-161-207-31-6-6-367-137	6,85cdef	11,65cdefgh	12,85bcdefg
T29	BPX-413E-02-161-207-31-6-20-325-104	8,00def	14,95h	16,60fghi
T30	BPX-413E-02-161-207-31-6-20-325-110	7,50cdef	12,50efgh	15,00defghi
T31	BPX-413E-02-161-207-31-6-20-325-123	5,00bcdef	7,90abcde	9,850abcd
T32	BPX-413E-02-161-207-31-6-20-325-124	3,70abc	8,15abcdef	9,15abc
T33	BPX-413E-02-161-207-31-6-6-367-101	7,75cdef	12,70efgh	17,65ghi
T34	BPX-413E-02-161-207-31-6-6-367-104	6,30bcdef	6,55abc	8,25ab

* Significant by the F test at 5% significance level. Means followed by the same letter in the column do not differ by the Duncan test ($P < 0,05\%$).

The presence of the gene *Mi* in TOM-684 line did not decrease or annulled the distance travelled by the mite, since its effects were similar to those presented by the susceptible line TOM-584 in the measured times. The fact that TOM-584 and TOM-684 genotypes have the same behavior indicates that the gene *Mi* is not effective in conferring repellency to spider mite (Table 3). And, similarly to TOM-584, TOM-684 also did not differ from 'Santa Clara' until 20 minutes of evaluation (Table 3 and Table 4, C2 and C3 contrasts).

The repellency conferred by the genotypes rich in AA or ZGB to spider mite of the *Tetranychus urticae* genus in all measured times surpassed all controls, including TOM 684 (Table 3). In a similar study, Marchese (2013) did not observe differences between the susceptible controls, whether or not they carried the gene *Mi*. On the other hand, Godzina et al. (2011) reported slow development of mites in relation to their development in tomato plants in the presence of the gene *Mi*. Under field conditions, the resistance conferred by the gene *Mi* was incomplete dominance type, and the

heterozygote presented intermediate resistance level.

The introduction of the gene *Mi* in tomato lines can be considered as a great advantage in providing to the plant a new resistance mechanism against the attack of nematodes of the *Meloidogyne* genus. Associated with high AA or ZGB contents, gene *Mi* could enhance the spectrum of resistance to other pests.

Homozygous genotypes rich in AA (TOM-687 and TOM-688) presented no significant differences between each other, and numerically, they were very similar at all evaluation times (Table 3 and Table 4, contrast C4). Genotypes rich in ZGB (ZGB 703 and ZGB 704) also did not differ during evaluations (Table 3 and Table 4, contrast C5). There were no differences between the genotypes rich in AA and the genotypes rich in ZGB (Table 3), indicating similar effect of the allelochemicals in preventing or decreasing the distance traveled by the mite in the leaflet, and these results were also found by other authors (OLIVEIRA et al., 2012; NEIVA et al., 2013).

Table 4. Contrasts of interest used to compare genotype and/or groups of genotypes with different zingiberene content (ZGB) and acylsugar content (AA). UFLA: Lavras, 2014.

Id.	Contrast of interest	Estimates		
		20 min	40 min	60 min
C1	T1-T2	1,00 ^{ns}	4,40*	6,35*
C2	T1-T3	2,80 ^{ns}	4,85*	7,85*
C3	T2-T3	1,80 ^{ns}	0,45 ^{ns}	1,50 ^{ns}
C4	T4-T5	1,35 ^{ns}	0,05 ^{ns}	-0,05 ^{ns}
C5	T6-T7	0,80 ^{ns}	0,35 ^{ns}	0,15 ^{ns}
C6	(T1+T2)/2-(T4+T5)/2	11,42*	13,22*	13,50*
C7	(T1+T2)/2-(T6+T7)/2	11,85*	14,32*	13,95*
C8	(T1+T2)/2-(T8+T9+T10+...+T34)/27	13,98*	16,58*	19,81*
C8	(T4+T5)/2-(T8+T9+T10+...+T34)/27	2,55*	3,36*	6,31*
C10	(T6+T7)/2-(T8+T9+T10+...+T34)/27	2,13*	2,26*	5,86*

^{ns} and * not significant and significant by the t test at 5% probability, respectively.

