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Selection of processing tomato genotypes resistant to two spotted spider mite

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ABSTRACT

One of the major problems in cultivation of tomato is the occurrence of pests and diseases. The objective of the research was to select genotypes from the second generation of the first backcross (F_2BC_1) between the tomato cultivar for processing *S. lycopersicum* (cv. Redenção) and the wild access *S. habrochaites* var. *hirsutum* (PI-127826), with high levels of zingiberene (ZGB), resistant to the two spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). From the F_2BC_1 population. The ZGB content was quantified and five plants with high zingiberene selected (RVTZ 2011-079-117, RVTZ 2011-079-185, RVTZ 2011-079-335, RVTZ 2011-079-345 and RVTZ 2011-079-503) and four with low zingiberene content (RVTZ 2011-079-417, RVTZ 2011-331-460, RVTZ 2011-079-538 and RVTZ 2011-079-548) were selected. Genotypes selected for high and low ZGB content and the parentals *S. habrochaites* var. *hirsutum* access PI-127826 and 'Redenção' were evaluated for repellency to the two spotted spider mite. There was a significant and inverse correlation between ZGB content and average distances travelled by mites on tomato leaflets. The genotypes of the F_2BC_1 generation with high zingiberene levels RVTZ 2011-079-117, RVTZ 2011-079-185, RVTZ 2011-079-335, RVTZ 2011-079-345 and RVTZ 2011-079-503 are promising for progress in achieving resistant lines to arthropod pests. Among them RVTZ 2011-079-117 stands out for resistance to two spotted spider mite.

Keywords: *Solanum lycopersicum*, *Solanum habrochaites* var. *hirsutum*, *Tetranychus urticae*, genetic breeding, zingiberene.

RESUMO

Seleção de genótipos de tomateiro para processamento, resistentes ao ácaro-rajado

Um dos principais desafios da cadeia produtiva de tomates é a incidência de problemas fitossanitários, os quais resultam em grande aplicação de agrotóxicos, podendo causar problemas ao ambiente e à saúde de agricultores e consumidores. O objetivo deste trabalho foi selecionar genótipos de segunda geração do primeiro retrocruzamento (F_2RC_1) entre a cultivar de tomateiro para processamento *S. lycopersicum* cultivar Redenção e o acesso silvestre *S. habrochaites* var. *hirsutum* (PI-127826), com elevados teores de zingibereno (ZGB), resistentes ao ácaro *Tetranychus urticae*. Da população F_2RC_1 foi quantificado o teor de ZGB e selecionadas cinco plantas com alto teor deste aleloquímico (RVTZ 2011-079-117, RVTZ 2011-079-185, RVTZ 2011-079-335, RVTZ 2011-079-345 e RVTZ 2011-079-503) e quatro com baixo teor (RVTZ 2011-079-417, RVTZ 2011-331-460, RVTZ 2011-079-538 e RVTZ 2011-079-548). Genótipos selecionados para altos e baixos teores de ZGB e, os parentais *S. habrochaites* var. *hirsutum* acesso PI-127826 e 'Redenção' foram avaliados quanto à repelência ao ácaro *T. urticae*. Houve correlação significativa e inversamente proporcional entre teor de ZGB e as distâncias médias percorridas pelos ácaros em folíolos de tomateiro. Os genótipos da geração F_2RC_1 com altos teores de zingibereno RVTZ 2011-079-117, RVTZ 2011-079-185, RVTZ 2011-079-335, RVTZ 2011-079-345 e RVTZ 2011-079-503 são promissores para avanços na obtenção de linhagens resistentes a artrópodes-praga. Dentre eles, o genótipo RVTZ 2011-079-117 destacou-se para resistência ao ácaro-rajado.

Palavras-chave: *Solanum lycopersicum*, *Solanum habrochaites* var. *hirsutum*, *Tetranychus urticae*, melhoramento genético, zingibereno.

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The tomato (*Solanum lycopersicum*) is one of the most cultivated vegetables used for fresh consumption throughout the world. It also has large-scale use in the agro-industry for food. Tomato for fresh consumption is cultivated in small and medium-sized properties, while tomato for processing is typically cultivated in large areas (Gameiro *et al.*, 2007). With industries in all consumer centers the processing of tomato is of global importance (Ubierna *et al.*, 2010). It is one of the main industrialized vegetable in the world.

