

Article

Characterization of Secondary Metabolites Responsible for the Resistance of Local Tomato Accessions to Whitefly (*Bemisia tabaci*, Gennadius 1889) Hemiptera in Tanzania

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Abstract: Plants have developed mechanisms to cope with stresses in their environments as they grow in diverse settings. Such means include releasing plant defense compounds upon attacks by pests or other stressors. Plants with these characteristics are essential as a plant germplasm source for breeding resistance against herbivores and insect pests. Therefore, this study aimed to screen germplasms for whitefly resistance and characterize the secondary metabolites responsible for this. Thirty local tomato accessions were screened for resistance against whiteflies (*Bemisia tabaci* Gennadius) in the screen house located at Tanzania Plant Health and Pesticides Authority (PTHPA) between January and April 2021. From this screening, seven local tomato cultivars: TZA3729, TZA5554, TZA5545, TZA5562, TZA5552, TZA3177 and TZA5157, showed resistance, and one accession (TZA5496) that showed susceptibility to whiteflies (negative control) and accession V1030462 that was a standard (positive control) were selected for the subsequent experiments. The experiment was conducted in July–October 2021 in the screen house at TPHPA and repeated in January–April 2022 using a completely randomized block design with three replications. From this experiment, three accessions: TZA3729, TZA5562 and TZA5157, showed resistance against whiteflies. However, accession TZA3729 was more resistant than TZA5562 and TZA5157 when compared to the resistant accession V1030462. Therefore, these accessions were further screened for secondary metabolites responsible for resistance against herbivores and insect pests—in this case, whiteflies. The GS-MS methanol extract results showed accession TZA3729 to possess a wide array of secondary plant metabolites responsible for plant self-defenses, such as diterpenes, Tetraterpenes, alkaloids, carotenoids and fatty acid esters. Therefore, the study recommends accession TZA3729 as a source of tomato plant germplasm for breeding tomatoes resistant to whiteflies.

Keywords: tomato accessions; secondary metabolites; resistance; susceptible tomato cultivars; GC-MS



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1. Introduction

Tomato is an important food and cash crop produced globally for its fruit [1,2] and the second most important economic horticultural crop after potatoes globally [3]. Tomato fruits are rich in iron; phosphorus and vitamins A, B and C, essential for a healthy life [1]. In addition, tomatoes contain lycopene, flavonoids, ascorbic and phenolic acids, compounds necessary to prevent chronic diseases and improve health [4]. Despite its importance, tomato production in Tanzania is estimated at 19 tons/Ha [5], which is very low as compared to the global production of 34 tons/Ha [1]. The most factors contributing to low tomato production are harsh weather, poor soil fertility, pest and diseases [6], with insect pests being the major contributor [7,8].

Whitefly is among the insect pests threatening tomato production [9]. This insect pest is winged, and it is in the order Hemiptera and primarily resides under the leaf surface of the host plant [10]. The adults and nymphs extract the plant nutrients through feeding on the plant phloem of more than 1000 plant species [11,12]. *Bemisia tabaci* also produces honeydew, a sugar substance that encourages the growth of black sooty mold on the vegetative plant parts. This mold interferes with the plant photosynthesis, consequently reducing production and product quality [10]. In addition to these effects, whiteflies vector >350 pathogenic plant viruses that cause diseases of economic importance in vegetables and other crops [2,13]. Generally, a pest can wipe out all the crops in tropical and subtropical regions of the world, resulting in 100% economic loss [14], which is quantified into more than a hundred million dollars annually [4].

Efforts towards whitefly control are much based on the use of synthetic pesticides [15], which proved ineffective due to the high ability of this pest to develop pesticide resistance [16]. Furthermore, tomato production farmers lack appropriate pesticide application knowledge, which leads to improper pesticide usage. As a result, it affects the non-target organisms and pollutes the environment and residue of the products that affect consumers [17], making pesticides unsuitable for crop production [15].

