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RESISTANCE OF ROUND TOMATO GENOTYPES WITH DETERMINATE GROWTH HABIT TO TWO-SPOTTED SPIDER MITES AND SILVERLEAF WHITEFLY

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ABSTRACT

The requirement for round tomato hybrids, with determinate growth habit, for consumption in natura has considerably increased in Brazil. Despite all potential, tomato cropping presents as main obstacle the occurrence of pests during its cycle. The wild species *Solanum pennellii* has been used in order to obtain resistant genotypes. However, little research has been conducted to date aiming at developing round tomato genotypes that are resistant to pests and present determinate growth habit. Therefore, the experiment aimed at selecting determinate genotypes that are rich in acylsugars and at checking their levels of resistance to two of the most important pests of the crop (two-spotted spider mite and silverleaf whitefly). Thirteen treatments were evaluated, 10 UFU057-F_2BC_1 generations, and three check treatments that comprised the wild species *S. pennellii* and two cultivated tomatoes (cv. Santa Clara and UFU057). The genotypes UFU057-F_2BC_1#112 and UFU057-F_2BC_1#101 stood out, showing the highest acylsugar content (17.1 and 18.4 nmols·cm^{-2} of leaf area, respectively). The same genotypes presented an elevated level of resistance to *Bemisia tabaci* with low values of oviposition, number of nymphs and adults of the fly. Regarding the two-spotted spider mite, the genotype UFU057-F_2BC_1#112 did not allow much advance of the mites along the surface of the leaflet, thus, it is concluded that this genotype with determined growth habit is promising for the tomato genetic breeding program, presenting high acylsugar contents and moderate to elevated level of resistance to *Tetranychus urticae* and *Bemisia tabaci*.
Additional keywords: Allelochemicals, arthropod pests, *Bemisia tabaci* biotic stress, *Tetranychus urticae*

RESUMEN

Genotipos de tomate redondo con hábito de crecimiento determinado y resistencia al ácaro de dos manchas y la mosca blanca
La demanda de híbridos de tomate redondo, con hábito de crecimiento determinado y de consumo como fruto fresco, ha aumentado considerablemente en Brasil. A pesar de su potencial, el cultivo de tomate tiene como principal obstáculo la presencia de plagas durante el ciclo productivo. La especie silvestre *Solanum pennellii* se ha utilizado para obtener genotipos resistentes. Sin embargo, son pocos los estudios orientados a desarrollar genotipos de tomate redondo, de crecimiento determinado, resistentes a plagas. Por ende, esta investigación tuvo como objetivo seleccionar genotipos, ricos en acilazúcares y evaluar los niveles de resistencia al ácaro de dos manchas y la mosca blanca. Se evaluaron 13 tratamientos: 10 en la generación UFU057-F_2BC_1 y 3 de control, compuestos por la especie silvestre *S. pennellii* y dos tomates cultivados (cv. Santa Clara y UFU057). Los genotipos UFU057-F_2BC_1#112 y UFU057-F_2BC_1#101 presentaron el mayor contenido de acilazúcares, con 17,1 y 18,4 nmols·cm^{-2} de área foliar, respectivamente, y a su vez presentaron una elevada resistencia a la mosca blanca, mostrando valores bajos para oviposición, número de ninfas y adultos de la plaga. Respecto al ácaro de dos manchas, el genotipo UFU057-F_2BC_1#112 no permitió mucho avance del ácaro en la superficie de los folíolos, por lo que se concluye que este genotipo es prometedor para el programa de mejoramiento genético del tomate, al presentar un alto contenido de azúcar acílico, con medio a elevado nivel de resistencia a *Tetranychus urticae* y *Bemisia tabaci*.
Palabras clave adicionales: Aleloquímicos, artrópodos plaga, *Bemisia tabaco*, estrés biótico, *Tetranychus urticae*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most cultivated and consumed vegetable all over the world, presenting a high socioeconomic and nutritional importance (Alvarenga et al., 2013),

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generating job and having an extreme importance for the vegetable seeds market, with an estimated value of $ 200 millions in Brazil (Treichel, 2016). Tomato fruit presents not only good palatability, but also vitamins A and C, calcium, potassium, and it is rich in lycopene, which is an important antioxidant (Agarwal and Rao, 2000).