The distance travelled by the mites in BPX413E clones selected for high acylsugar and zingiberene contents was shorter in relation to controls with low allelochemicals content ('Santa Clara' and TOM 584) in all the measured times (Table 4, C8). This result was expected when

compared with clones and with TOM-684 line (carrying the gene *Mi*), since this line (TOM-684) has the same resistance level as TOM-584, as discussed above. Thus, the gene *Mi* present in TOM 684 is not effective in repelling mite; however, it is

another tool to be used in breeding programs aiming at pest resistance.

In general, all the BPX-413E clones selected presented, in the evaluation times (20, 40 and 60 minutes), distances covered by the mite equal or smaller than treatments rich in AA alone or ZGB, especially with regard to mean distances covered by the mites after 40 minutes (Table 4, C8, C9 and C10). Similar results were found by Silva et al. (2009) and Maluf et al. (2001), which proves that resistance of *T. urticae* mites mediated by the allelochemical was higher in 40 and 60 minutes.

The highest repellency level presented by the clones indicates the existence of synergistic effect of allelochemicals for resistance to spider mite, and demonstrates the advantage of investing in simultaneous selection for both allelochemicals. In similar works carried out by Silva (2009) and Maluf et al. (2001), synergistic effect for mite was not detected, perhaps, for they have tested double heterozygote genotype both for AA and for ZGB, contrary to what was demonstrated by Silva et al. (2009) in tests for resistance to *Tuta absoluta*. Several studies have confirmed the efficiency of allelochemicals in resistance and/or repellency to spider mite (RESENDE et al., 2002, GONÇALVES et al., 2006, SILVA et al., 2009, MALUF et al., 2010). However, none of them have found the effect of homozygous treatments rich in both allelochemicals. The only work in this sense was carried out by Silva et al. (2009); nevertheless, the

synergistic effects were evaluated in heterozygote hybrids for both AA and ZGB. The effects on non-segregating lines for both AA and ZGB were not evaluated.

CONCLUSIONS

The gene *Mi* was not effective in conferring repellency to spider mite, although it may be advantageous for tomato breeding programs aiming at resistance to other pests.

Zingiberene (ZGB) or acylsugar (AA) were efficient and equivalent in promoting repellency to mite of the *Tetranychus urticae* genus.

Zingiberene and acylsugar combined in tomato lines presented synergistic effect, which increased repellency to mite.

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RESUMO: O tomate (*Solanum Lycopersicum*) é um produto destinado à alimentação humana, seja na forma industrializada ou “in natura”. A expansão da área de cultivo dessa cultura favoreceu, entretanto, o surgimento de pragas e de doenças de difícil controle, que afetam significativamente a sua produção. Assim, o objetivo, neste trabalho, foi quantificar e comparar os efeitos do gene *Mi*, do alto teor de AA (isoladamente), do alto teor de ZGB (isoladamente) e dos altos teores de AA e ZGB (simultaneamente) na repelência de linhagens de tomateiro ao ácaro-rajado *Tetranychus urticae*, e detectar possíveis efeitos sinérgicos da presença dos dois aleloquímicos relativamente à presença de só um deles, isoladamente. O delineamento utilizado foi inteiramente casualizado, com 4 repetições. Foram retirados 4 folíolos expandidos de tamanhos semelhantes no terço superior das plantas em estágio fenológico de pré-florescimento. O bioensaio foi realizado no interior de câmara fria, à temperatura de 16±1 °C e umidade relativa de 64±4%. A resistência conferida pelos genótipos ricos em AA ou ZGB, na repelência ao ácaro-rajado do gênero *Tetranychus urticae*, em todos os tempos avaliados, superou todas as testemunhas, inclusive o TOM 684. O gene *Mi* não alterou na resistência. Houve efeito sinérgico entre AA e ZGB.

PALAVRAS-CHAVE: *Tetranychus urticae*, *Solanum lycopersicum*. Melhoramento genético

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