Previous works have stressed the need to develop and adopt sustainable farming to promote environmentally friendly pest control practices [18]. The use of tomato cultivars resistant to whiteflies is a great option and an essential element in Integrated Pest Management (IPM) [19]. Several wild tomato relatives are reported to be resistant to whiteflies, with characteristics related to the possession of glandular trichomes that produce and exude allelochemicals or secondary metabolites—for instance, *Solanum galapagense* with type VI trichomes [20]. Other wild tomato species with resistance to *Bemisia tabaci* are *Solanum pimpinellifolium*, *S. habrochaites*, *S. hirsutum* and *S. pennellii* Correll D'Arcy [21]. However, these already existing wild tomato relatives are not enough to provide the required germplasm for breeding tomato varieties to meet the demand for varieties resistant to whiteflies. This is because these wild tomato cultivars are not commercially produced to increase their multiplication and availability [22]. This shortage of wild tomato relatives resistant to insect pests necessitates the continued screening of wild tomato accessions to identify accessions with resistance against insect pests such as whiteflies. It is vital and the first step (pre-breeding activity) in developing resistant cultivars to control herbivore insects [3]. Therefore, the current study was to screen thirty local tomato accessions for resistance against whiteflies, aiming at coming up with resistant accession(s) that will add to the already known resistant accessions for breeding purposes that can lead to an increased supply of the resistant varieties, especially in areas prone to this pest.

2. Materials and Methods

2.1. Experimental Site, Plant Materials and Experimental Design

The study was conducted at Tanzania Plant Health and Pesticides Authority (TPHPA). From January to April 2021, thirty seeds of local tomato accessions obtained from the National Plant Genetic Resource Center located at TPHPA were screened for resistance against whiteflies in the screen house. During the screening, these selected accessions, seeds of *Solanum pimpinellifolium* accession number V1030462 (a resistant variety), were planted in 60-plug seedling trays with peat moss soil and fertilized for ten-day intervals. After four weeks from seed sowing, the seedlings were transplanted in pots in a screen house at TPHPA. The experiment was laid out in a Completely Randomized Design (CRD), each replicated three times.

Two weeks after transplanting, adult whiteflies previously reared on tomatoes in another nearby screen house at TPHPA were identified by morphological features and introduced to the tomato plants (20 adults/plant) as a choice bioassay. Then, two weeks later, the assessment of tomato plants infested (inoculated) with the whitefly pest began. The evaluation was divided into two phases; the first phase was the month after whitefly inoculation, and the second one was the second month post-inoculation.

The two phase evaluations intend to rule out the peak whitefly activities to govern whitefly management. During the assessment, we recorded the number of adult whiteflies, the number of eggs/nymphs per leaflet and the number of leaves deposited with honeydew weekly for two months.

From this screening, seven (7) accessions that showed resistance to whiteflies, one that showed high susceptibility to whiteflies and *Solunum pimpinellifolium*, which is a resistant accession, were selected for further screening between July and October 2021 and repeated in January and April 2022. The screening was carried out as described above in the screening of the thirty local tomato accessions.

2.2. Identification of Metabolites Responsible for Whitefly Resistance

The three identified resistant local accessions of tomatoes were screened for the level of metabolites accountable for inducing resistance, such as flavonoids, terpenes, alkaloids and anthocyanins. The study identified three local tomato accessions that are resistant to whiteflies in the second screening (accessions TZA3729, TZA5562 and TZA5157) as compared to the positive and negative controls, V1030462 and TZA5496, respectively. Such accessions were planted in the screen house at TPHPA. One month following transplanting, the leaves of each accession were picked separately, washed with tape water and dried under shade until they dried, as both dried and fresh plant materials are reported to be used as a source for the extraction of secondary plant metabolites [23]. Therefore, the dried leaves were ground into a refined product by blender and sieved through a 1-mm mesh to get a fine powder. Then, single extraction using methanol solvent was performed to prepare the products for the GC-MS analysis, where GC (Agilent Technologies7890 GC System) mounted to MS (Agilent Technologies 5975C Inert XL EI/CIMSD) was used.