The species may be considered one of the most susceptible to arthropod pests, due in part to its narrow genetic base as a consequence of the breeding and domestication processes (Villand et al., 1998). This experiment focused on the two-spotted spider mites *Tetranychus urticae* Koch 1836, and on the silverleaf whitefly *Bemisia tabaci* (Gennadius), since they are key pests for tomato cropping and they are capable of causing multiple damages to plants. Spider mites have been causing considerable loss to Brazilian production since the 70's. (Silveira et al., 2011). Furthermore, whitefly is a huge problem too, for it attacks the fruit, reducing tomato market price, and it spreads a complex of begomovirus to plants (Dinsdale et al., 2010).

The main recommendation for arthropod pest control has been the chemical method by means of insecticides and acaricides (Silveira et al., 2011). Nonetheless, farmers and researchers have been using this method incorrectly, culminating in the increase of individuals that are resistant to chemicals. For this reason, it is essential to search for alternative methods to kill pests.

Plant breeding for producing genotypes resistant to arthropod pests has been mainly used nowadays (Gonçalves et al., 2010; Maluf et al., 2010). The use of wild species of tomato, such as *Solanum pennellii*, has allowed to obtain lines (Gonçalves et al., 2010; Maciel et al., 2017) and hybrids with a broad spectrum of resistance to the main pests that affect tomato cropping (Maluf et al., 2010). The wild genotypes present distinctive characteristics, such as glandular trichome (Simmons and Gurr, 2005), synthesis of allelochemicals (Carter et al., 1989) and enzyme expression (Howe et al., 1996), which contribute to induce resistance to pests. On the other hand, a type of allelochemical named acylsugar is found in the wild genotype *S. pennellii*, and, when it is combined with glandular trichome, induces a broad spectrum of resistance in the plant (Gonçalves et al., 2007).

Tomato plants, presenting determined growth habit are more compact than those with undetermined growth, so do not have the need for cultural practices such as tillage, unfolding and thinning of fruits. This way the production costs can be reduced. Currently, information regarding the development of tomato genotypes for consumption *in natura*, resistant to pests and with determined growth habit is scarce.

The objective of the experiment was to select round tomato genotypes, with determinate growth habit, for consumption *in natura* that are rich in acylsugars and present satisfactory levels of resistance to the two-spotted spider mite and silverleaf whitefly.

MATERIAL AND METHODS

The experiments were conducted at Estação Experimental de Hortaliças (Experimental Horticulture Station, 18º42' S, 47º29'W, 873 meters above sea level) and at Laboratório de Análise de Sementes e Recursos Genéticos (Genetic Resources Laboratory) of the Federal University of Uberlândia (Lagen-UFU), in Monte Carmelo city, Minas Gerais, from April 2013 to September 2016.

The genetic materials came from UFU germplasm bank, and four segregating populations (F_2BC_1) were evaluated for the experiments. They were obtained from an interspecific cross between the pre-commercial line UFU057 (*Solanum lycopersicum* L.) and the wild access LA-716 (*S. pennellii*), followed by one backcrossing. The genotype UFU057, which was also used as a susceptible check treatment, is a round line with large fruit size (> 300 g), low level of acylsugars and susceptible to pests (Peixoto et al., 2019). In addition, it is homozygous recessive (spsp) for the *self-pruning* gene and presents a genotypic background similar to the cultivar Santa Clara. On the other hand, the wild access LA-716 (*S. pennellii*), which was used as a resistant check treatment, has a high level of acylsugar, small fruit size (15 g) and is resistant to entomological organisms (Maluf et al., 2010). It is a genotype with indeterminate growth habit and homozygous dominant (SPSP) for the *self-pruning* gene. The segregating populations were UFU057B#13, UFU057B#28, UFU057B#41 and UFU057B#53.

The seeds were sowed on May 2015 in a

polystyrene seed trays. Thirty-five days after sowing, the seedlings were transplanted into 5 L pots filled with commercial substrate of coconut fiber.