Frequent occurrences of phytosanitary problems affect the growth and development of tomato, placing it at a high risk for losses during the production cycle. Among the major arthropod pests, two spotted spider mites (*Tetranychus urticae*) are one of the most conspicuous (Silva *et al.*, 2009).

The two spotted spider mite, although considered a secondary pest of tomato, is becoming increasingly important as the tomato plant provides favorable conditions for their reproduction and development (Lucini *et al.*, 2016). In high infestation conditions, the major damages caused to the culture are drying of leaves and premature ripening of the fruits (Attia *et al.*, 2013). Chemicals, insecticides, and miticides are the most widely used form of pest control. However, intensive use of agrochemicals can damage the environment and affect the health of farmers and consumers (Silva *et al.*, 2009). An alternative to this is the introduction of genetic resistance in plants through crosses with wild genotypes rich in allelochemicals, compounds produced during secondary metabolism (Maluf *et al.*, 2001; Silva *et al.*, 2009; Neiva *et al.*, 2013; Lima *et al.*, 2015, 2016).

The resistance to arthropod pests in wild tomato plants has often been associated with the production and exudation of allelochemicals by glandular trichomes present in stems, leaves, and fruits (Lucini *et al.*, 2016). Acyl sugar is the major allelochemical produced by the wild species *Solanum pennellii* (Dias *et al.*, 2016) and *Solanum galapagense*, while the species *Solanum habrochaites* produces two allelochemicals, 2-tridecanone and zingiberene (Baldin *et al.*, 2005; Oliveira *et al.*, 2012; Lima *et al.*, 2015).

Generally, studies about the mechanisms and resistance levels in parental genotypes of *S. habrochaites* var. *hirsutum* in relation to twostriped mite (Weston *et al.*, 1989) and whitefly (Fancelli *et al.*, 2005) are related to the presence of trichomes in which sesquiterpene zingiberene is produced. The production of segregating generations can allow separation and evaluation of multiple mechanisms of resistance that may occur in plants. Furthermore, biological and behavioral bioassays are important in breeding programs in order to determine the degree of resistance of the resulting genotypes in relation to these herbivores (Carter & Snyder, 1985).

The zingiberene (ZGB) is among the most studied allelochemicals, providing satisfactory levels of resistance to the tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) (Lima *et al.*, 2015), whitefly (Baldin *et al.*, 2005), two spotted spider mite (Silva *et al.*, 2009), and red spider mite *Tetranychus evansi* (Acari:Tetranychidae) (Maluf *et al.*, 2001). It is a sesquiterpene whose production occurs mainly in glandular trichomes, of which types IV and VI are present in large quantities in the leaf epidermis of the wild genotype *S. habrochaites* var. *hirsutum* PI-127826 (Gonçalves *et al.*, 2006).

The objective of this study was to select genotypes from the second generation of the first backcross (F_2BC_1) between the tomato cultivar *S. lycopersicum* 'Redenção' and the wild access *S. habrochaites* var. *hirsutum* PI-127826, with high levels of ZGB that helps plants to resist to two spotted spider mite attack.

MATERIAL AND METHODS

The experiments were conducted during 2013 and 2014 in the Vegetable Crops Sector of the Department of Agriculture at the Universidade Estadual do Centro-Oeste (UNICENTRO) (25°23′00″S; 51°29′39″W; altitude 1.024 m). The climate is mesothermal humid subtropical (Cfb), with moderate summers and frequent and severe frosts in winter.

The segregating F_2 genotypes were obtained from the interspecific cross between the *S. lycopersicum* cultivar 'Redenção,'strain with low levels of ZGB and characteristics for processing, and the wild species *S. habrochaites* var. *hirsutum* access PI-127826, genotype with high levels of ZGB.

