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Selection of tomato genotypes for processing with high zingiberene content, resistant to pests

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ABSTRACT

Tomato cultivars resistant to arthropod pests are an important tool to reduce the use of pesticides. Resistance sources can be found in wild *Solanum* species such as *S. habrochaites*, which shows high levels of zingiberene (ZGB). This study aimed to evaluate the resistance of a tomato F₂ progeny to spider mite and whitefly by evaluating the ZGB content, in laboratory, in plants from the F₂ population of the interspecific crossing *Solanum lycopersicum* cultivar Redenção x *Solanum habrochaites* var. *hirsutum* (PI-127826), F₁ plants, susceptible plants (cultivar Redenção) and plants of the wild species (PI-127826). From the F₂ population, six plants with high content and three with low content of ZGB were selected. For evaluating the impact of ZGB on the spider mite behavior, the distance walked by spider mites from a central point was measured on the selected plants and their parents. For whitefly, the number of eggs and nymphs were quantified. Spider mites travelled shorter distances in plants with high ZGB content. Also, the number of whitefly eggs and nymphs was lower in these plants. Higher ZGB contents affected negatively both the spider mite and the whitefly behavior.

Keywords: *Solanum lycopersicum*, *Solanum habrochaites*, allelochemicals, *Tetranychus urticae*, *Bemisia tabaci*.

RESUMO

Seleção de genótipos de tomateiro para processamento com alto teor de zingibereno resistentes a pragas

Cultivares de tomateiros resistentes a artrópodos-praga são uma importante ferramenta para a redução do uso de agrotóxicos nas lavouras. As fontes de resistência podem ser encontradas em espécies silvestres de *Solanum*, como o *S. habrochaites*, que apresenta altos teores de zingibereno (ZGB). Com o objetivo de avaliar a resistência ao ácaro-rajado e à mosca-branca em progêneses F₂ de tomateiro, foi quantificado o teor de ZGB em laboratório, em plantas da população F₂ obtidas a partir do cruzamento interespecífico de *Solanum lycopersicum* cultivar Redenção x *Solanum habrochaites* var. *hirsutum* (PI-127826), em plantas F₁, cultivar suscetível (Redenção) e em plantas da espécie silvestre (PI-127826). Da população F₂ foram selecionadas seis progêneses para alto teor e três para baixo teor de ZGB. Para avaliar o impacto de ZGB sobre o comportamento do ácaro-rajado, mediu-se a distância percorrida pelo ácaro a partir de um ponto central nas plantas selecionadas e nos genitores. Já para mosca-branca foram quantificados número de ovos e ninfas em folíolos. Os resultados demonstraram que em plantas com alto teor de ZGB os ácaros percorreram as menores distâncias. Já para mosca-branca foi encontrado menor número de ovos e ninfas nessas plantas. Assim, pode-se concluir que teores mais altos de ZGB afetam negativamente o comportamento do ácaro-rajado e da mosca-branca.

Palavras-chave: *Solanum lycopersicum*, *Solanum habrochaites*, aleloquímicos, *Tetranychus urticae*, *Bemisia tabaci*.

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The tomato (*Solanum lycopersicum*) for processing is grown in most Brazilian regions all year long. Farmers grow 21.3 million hectares, with a production of 1.87 million tons. Brazil is the fifth largest producer worldwide representing about 5% of the world production. Also, this crop represents a great socio-economic importance in the Brazilian productive areas (WPTC, 2014).

Consumption of tomato-derived products has been increasing considerably in the latest years, the advancement of urbanization and the greater inclusion of women into the labor market being fundamental for this increase (Camargo *et al.*, 2006). The increase of consumption requires a corresponding increase of production but pests have led to lower-than-expected productivity, maintaining the

high levels of tomato-derived products imports in Brazil (Nascimento *et al.*, 2013).

Regarding pests which attack the tomato crop, the whitefly *Bemisia tabaci* Gennadius Biotype B (Hemiptera: Aleyrodidae) is considered a key pest due to its high damage potential and capacity to reduce yields under specific weather conditions. For mites of the genus *Tetranychus*, especially for *T.*

urticae (Acari: Tetranychidae), despite being considered secondary pests, may cause serious damage under high infestation (Toscano *et al.*, 2004; Maluf *et al.*, 2007).