The experiment consisted of 550 plants: 400 belonged to the segregating populations (100 for each one), 50 to the wild genotype and recurrent parent (*S. pennellii*), 50 to the donor parent (UFU057) and 50 to the cultivar Santa Clara. The plants were cultivated in a bow-type greenhouse, measuring 7 x 21 m with the ceiling of 4 m, covered with a transparent polyethylene film of 150 μ, additivated against ultraviolet rays and curtain side of white and anti-aphid screen.

Leaflets were collected from the upper third of the non-infested plants, 75 days after sowing, in order to quantify the acylsugar content. Seeking the allelochemical extraction, samples comprising 6 leaf discs (4.2 cm^2) were collected in plants of each population. The samples were placed inside test tubes and sent to UFU at Lagen, where they were evaluated with respect to acylsugar content according to the methodology proposed by Resende et al. (2002) and adapted by Maciel and Silva (2014).

Among the four segregating populations, the 25 F_2BC_1 genotypes that stood out for acylsugar content were selected and re-evaluated with respect to allelochemical content, according to the same methodology aforementioned, and 10 of them with the highest content (UFU-F_2BC_1#8, #112, #13, #101, #74, #77, #139, #110, #73 and #4) were selected and, along with the wild access *S. pennellii*, the cv. Santa Clara and the genotype UFU057, were cloned using rooting axillary shoots. The buds had homogenous size and were cultivated on polystyrene trays and covered with substrate of coconut fiber. At 17 days after shoot cloning, the seedlings were transplanted into 5 L pots with the same substrate.

The experiment was set up in a randomized block design, with four replications and 13 genotypes, comprising 52 plots of five plants each and culminating in 260 plants on the experiment. The temperature and relative humidity were 18.7-34.0 °C and 72-95 % (min-max), respectively.

Thirty days after transplanting, the leaf acylsugar content of the clones was quantified following the same procedure as mentioned before.

To determine the resistance to two-spotted spider mite (*T. urticae*), mites were previously collected from tomato plants naturally infested in Monte Carmelo city in January 2016 and reared onto susceptible tomato plants of the cv. Santa Clara (*S. lycopersicum*), that is susceptible to this pest. The structure used to rear the mites was a 6 x 4 x 2 m greenhouse with the same characteristics of the one previously described. The resistance to spider mites was analyzed according to the test that Weston and Snyder (1990) proposed, measuring the distance covered by the mites on the surface of the genotype leaflets for 5, 10, 15 and 20 minutes. Timing started after 5 mites were placed on the head of a metallic thumbtacks attached to the center of the leaflets.

To measure the genotypes resistance to whitefly, leaves with nymphs and eggs were previously collected from tomato plants naturally infested in Monte Carmelo city during the month of January 2016. The insects were bred in the same greenhouse used to rear two-spotted spider mites. Later, they were released on the treatments, and the genotypes evaluated 90 days after shoot cloning with respect to the number of eggs and nymphs per leaf area, using five leaflets that were collected from the upper third of the plants with the aid of a stereoscopic microscope (40x), according to Maluf et al. (2010). A mirror was used to count the number of adults before they leave the surface of the plants.

After verifying the assumptions, an analysis of variance (Anova) was performed along with the average test of Scott-Knott for the acylsugar content of the leaves and for the presence of eggs, nymphs and adults of whitefly. In the case of the bioassay test of resistance to mite, or repellency test, the rate of advance of mites with time was adjusted by means of linear regression models. The relative superiority of the genotypes for the acylsugar content was calculated in relation to the cv. Santa Clara or UFU057, using the equation RS (%) = 100 [(C1/C2) -1)], where RS is the relative difference of the concentration of acylsugar present in the F_2BC_1 genotypes in relation to the cv. Santa Clara or UFU057, C1 is the concentration of acylsugar in the leaflets of the F_2BC_1 genotypes, and C2 is the concentration of acylsugar in the leaflets of the cv. Santa Clara or UFU057. The analyses were done using the statistical software Genes (Cruz, 2013).