Quantification of the ZGB content of F_2 generation was performed on leaf discs collected from young fully expanded leaflets located in the upper third of the plants totaling 6 cm^2 of leaf area (Freitas *et al.*, 2000). For ZGB extraction, 2 mL of distilled hexane was added to the leaf discs in open glass test tubes and shaken for 40 seconds. Subsequently the samples were quantified by spectrophotometric reading (Cary 100 UV-Vis) with absorbance at wavelength 270 nm. The obtained values were then calculated in mmol/cm^2 of leaf area. The ZGB concentrations in the leaflets are directly proportional to the absorbance; higher absorbance values reflect greater ZGB contents.

To obtain the F_2BC_1 population, backcrossing was conducted in selected plants of the F_2 generation (with high levels of ZGB) with the recurring female cultivar 'Redenção'. The obtained fruits from backcross were collected and the F_1BC_1 seeds retrieved. The F_2BC_1 population was obtained through self-fertilization of the F_1BC_1 generation.

In F_2BC_1 generation, by using the same methodology used in the quantification of ZGB in the F_2 generation, from a total of 600 evaluated plants, there were five with high ZGB content (RVTZ 2011-079-117, RVTZ 2011-079-185, RVTZ 2011-079-335, RVTZ 2011-079-345, and RVTZ 2011-079-503) and four with low content which were selected (RVTZ 2011-079-417, RVTZ 2011-331-460, RVTZ 2011-079-538, and RVTZ 2011-079-548). Plants with contrasting ZGB contents were cloned by rooting of axillary shoots in polystyrene trays consisting of 128 cells filled with commercial substrate Mecplant®. Sowing of the cultivar 'Redenção' was held concurrently with cloning, and the sowing of wild species *S. habrochaites var. hirsutum* access PI-127826 was carried out fifteen days earlier. Difference in emergence and growth was the reason for staggered sowing.

At 21 days after cloning, when plants had formed roots and the parentals had between three and five true leaves, the genotypes with contrasting ZGB content (high ZGB content, *S. habrochaites* var. *hirsutum* PI-127826, and low ZGB content, *S. lycopersicum* 'Redenção') as well as the control, were transplanted in low density polyethylene jars of 10 dm^3 containing sieved soil.

To evaluate the plant repellency to *T. urticae*, an adapted methodology was used (Weston & Snyder, 1990). Mites were collected in snap bean plants and placed for breeding in tomato plants, cultivar Santa Cruz Kada® in greenhouse. A randomized block design with four replications was used to evaluate the selected tomato genotypes of F_2BC_1 for high and low ZGB content, to the parentals *S. habrochaites* var.

hirsutum access PI-127826 and to the commercial cultivar 'Redenção'. Each replication consisted of one plant. About 40 days after transplanting, three fully expanded young leaflets of similar size were collected, from upper third of each plant.

Each of the leaflets was fixed at the central region, with the abaxial side up, on a sheet of A4 paper (210 x 297 mm) and placed on expanded polystyrene boards with the aid of a metallic tack (9 mm diameter). On each metal thumbtack, with help of a fine brush, ten adult female mites obtained from a laboratory rearing were released. The distance covered by the mites (mm) from the center of the tack was measured after 20, 40 and 60 min of exposure. The maximum gap between the center of the thumbtack and longest distance from the end of the leaflet was taken as distance traveled by the mite after it left the leaflet. Mites that remained on the metal thumbtack were considered to have traversed a zero distance. We considered small distances covered by mites as indicative of greater level of repellency.

The data obtained in the tests were submitted to the Shapiro-Wilk normality test and the Lèvene test of homogeneity of variances. Obtained results were subjected to transformation by square root ($x^{1/2}$). Data were submitted to analysis of variation and averages grouped by Scott-Knott test, with $p<0.05$, using the statistical program Sisvar (Ferreira, 2008).

Pearson correlations were estimated between the results of the test of resistance to pests and the ZGB levels of the genotypes using the statistical program Assistat 7.7 Beta (Silva, 2014). Contrasts of interest were estimated for each variable, including control and F_2BC_1 genotypes with contrasting levels of ZGB, through software Sisvar (Ferreira, 2008).