During extraction, 2 g of the sample powder was placed in 50-mL centrifuge tubes and soaked in methanol solvent for 24 h while shaking on an orbital shaker. Then, the extracts were decanted and filtered by cotton wool through a funnel and allowed to evaporate through a rotary evaporator. The resulting product (slurry-like) was added with 0.6 g magnesium sulfate, 0.3 g sodium acetate and 0.2 g sodium citrate simultaneously to remove water, fatty acids, carbohydrates and chlorophyll, respectively, and then, the tubes were centrifuged for 30 min. The mixture was allowed to settle and separated into layers. The upper layer was shifted in a 15-mL centrifuge tube with 0.2 g magnesium sulfate, and 0.1 g florosil was added. Then, the mixture was shaken for one minute and centrifuged for 30 min. Lastly, 1 mL of the upper layer containing florosil solvent (for cleaning the extract) was transferred into vials for GC-MS (instrumental) analysis.

All the leaf extracts were MS analyzed using a GC column with a length of 25 m, a diameter of 320 mm and an injection volume of 1 mL. Using a heating rate of 5 °C/s, the temperature was raised to 150 °C and then to 180 °C from a starting temperature of 50 °C. Helium was used as a carrier gas, with a flow rate of 1.2 mL/min and a constant linear velocity of 47.661 cm/s. The detector temperature was kept constant at 250 °C. The chemical constituents of the plant extracts were identified by comparing the retention indices and mass fragmentation patterns of the samples to those found in the National Institute of Standards and Technology (NIST) library.

3. Statistical Analysis

The collected data were subjected to a one-way analysis of variance (ANOVA) using GenStat (15th edition). Treatment means separation was done by Tukey's test at a 5% significance level.

A correlation analysis was done using Jamovi Version 1.2.27 software between the number of adult whiteflies and the number of eggs/nymphs and the yield and between the number of adults and nymphs on the number of leaves deposited with honeydew to work out the resistance or susceptibility of a particular accession. In addition, the identified resistant local accessions of tomatoes were screened for the level of metabolites responsible for inducing resistance, such as flavonoids, terpenes, alkaloids and anthocyanins.

4. Results

From Figure 1, the results on the adult whiteflies recorded in the first and second months of the experiment in the first season (2021) differed significantly ($p \leq 0.001$), with accession TZA3729 and V1030462 having the least number of adult whiteflies/leaflet: 5.3 and 6, respectively, followed by accession TZA5562 with 9 adult whiteflies per leaflet. The negative control (accession TZA5496) had 73 adult whiteflies, which is the highest mean.