RESULTS AND DISCUSSION

The F_2BC_1 genotypes showed a significant difference ($P≤0.05$) in acylsugar content. The wild access LA-716 (*S. pennellii*) differed from the susceptible check treatments (cv. Santa Clara and UFU057) with an acylsugar content that was 161.3 and 217.6% higher than those treatments, respectively, corroborating studies where tomatoes of Santa Cruz type were used (Maluf et al., 2010). Similarly, UFU057-F_2BC_1#112 and UFU057-F_2BC_1#101 acylsugar content stood out, differing statistically from the cv. Santa Clara and UFU057. Their acylsugar content was 37.9 and 48.4% superior, respectively, when compared to the cv. Santa Clara, and 67.6 and 80.4% superior, respectively, when compared to UFU057. Nevertheless, their acylsugar contents were much lower than the resistant *S. pennellii* treatments (Table 1).

Table 1. Acylsugar content and relative superiority of the genotypes compared to the cv. Santa Clara or UFU057

Genotype	Acylsugar (nmol·m^{-2} of leaf area)	Relative superiority over cv. Santa Clara / UFU057 (%)
T1= UFU057-F_2BC_1#8	13.2 c	6.5 / 29.4
T2= UFU057-F_2BC_1#112	17.1 b	37.9 / 67.6
T3= UFU057-F_2BC_1#13	11.6 c	-6.5 / 13.7
T4= UFU057-F_2BC_1#101	18.4 b	48.4 / 80.4
T5= UFU057-F_2BC_1#74	11.9 c	-4.0 / 16.7
T6= UFU057-F_2BC_1#77	13.0 c	4.8 / 27.5
T7= UFU057-F_2BC_1#139	12.8 c	3.2 / 25.5
T8= UFU057-F_2BC_1#110	13.1 c	5.6 / 28.4
T9= UFU057-F_2BC_1#73	11.3 c	-8.9 / 10.8
T10= UFU057-F_2BC_1#4	12.1 c	-2.4 / 18.6
T11= *S. pennellii*	32.4 a	161.3 / 217.6
T12= Santa Clara	12.4 c	-
T13= UFU057	10.2 c	-
CV (%)	9.3	

Averages followed by the same letter do not differ statistically from each other according to the Scott-Knott test ($P≤0.05$)

In tomato genotypes of Santa Cruz type, there are reports of hybrids with satisfactory levels of resistance to whitefly (Maluf et al., 2010). Also, in this type of tomato, Gonçalves et al. (2010) obtained lines rich in acylsugar from an interspecific cross with *S. pennellii* and reported satisfactory levels of resistance to *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae).

It is important to note, however, that there are reports about unknown factors controlling the presence of acylsugar that can be lost across generations, which makes it difficult to obtain genotypes similar to the wild species *S. pennellii* (Gonçalves et al., 2007; Gonçalves et al., 2010; Maluf et al., 2010).

Evaluating the resistance to silverleaf whitefly, there were significant differences ($P≤0.05$) among the genotypes with respect to oviposition and the presence of nymphs and adults (Table 2).

Eggs of whitefly were not found on the wild access *S. pennellii*. This genotype showed the lowest averages for number of nymphs and adults of whitefly, indicating that the insect does not have preference for that genotype. On the contrary, the cv. Santa Clara presented the highest values for oviposition, presence of nymphs and adults (Table 2). In relation to the number of eggs per leaf area on the F_2BC_1 segregating genotypes, the insect showed no interest for when compared to the cv. Santa Clara. In the number of nymphs, the genotypes UFU057-F_2BC_1#112, UFU057-F_2BC_1#101 and UFU057-F_2BC_1#139 showed the lowest index of preference. It is important to emphasize that the treatments UFU057-F_2BC_1#112 and UFU057-F_2BC_1#101 were also classified as rich in acylsugar (Table 1).