RESULTS AND DISCUSSION

There were significant differences among genotypes for average distances traveled by mites on abaxial surface of leaflets (Table 1). At 20 and 60 min, two of the genotypes evaluated with high ZGB content (RVTZ 2011-079-345 and 2011-079-503 RVTZ) demonstrated the lowest distance traveled by the mites, which corresponded to the control with high content of ZGB, PI-127826: 1.95, 1.30, and 1.22 mm for 20 min and 2.56, 2.66 and 2.70 mm for 60 min, respectively. At 40 min, all genotypes with high ZGB content (RVTZ 2011-079-345, RVTZ 2011-079-503, RVTZ 2011-079-185, RVTZ 2011-079-117, and RVTZ 2011-079-335) showed results that did not differ from the wild parental PI-127826, with 1.80, 2.22, 3.64, 3.83, 6.28 and 2.22 mm, respectively. At all evaluated exposure times, genotypes selected for high levels of ZGB had obtained lower distances traveled by the mites in comparison with the parental 'Redenção'.

There was a significant and inversely proportional correlation between the ZGB content and the average distances traveled by the mites on the tomato leaflets. The group of plants F_2BC_1 with high ZGB content did not differ from wild type control PI-127826 (Table 1). However, the cultivar 'Redenção', presented the highest average distances traveled by mites in the leaflets, followed by the group with low ZGB content.

The bioassay made with two spotted spider mite allowed clearly observe the sharp contrast between *S. lycopersicum* 'Redenção' and genotypes with low ZGB content when compared to selected genotypes for high content of allelochemical (Table 1).

The results obtained in this study are in agreement with those presented by Gonçalves *et al.* (2006) and Lima *et al.* (2016). These authors found a positive correlation between ZGB content and resistance to *T. evansi* and *T. urticae*, and to *Tuta absoluta*

Table 1. Mean distance travelled (mm) by two spotted spider mites after 20, 40 and 60 minutes of exposure to the abaxial surface of leaflets in tomato genotypes *S. lycopersicum* cv. 'Redenção', *S. habrochaites* var. *hirsutum* PI-127826 and selected F_2BC_1 plants with high and low zingiberene (ZGB) content. Guarapuava, UNICENTRO, 2015.

Genotype	Zingiberene content (Abs)[1]	Travelled distance (mm)		
		20 min	40 min	60 min
S. habrochaites PI-127826	0.338	1.22 a[2]	2.22 a	2.70 a
RVTZ 2011-079-117 (high)	0.216	2.46 b	3.83 a	5.24 b
RVTZ 2011-079-185 (high)	0.346	3.10 b	3.64 a	4.83 b
RVTZ 2011-079-335 (high)	0.216	4.71 b	6.28 a	7.72 b
RVTZ 2011-079-345 (high)	0.197	1.95 a	1.80 a	2.56 a
RVTZ 2011-079-503 (high)	0.285	1.30 a	2.22 a	2.66 a
RVTZ 2011-079-417 (low)	0.038	15.19 c	16.26 b	17.26 c
RVTZ 2011-331-460 (low)	0.039	21.63 c	21.88 b	22.95 c
RVTZ 2011-331-538 (low)	0.029	14.94 c	19.77 b	19.71 c
RVTZ 2011-331-548 (low)	0.025	22.86 c	26.14 c	27.39 d
S. lycopersicum cv. Redenção	0.103	33.58 d	32.46 c	34.90 d
CV (%)	84.56	28.63	24.87	24.94
Correlation with ZGB content		-0.82 **	-0.83 **	-0.83 **
Comparison of contrast of interest estimative		**20 min**	**40 min**	**60 min**
Genotypes high vs. genotypes low		-14.60 **	-15.96 **	-15.98 **
PI-127826 vs. genotypes high		-2.83 ns	-2.84 ns	-3.15 ns
PI-127826 vs. genotypes low		-17.44 **	-18.79 **	-19.13 **
Redenção vs. genotypes high		29.53 **	27.41 **	29.05 **
Redenção vs. Genotypes low		14.92 **	11.45 **	13.07 **

[1]ZGB content at 270 nm; [2]Means followed by same letters in the column belong to the same group, Scott-Knott test, p<0,05; **significant correlation, Student t test, p<0,01; **significant contrast by Scheffé test, p<0.01; nsnot significant.