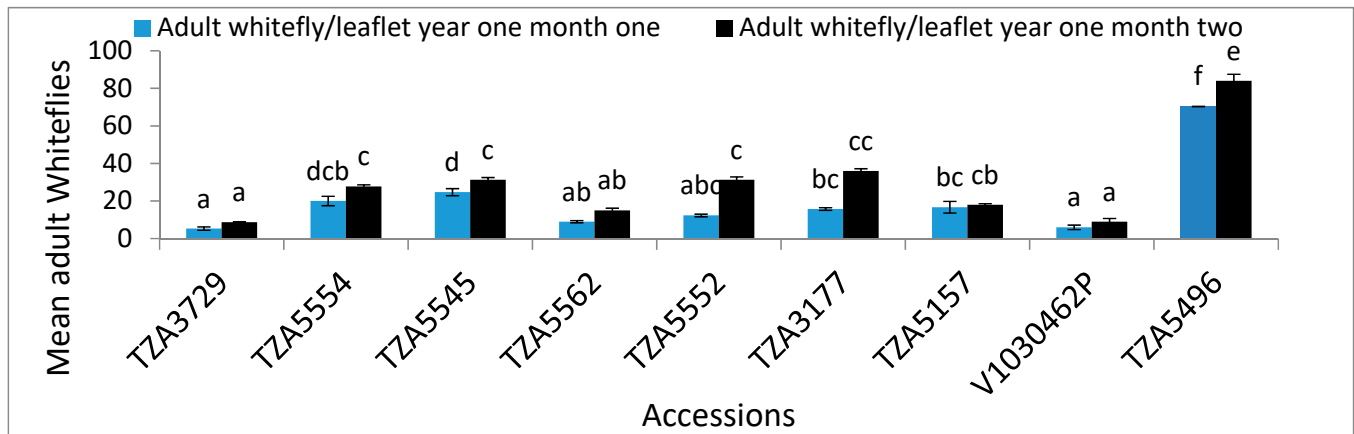


Figure 1. Mean adult whiteflies/leaflet in the first and second months of the first season assessment ($p = 0.001$). Bars followed by the same letter(s) are not significantly different at $p = 0.05$.

As well, accessions recorded differed mean egg/nymph ($p \leq 0.001$), where accessions V1030462 and TZA3729 had the least eggs/nymphs, with one and two eggs/nymphs, respectively, followed by accessions TZA5562 with 9 and accession TZA5157 with 9.3 mean egg/nymph per leaflet, as indicated in Figure 2.

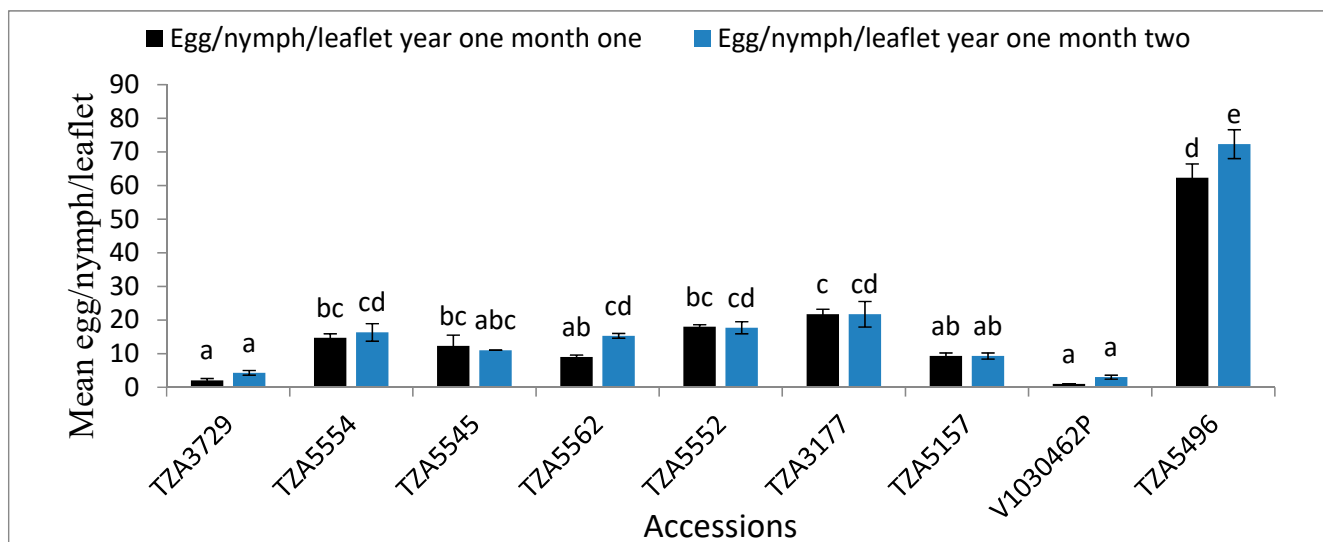


Figure 2. Mean egg/nymph/leaflet in the first and second months of the first season assessment ($p = 0.001$). Bars followed by the same letter(s) are not significantly different at $p = 0.05$.

In Figure 3, accessions TZA3729 and TZA5562 have the least mean number of leaves deposited with honeydew (0.3), followed by accessions V1030462 and TZA5157 with 0.6 and 1, respectively. Such means differed significantly ($p \leq 0.001$).