Pest management in tomato is usually carried out through chemical control, which may harm the farmer, the environment and the consumer (Silva *et al.*, 2009). Breeding programs aim to develop cultivars resistant to these pests, the introgression of genes of wild species into commercial genotypes being used for this purpose. According to Weston *et al.* (1989), the wild species *Solanum habrochaites* var. *hirsutum* has been used due to the presence of sesquiterpenes called zingiberene (ZGB), which provide resistance of plants to pests. Some studies showed that high contents of this substance in tomato leaflets promote resistance to whitefly (Freitas *et al.*, 2002; Neiva *et al.*, 2013), as well as to tomato leafminer [*Tuta absoluta* (Lepidoptera: Gelechiidae)] (Azevedo *et al.*, 2003; Oliveira *et al.*, 2012) and spider mite (Maluf *et al.*, 2001, 2007; Gonçalves *et al.*, 2006).

According to Freitas *et al.* (2002), the ZGB content has monogenic inheritance with incomplete dominance towards lower content. However, the occurrence of modifier genes not ruled out. Freitas *et al.* (2002) also state that the inheritance has high heritability ($h^2 = 67.8\%$). These estimates allow greater success in breeding programs due to higher chances of gains in selections for high content of ZGB.

This study aimed to select plants with high ZGB content, resistant to spider mite and whitefly.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse, in the Vegetable Crops Sector of the Department of Agronomy of the Centro-Oeste State University, in Guarapuava, Paraná State, Brazil. Interspecific cross *Solanum lycopersicum* cultivar Redenção x *Solanum habrochaites* var. *hirsutum* (PI-127826) was carried out. The cultivar Redenção for industrial processing with

resistance to *geminivirus* and tospovirus (Ferraz *et al.*, 2003) being used as female genitor. Female-parent flowers were emasculated before anthesis, and open flowers of accession PI-127826 were collected for pollen extraction. Pollen extracted was, then, put in contact with the style stigma of the emasculated flowers. Pollinated flowers received marking to identify the crosses. F₁ seeds were extracted from the fruits obtained, sown in styrofoam trays and the seedlings transplanted into 10 dm³ capacity pots. The flowers of F₁ plants were self pollinated and from them, fruits containing F₂ seeds were collected.

All the plants used in the experiments, described as follows, were maintained in a greenhouse in 10 dm³ capacity pots, containing a mixture of commercial substrate and soil in the proportion of 1:1, fertilized with N-P-K formulation 04-14-08 and limestone, according to the recommendation of Boletim 100 (Instituto Agronômico de Campinas, 1996), with daily irrigation.

Experiment 1 - Quantification of ZGB in the leaflets - the authors quantified the ZGB contents in the leaflets of 433 plants of F₂ population, 40 F₁ plants, 40 plants of cultivar Redenção and 40 plants of the accession PI-127826 at about 35 days after planting, according to the methodology proposed by Freitas *et al.* (2000). Six leaf discs of young expanded leaflets were collected from the upper third of the plants, totaling an area of 6 cm², which were put in labeled test tubes. Then, 2 mL of hexane were added in each tube, stirred up in a magnetic stirrer (Vortex) for 40 seconds to promote the extraction of ZGB. After stirring, the leaf discs were removed and the solution obtained was submitted to absorbance reading using a spectrophotometer (Cary 60 UV-VIS), wavelength of 270 nm, the absorbance being directly proportional to the amount of ZGB in leaf extract.

Based on the absorbance reading, the authors selected plants of F₂ generation with contrasting ZGB contents (six with high content and three with low content) to be submitted to tests of resistance to spider mites and whitefly (experiments

2 and 3). To select contrasting genotypes within the segregating population, the authors used as criterion the average value of absorbance obtained from parents [*S. habrochaites* (high content of the allelochemical) and cultivar Redenção (low content)]. Contents of ZGB in selected plants were confirmed with three replications.

The selected plants were cloned through rooting of axillary shoots from the plants in styrofoam trays containing commercial substrate. Subsequently, they were transplanted into 5 dm³ capacity pots, and kept in a greenhouse until the phenological stage of pre-flowering, with daily irrigation and without phytosanitary control.