(Lepidoptera: Gelechiidae) in genotypes originated from the interspecific cross between *S. habrochaites* var. *hirsutum* and *S. lycoperscicum* TOM-556 and 'Redenção', respectively.

In most studies on tomato resistance, repellency estimated time is given by the mite permanence on the surface of a tack when exposed to allelochemicals. In the present study, the results confirmed this information, and the genotypes with high ZGB content have higher degree of repellency in bioassays.

Silva *et al*. (2009) found a higher degree of repellency to spider mite in double heterozygotic genotypes with a high ZGB content and acyl sugars, compared to the commercial controls. Maluf *et al*. (2001) considered ZGB the main factor responsible for the resistance to *T. evansi* in interspecific hybrids originating from the crossing between *S. habrochaites* var. *hirsutum* x *S. lycopersicum*.

Toscano *et al*. (2002) observed that the genotype PI-127826 have trichomes type I, IV, Va, VIc and VII. Glandular trichomes type IV along with the type VI, are responsible for production of the allelochemical ZGB (Gonçalves *et al.*, 2006). These trichomes are present in low concentration in *S. lycopersicum* 'Redenção' (Lucini *et al.*, 2015). According to Alba *et al.* (2009), glandular trichomes type IV are responsible to reduce oviposition of females and increase repellency and mortality of adults of two spotted spider mites on leaflets of tomato plants, by producing allelochemicals such as acylsugars. Figueiredo *et al.* (2013) showed that high densities of glandular trichomes on abaxial surface of strawberry leaflets decrease mobility of adults of two spotted spider mites. These trichomes promote resistance or no preference or antixenosis type, in which plants do not present attractive to the pest for basic functions such as shelter, food, oviposition and other essential activities (Lucini *et al.*, 2015).

High resistance levels against two spotted spider mite in genotypes with high levels of allelochemicals is a favorable condition within the integrated pest management, because it facilitates the control, reducing the amount of chemicals used and simultaneously contributing to the reduction of production costs (Alba *et al.*, 2009). The role of ZGB and other alellochemicals in promoting resistance to pests has been confirmed in other studies. Silva *et al.* (2009) found that heterozygotic tomato genotypes rich in ZGB and double heterozygotic genotypes with high levels of ZGB and acyl sugars had fewer whitefly eggs, lower whitefly nymphs survival and lower distances traveled by two spotted spider mites on the leaflets, compared to commercial controls, suggesting a kind of no preference resistance for feeding and/or antibiosis. Neiva *et al.* (2013) obtained higher resistance to whitefly in tomato lines rich in acyl sugars, ZGB and 2-tridecanone, in comparison to the genotypes with low contents of these compounds. Rakha *et al.* (2017) observed that wild tomato genotypes with high density of type IV glandular trichomes were less preferred by two spotted spider mites adults for oviposition, feeding or shelter, and suffered less damage on leaves in comparison with a commercial cultivar, used as a control.

This study demonstrated that the indirect selection of genotypes with high levels of ZGB presents efficiency, enabling accelerating the process of selection of genotypes resistant to arthropod pests. According to Gonçalves *et al.* (2006), selecting through allelochemical content can be more efficient in obtaining genotypes with higher arthropod resistance levels in tomato plants when compared with bioassays using insects.

Tomato genotypes F_2BC_1 with high levels of ZGB, RVTZ 2011-079-117, RVTZ 2011-079-185, RVTZ 2011-079-335, RVTZ 2011-079-345 and RVTZ 2011-079-503 presented shorter average distances traveled by mite *T. urticae* on the abaxial surface of the leaflets. Thus, these genotypes can be used in future backcrossing using the cultivar 'Redenção' as a recurrent parent, thus aiming to increase the agronomic characteristics and maintain the resistance to arthropod pests.

In conclusion, genotypes of F_2BC_1 generation with high zingiberene levels RVTZ 2011-079-117, RVTZ 2011-079-185, RVTZ 2011-079-335, RVTZ 2011-079-345 and RVTZ 2011-079-503 are promising for improvements in achieving lines with background for processing, resistant to arthropod pests. Among them, we highlight that RVTZ 2011-079-117 represents an important technological breakthrough for tomato production, and is available for breeding programs which can be used in developing lineages.

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