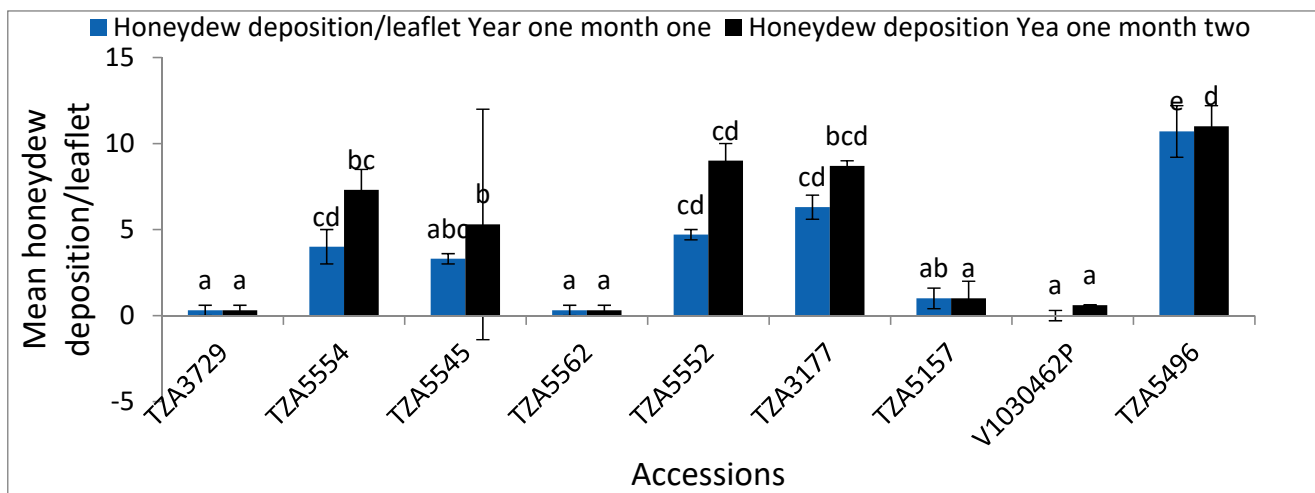


Figure 3. Mean honeydew deposition/leaflet in the first and second months of the first season assessment ($p = 0.001$). Bars followed by the same letter(s) are not significantly different at $p = 0.05$.

The same trend was recorded in the second season of the experiment, where accessions that showed a high performance in the first season (July–October 2021) also performed better in the second season (January–April 2022) of the experiment. In this season, the accessions also showed differing mean adult whiteflies in the first and second months of the assessment ($p \leq 0.001$). For example, from Figure 4, accession V1030462 had the least adult whiteflies in both the first and second months of the second season: 1.7 and 3, respectively, preceded by accession TZA3729 with 6.3 and 6, respectively, and then accessions TZA5562 and TZA5157 with 7.3 and 14.3 and 7.7 and 9 adults whiteflies/leaflet, respectively.