Experiment 2 - Resistance to spider mite

The resistance to spider mite *T. urticae* was quantified through a bioassay proposed by Weston & Snyder (1990). The mites used came from the nursery maintained in the Entomology Laboratory of the Agronomy Department of the Midwest Paraná State University, at temperature of 25±2°C, humidity of 70±10% and photofase of 12 h, in jack bean plants (*Canavalia ensiformis*). The bioassay was carried out approximately 60 days after transplanting F₂ population, at room temperature and humidity in laboratory. The experimental design was completely randomized, with 12 treatments, corresponding to six plants with high content of ZGB, three plants with low content, parents (Redenção and PI-127826) and F₁, with four replications totalizing 48 leaflets (one leaflet per plot). Four young expanded leaflets were collected from the upper third and fixed with the aid of a metal thumbtack (9 mm in diameter) in the central region of adaxial leaf surface, on an offset sheet of paper, on a styrofoam plate. Leaflets were distributed randomly on a styrofoam plate, forming a replication. Ten female mites were collected from the controlled nursery and transferred to the center of each thumbtack, with the aid of a fine brush. The average distances 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. Shorter distances traveled

by mites were considered indicative of higher levels of resistance. The authors considered zero the distance traveled by the mites which remained on the thumbtack and they considered the greater distance from the tack to the leaf edge when the spider mite left the adaxial leaf surface.

Experiment 3 – Resistance to whitefly - for the evaluations of resistance to whitefly (*Bemisia tabaci* Biotype B), one population of this insect was maintained in a greenhouse in the Sector of Vegetable Crops of Midwest Paraná State University, in sweet potato (*Ipomoea batata*). Approximately 35 days after cloning, the contrasting plants selected in relation to ZGB content, along with parents and F₁ plants, were submitted to infested environment. The experimental design was completely randomized, with 12 treatments, corresponding to six F₂ plants with high content of ZGB, three F₂ plants with low content, parents (Redenção and PI-127826) and F₁, with four replications totalizing 36 plants (one plant per plot). After 48 hours of submission to the infested environment, these plants were removed and taken to another greenhouse, where the first evaluation was carried out. This evaluation consisted of the collection of three leaflets, one from the upper third, one from the middle third and another from the lower third of each plant. The leaves from where the leaflets were collected were identified in order to be collected leaflets of the same leaves evaluated, in the next evaluation. In the evaluation, the authors counted the number of eggs in 2 cm² leaf area of the leaflet abaxial face, with the aid of a stereoscopic microscope. Twenty days after infestation, leaflets were collected again and evaluated for oviposition and number of the last instar nymphs, in 2 cm² of leaf area of leaflet abaxial faces, also with the aid of stereoscopic microscope.

The data obtained in these experiments were subjected to analysis of variance and the averages were compared through Scott-Knott test, using the statistical program SISVAR (Ferreira, 2008). Pearson correlations

were estimated to verify the association between ZGB contents and resistance of the plants. The significance of correlations was calculated by the t test, with the aid of *Assistat* software.

For carried out trials, the authors estimated contrasts of interest among parental and plant groups with contrasting ZGB contents. Statistical software SISVAR (Ferreira, 2008) was also used for this analysis.

RESULTS AND DISCUSSION

Plants selected in experiment 1 for high and low ZGB content are shown in Table 1.

Table 1. Mean distance travelled (mm) by *T. urticae* and estimate of the contrasts of interest among tomato progenies after 20, 40 and 60 minutes of exposure to the adaxial surface of leaflets genotype *S. lycopersicum* cultivar Redenção, *S. habrochaites* var. *hirsutum* (PI-127826), F₁ generation and selected F₂ plants with high and low ZGB content (distâncias médias percorridas (mm) por *T. urticae* e estimativa dos contrastes de interesse entre as progênies de tomateiro após 20, 40 e 60 minutos de exposição à superfície adaxial dos folíolos de genótipos *S. lycopersicum* cultivar Redenção, *S. habrochaites* var *hirsutum* (PI-127826), geração F₁ e progênies selecionadas da geração F₂ com alto ou baixo teor de ZGB). Guarapuava, UNICENTRO, 2014.