Table 2. Oviposition, number of nymphs and adults of *Bemisia tabaci* on the upper third of round tomato genotypes (measured 60 days after the infestation)

Genotype	Oviposition (eggs per cm^2 leaflet)	Nymphs per leaflet	Adults per leaflet
T1= UFU057-F_2BC_1#8	3.5 d	33.2 b	4.0 d
T2= UFU057-F_2BC_1#112	1.6 e	8.1 g	2.2 e
T3= UFU057-F_2BC_1#13	1.6 e	21.3 e	8.9 a
T4= UFU057-F_2BC_1#101	3.8 d	9.9 f	3.6 d
T5= UFU057-F_2BC_1#74	7.0 b	27.2 c	5.1 c
T6= UFU057-F_2BC_1#77	1.5 e	27.7 c	9.6 a
T7= UFU057-F_2BC_1#139	0.9 f	10.8 f	3.9 d
T8= UFU057-F_2BC_1#110	4.6 c	51.5 a	3.6 d
T9= UFU057-F_2BC_1#73	1.8 e	24.4 d	2.1 e
T10= UFU057-F_2BC_1#4	4.3 c	33.4 b	7.3 b
T11= *S. pennellii*	0.0 g	0.4 h	0.5 f
T12= Santa Clara	9.3 a	32.9 b	8.2 b
T13= UFU057	4.6 c	19.4 e	5.4 c
CV (%)	18.4	6.6	18.0

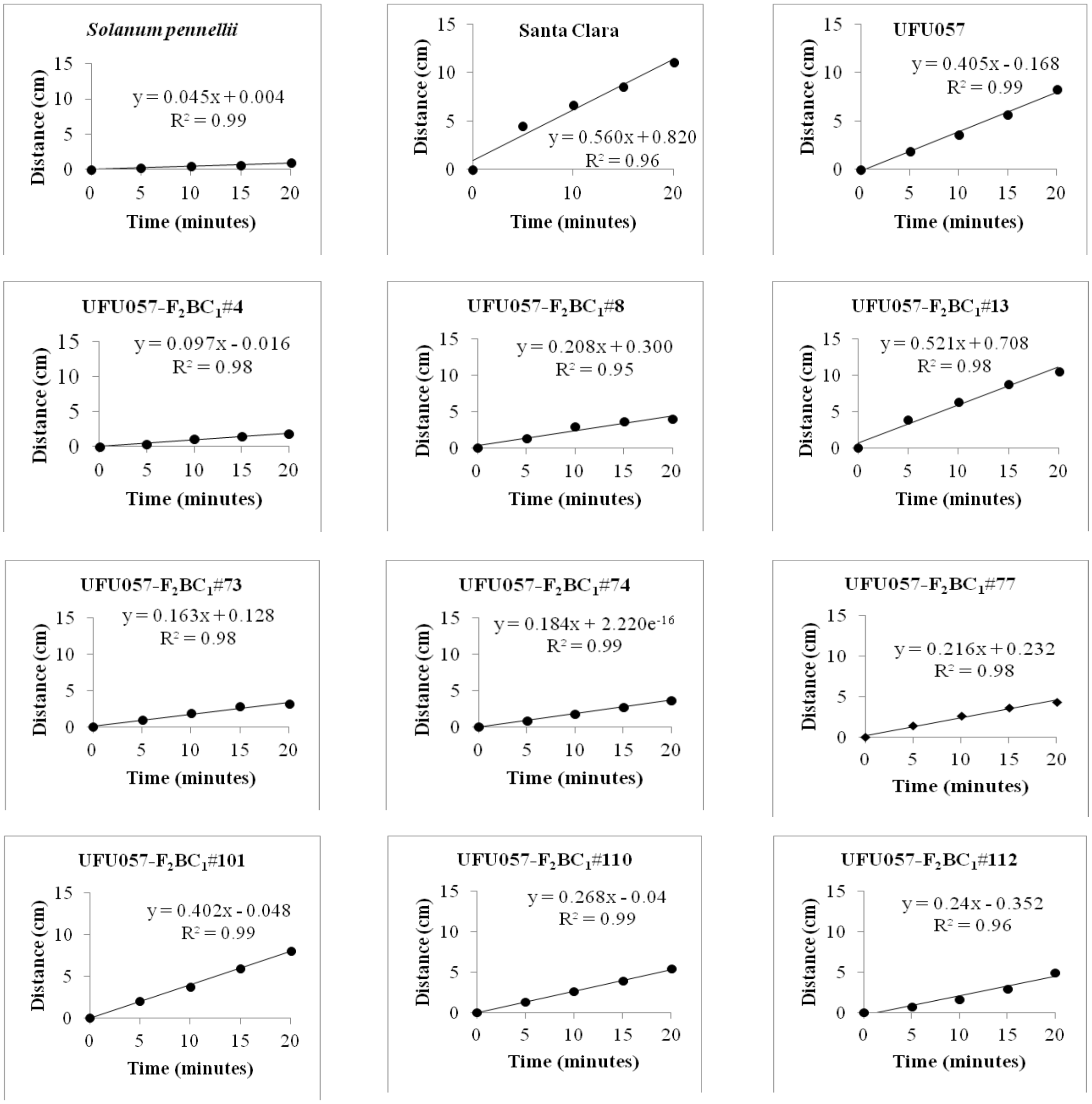
Averages followed by the same letter do not differ statistically from each other in the Scott-Knott test ($P \leq 0.05$)