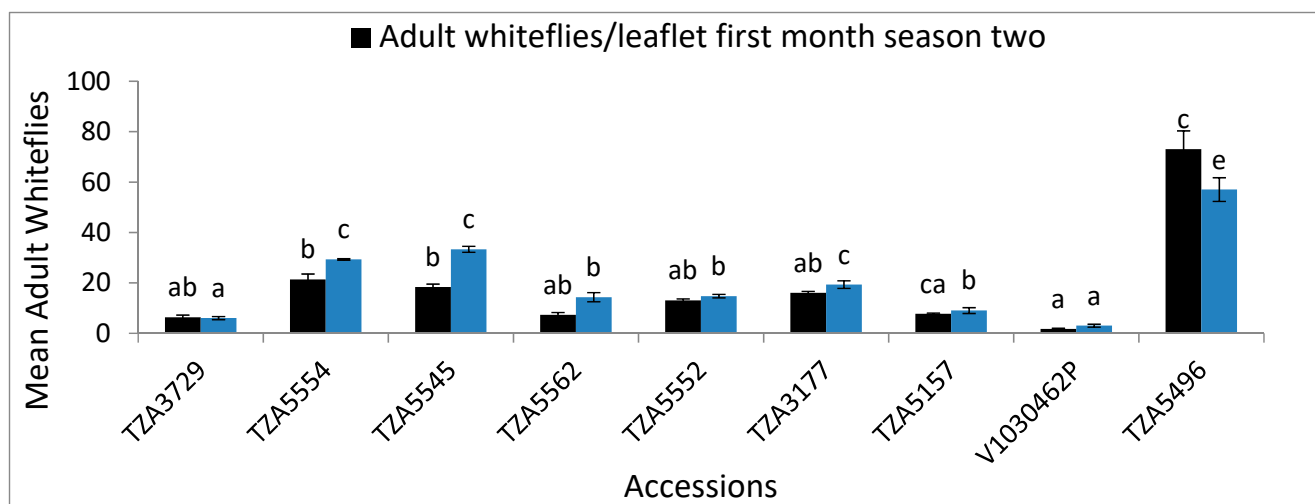


Figure 4. Mean adult whiteflies/leaflet in the first and second months of the second season assessment ($p = 0.001$). Bars followed by the same letter(s) are not significantly different at $p = 0.05$.

In the case of egg/nymph mean, accessions recorded differing egg/nymph means ($p \leq 0.001$), with accessions V1030462 and TZA3729 having the least means in both months: 1 and 2 in the first and 3 and 4 in the second months of the assessment, respectively, followed by accessions TZA5562 and TZA5157, as indicated in Figure 5.

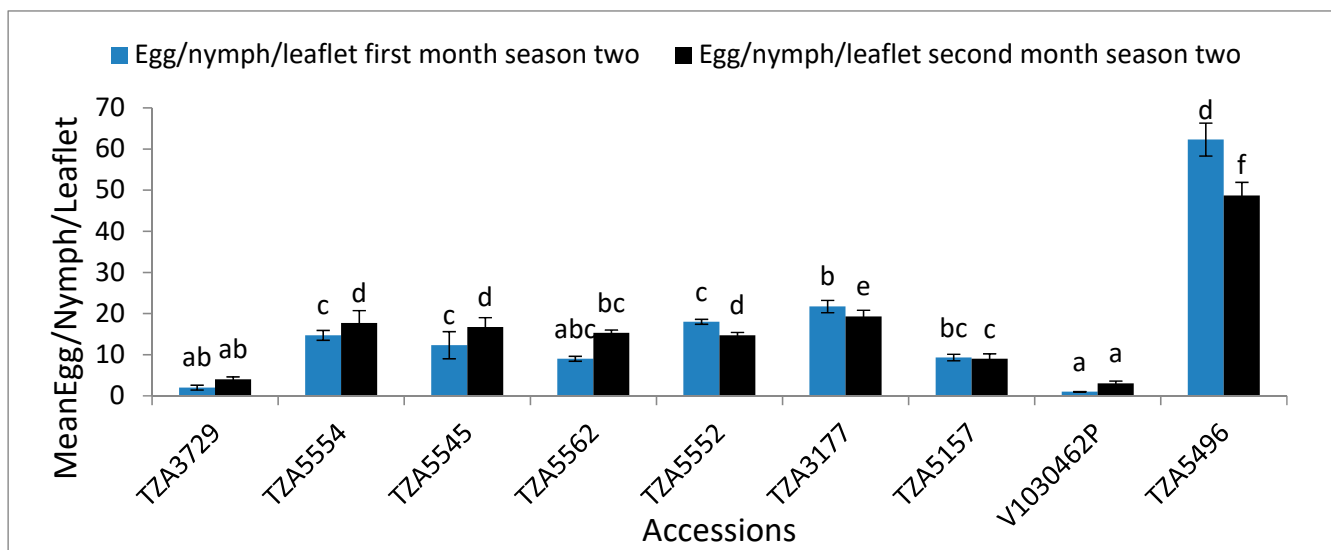


Figure 5. Mean egg/nymph/leaflet in the first and second months of the second season assessment ($p = 0.001$). Bars followed by the same letter(s) are not significantly different at $p = 0.05$.