Genotypes	Zingiberene content (Abs) ¹	Distance travelled (mm)		
		20 min	40 min	60 min
PI-127826	1.099	0.73 ² a	1.16 ² a	1.03 ² a
RVTZ pl#79 (high)	0.715	10.25 ² b	7.37 ² a	8.16 ² b
RVTZ pl#141 (high)	0.719	9.90 ² b	11.20 ² b	11.19 ² b
RVTZ pl#142 (high)	0.813	7.68 ² b	5.25 ² a	5.80 ² a
RVTZ pl#143 (high)	0.592	9.37 ² b	10.93 ² b	10.91 ² b
RVTZ pl#277 (high)	0.747	11.02 ² b	13.97 ² b	14.36 ² b
RVTZ pl#331 (high)	0.746	9.90 ² b	11.21 ² b	13.05 ² b
RVTZ pl#09 (low)	0.247	20.08 ² c	21.93 ² d	23.16 ² c
RVTZ pl#189 (low)	0.263	13.38 ² b	17.37 ² c	19.20 ² c
RVTZ pl#365 (low)	0.210	19.76 ² c	21.22 ² d	26.80 ² d
Redenção	0.084	21.35 ² c	25.27 ² d	28.01 ² d
F ₁ (Redenção X PI-27826)	0.145	8.16 ² b	16.00 ² c	19.13 ² c
Correlation		-0.78*	-0.89*	-0.91*
Identification of contrasts of interest		Estimate		
C1= plants (high ZGB) x plants (low ZGB)		-7.55**	-10.19**	-13.46**
C2= PI-127826 x plants (high ZGB)		-8.96**	-8.83**	-9.55**
C3= PI-127826 x plants (low ZGB)		-17.01**	-8.83**	-22.03**
C4= Redenção x plants (high ZGB)		11.66**	15.28**	17.43**
C5= Redenção x plants (low ZGB)		3.61	5.10	4.96

¹Zingiberene content at 270 nm; ²Means followed by the same letter in column belong to the same group by Scott-Knott, 5% (medias seguidas de letras iguais na coluna pertencem ao mesmo grupo, teste Scott-Knott, 5%); *significant by Student 5%; **significant by mean comparison test at 5% (significativo pelo teste de comparação a 5%).

Table 2. Mean number of eggs and nymphs of *B. tabaci* on the abaxial leaflet face and estimate of the contrasts of interest among tomato plants after 48 hours and 20 days in the environment infested with whitefly for genotypes *S. lycopersicum* cultivar Redenção, *S. habrochaites* var. *hirsutum* (PI-127826), F₁ generation and F₂ selected plants (número médio de ovos e ninfas de *B. tabaci* na face abaxial de folíolos e estimativa dos contrastes de interesse entre as progênies de tomateiro após 48 horas e 20 dias em ambiente infestado com mosca-branca nos genótipos *S. lycopersicum* cultivar Redenção, *S. habrochaites* var *hirsutum* (PI-127826), geração F₁ e progênies selecionadas da geração F₂). Guarapuava, UNICENTRO, 2014.

Genotypes	Zingiberene content (Abs) ¹	48 hours	20 days	
		Number of eggs	Number of eggs	Number of nymphs
PI-127826	1.099	9.00 ² a	10.89 ² a	11.22 ² a
RVTZ clone pl#79 (high)	0.715	7.00 ² a	5.44 ² a	8.44 ² a
RVTZ clone pl#141 (high)	0.719	10.44 ² a	10.11 ² a	14.33 ² a
RVTZ clone pl#142 (high)	0.813	9.00 ² a	9.66 ² a	4.89 ² a
RVTZ clone pl#143 (high)	0.592	10.55 ² a	3.77 ² a	11.33 ² a
RVTZ clone pl#277 (high)	0.747	19.33 ² a	3.00 ² a	12.78 ² a
RVTZ clone pl#331 (high)	0.746	10.89 ² a	5.89 ² a	5.33 ² a
RVTZ clone pl#09(low)	0.247	15.77 ² a	33.55 ² b	36.77 ² b
RVTZ clonepl#189(low)	0.263	32.66 ² b	48.11 ² b	39.00 ² b
RVTZ clone pl#365(low)	0.210	54.55 ² c	58.55 ² b	77.89 ² c
Redenção	0.084	54.33 ² c	52.55 ² b	59.55 ² c
F ₁ (Redenção x PI-127826)	0.145	10.44 ² a	6.66 ² a	14.33 ² a
Correlation		-0.65*	-0.70*	-0.71*
Identification of contrasts of interest		Estimate		
C1= plants (high ZGB) x plants (low ZGB)		-23.13**	-40.43**	-41.70**
C2= PI-127826 x plants (high ZGB)		-2.20	4.57	1.70
C3= PI-127826 x plants (low ZGB)		-25.33**	-35.85**	-40.00**
C4= Redenção x plants (low ZGB)		43.13**	46.24**	50.04**
C5= Redenção x plants (low ZGB)		20.00	5.81	8.33