Figure 1 shows the regression equations and the coefficient of determination that were obtained from the analyses of the repellency test to two-spotted spider mite. The high values of the determination coefficients show a high uniformity of mite advance. After 20 minutes, mites covered a short distance when they were put on the leaflets of the wild access *S. pennellii* (< 0.2 cm), as shown by the low slope of the line (0.045), what may indicate repellency. The result corroborates other experiments that used the wild genotype and Santa Cruz tomatoes as check treatments (Pereira et al., 2008; Maluf et al., 2010; Maciel et al., 2018). On the other hand, mites covered longer distance in the susceptible check treatments cv. Santa Clara and UFU057 (>5 cm).

The mite *Tetranychus urticae* moved faster in the UFU057, UFU057-F_2BC_1#101 and UFU057-F_2BC_1#13 genotypes, and in Santa Clara cultivar. With each minute increase in the observation time of the path taken by the two-spotted spider mite, it showed an average increase in the distance traveled of approximately 0.4 cm in both accesses UFU057 and UFU057-F_2BC_1#101, while in the UFU057-F_2BC_1#13 and Santa Clara genotypes the average distance increase per minute was 0.5 cm and 0.6 cm, respectively. As shown in the Figure 1, these two genotypes presented the highest slope of their lines among all genotypes (0.521 and 0.560, respectively).

Some authors (Pereira et al., 2008; Maluf et al., 2010) found tomatoes lines and hybrids of the Santa Clara type that were rich in acylsugar and resistant to two-spotted spider mite. In the present experiment, the genotypes UFU057-F_2BC_1#4, UFU057-F_2BC_1#73, UFU057-F_2BC_1#77, UFU057-F_2BC_1#112 and UFU057-F_2BC_1#139 stood out, and mites covered only a short distance on the surface of their leaflets (Figure 1). It is emphasized that the genotype UFU057-F_2BC_1#112 stood out in the bioassay of repellency test to mite and also presented high acylsugar content and resistance to silverleaf whitefly (Tables 1 and 2).

In general, the results of the experiment proved the possibility to obtain determinate genotypes, with high acylsugar content and resistance to pests, using wild access and consequent genes transgression.



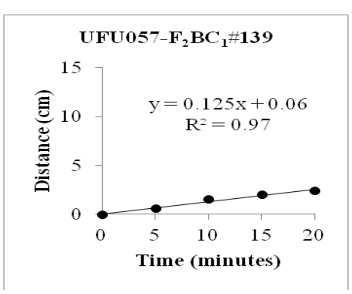


Figure 1. Regression equations of the average distance covered by *Tetranychus urticae* along the surface of the leaflets after 5, 10, 15 and 20 minutes

CONCLUSION

The genotype UFU057-F_2BC_1#112, with determinate growth habit and round fruits, stood out as a promising material for UFU's tomato breeding program, for showing high acylsugar content, moderate level of resistance to the two-spotted spider mite and elevated resistance to silverleaf whitefly.

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