Accessions V1030462 and TZA5157 were not deposited with any honeydew in the first month of the second assessment season. However, accession TZA5157 had one leaflet deposited with honeydew in the second month of the second season of the evaluation. On the other hand, accessions TZA3729 and TZA5562 recorded only 0.3 mean leaflets deposited with honeydew in both the first and second months in the second season of the assessment. However, accession TZA5496 had the maximum mean number of leaves deposited with honeydew in the first and second months of the assessment: 7.3 and 10.7, respectively, as shown in Figure 6.

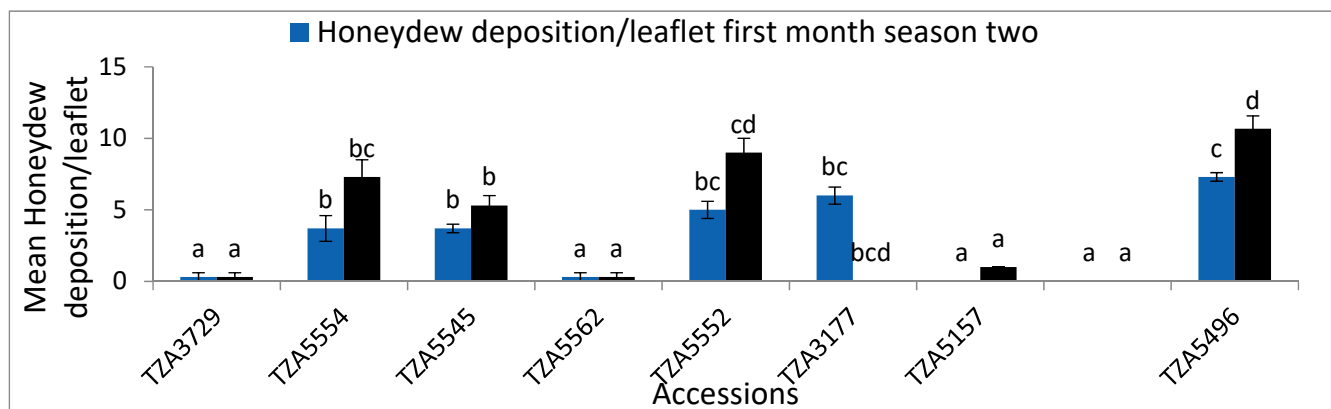


Figure 6. Mean honeydew deposition/leaflet in the first and second months of the second season assessment ($p = 0.001$). Bars followed by the same letter(s) are not significantly different at $p = 0.05$.

In terms of yield per plant, accessions that scored lower means of the other measured parameters (adult whitefly, egg/nymph and honeydew deposition) in the two seasons, as indicated in Table 1, had higher yields than those accessions with higher measured parameters. This scenario makes the yield differ significantly ($p \leq 0.001$), with accessions TZA3729 and V1030462 having the highest yields of 1.4 kg and 1.1 of the local tomatoes per plant in the first and second seasons, respectively, preceded by accessions TZA5562 and TZA5157 having 1.2 kg and 1 kg of the local tomatoes per plant in the first and second seasons and with accession TZA5496 having the least yield of 0.1 kg per plant in both seasons, as indicated in Figure 7.