¹Zingiberene content at 270 nm; ²Means followed by the same letter in column belong to the same group by Scott-Knott, 5% (medias seguidas de letras iguais na coluna pertencem ao mesmo grupo, teste Scott-Knott, 5%); *significant by Student 5%; **significant by mean comparison test at 5% (significativo pelo teste de comparação a 5%).

they showed averages lower than the plants with low content (contrast C1 and C2, Table 1). Significant and negative values of estimated contrasts C1 and C2 highlight opposite behavior between plants with high and low content for distance traveled by spider mite on the leaflet surface, allowing, therefore, inference that high contents of the allelochemical promote greater resistance to spider mite.

The plants selected for low content showed higher averages of distances traveled than the plants selected for high ZGB content, similar to accession *S. lycopersicum* cultivar Redenção and to F₁ (contrasts C1 and C5, Table 1). Positive and no significant values

estimated in contrast C5 prove similar drive of spider mite on the leaf surface in plants with low ZGB content and in cultivar Redenção, the susceptibility standard used.

In the evaluation carried out 20 minutes after releasing the mites, the plant RVTZ pl#09 and RVTZ pl#189, selected for low ZGB content, and F₁ population, showed similar averages to the plants selected for high content. This probably happens due to the time necessary for the spider mite notice the allelochemicals and its effect in relation to the distance traveled (Lucini *et al.*, 2015). This effect was only observed 40 and 60 minutes after the release of mites, when the authors noticed higher

distance traveled on the leaf surface of plants with low allelochemical contents, since high ZGB content inhibit the drive of spider mite (Table 1).

The plant RVTZ pl#142 stood out as one of the most promising lines, showing averages similar to accession PI-127826 in the evaluations of 40 and 60 minutes, showing greater resistance to spider mite.

The results are in accordance with Maluf *et al.* (2007) and Silva *et al.* (2009) showing that the high ZGB allelochemical contents provide resistance to spider mite. The study states that resistance provided by high ZGB content is directly related to high density of glandular trichomes on leaflet surface.

In experiment 3, in which the authors evaluated resistance to whitefly, significant difference between the plants in evaluations carried out was also observed (Table 2).

The plants selected for the high ZGB content did not differ from PI-127826, showing resistance to whitefly. On the other hand, the plants selected for low allelochemical contents showed results similar to the susceptible accession, cultivar Redenção (Table 2). This fact shows that the presence of high ZGB content in the leaflets acts effectively in the resistance of the plant to whitefly.

There were significant and negative correlation between oviposition and number of eggs and nymphs, and ZGB content (Table 2). Thus, the increase of ZGB content causes reduction in insect population on the plants. These results are similar to ones found by Freitas *et al.* (2002) and Neiva *et al.* (2013), in which high ZGB content provided resistance to whitefly.

These results are in accordance with that found by Azevedo *et al.* (2003); Maluf *et al.* (2007, 2010), Oliveira *et al.* (2012) and Neiva *et al.* (2013) who verified that high ZGB contents, together with high density of glandular trichomes, promote resistance of plants to spider mite, whitefly and tomato leafminer. According to Gonçalves *et al.* (2006), high ZGB content is directly linked to high density of glandular trichomes.

Genetic gains, related to resistance

to these arthropod pests, when selection for high ZGB content is done, can be expected in breeding programs which aim to obtain resistant cultivars, since heritability for ZGB content is high (67.8%) (Freitas *et al.*, 2002).

Indirect selection of plants in relation to ZGB content shows to be highly efficient in selecting plants resistant to spider mite and whitefly. Plants selected for high content will be retrocrossed with recurrent genitor (*S. lycopersicum* 'Redenção') for the continuity of the breeding program and to obtain commercial genotypes, resistant to arthropod pests.

The results obtained confirm that indirect selection of plants with high ZGB content is efficient to obtain tomato plants for processing, resistant to arthropod pests, as spider mite (*T. urticae*) and whitefly (*B. tabaci*) herein evaluated.

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