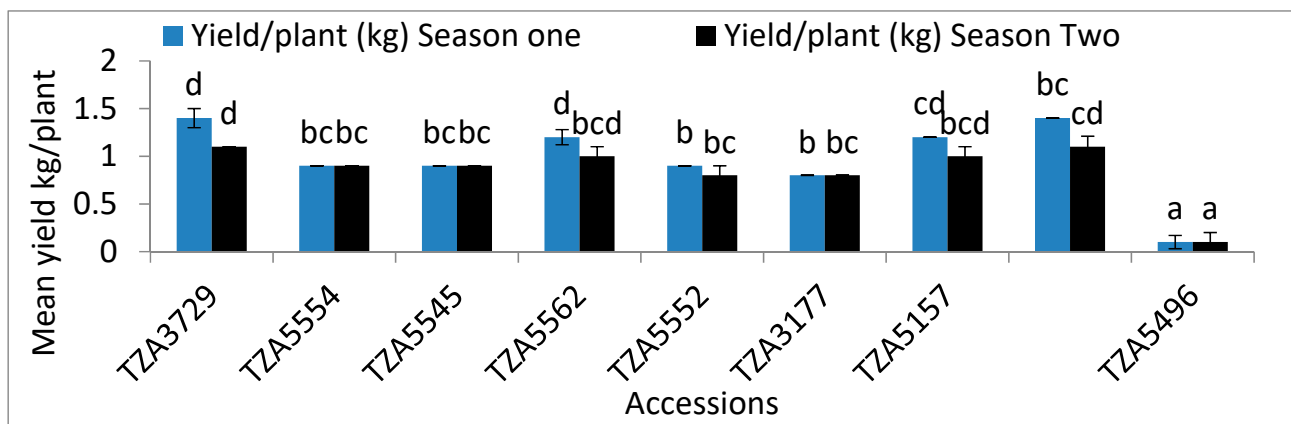


Figure 7. Mean yield kg/plant in the first and second seasons assessments ($p = 0.001$). Bars followed by the same letter(s) are not significantly different at $p = 0.05$.

Table 1. Performance of the seven selected local tomato accessions against whiteflies (*Bemisia tabaci*) in the two tomato-growing seasons, 2021 and 2022, in comparison to the resistant and susceptible accessions. Each value is a mean $\pm$ standard error of the three replicates. Means within the same column followed by the same letter(s) are not significantly different.

Season One (July–October 2021)								Season Two (January–April 2022)						
Assessment from 30 Days Post-Inoculation				Assessment from 60 Days Post-Inoculation				Assessment from 30 Days Post-Inoculation			Assessment from 60 Days Post-Inoculation			
Accession	AW/L	E/N/L	HD/L	AW/L	E/N/L	HD/L	Y/P	AW/L	E/N/L	HD/L	AW/L	E/N/L	HD/L	Y/P (kg)
TZA3729	5.3 $\pm$ 0.9 _a	2 $\pm$ 0.6 _a	0.3 $\pm$ 0.3 _a	8.7 $\pm$ 2 _a	4.3 $\pm$ 0.7 _a	0.3 $\pm$ 0.3 _a	1.4 $\pm$ 0.1 _d	6.3 $\pm$ 0.9 _{ab}	2 $\pm$ 0.58 _{ab}	0.3 $\pm$ 0.3 _a	6. $\pm$ 0.6 _a	4 $\pm$ 0.6 _{ab}	0.3 $\pm$ 0.3 _a	1.1 $\pm$ 0 _d
TZA5554	20 $\pm$ 2.5 _{dcd}	14.7 $\pm$ 1.2 _{bc}	4 $\pm$ 1 _{bcd}	27.7 $\pm$ 0.9 _c	16.3 $\pm$ 2.6 _{cde}	7.3 $\pm$ 1.2 _{bc}	0.9 $\pm$ 0.0 _{bc}	21.3 $\pm$ 2.2 _b	14.7 $\pm$ 1.2 _c	3.7 $\pm$ 0.9 _b	29.3 $\pm$ 0.3 _d	17.7 $\pm$ 3 _d	7.3 $\pm$ 1.2 _{bc}	0.9 $\pm$ 0.0 _{bcd}
TZA5545	24.7 $\pm$ 1.9 _d	12.3 $\pm$ 3.2 _{bc}	3.3 $\pm$ 0.3 _{abc}	31.3 $\pm$ 1.2 _c	11 $\pm$ 2.1 _{abcd}	5.3 $\pm$ 6.7 _b	0.9 $\pm$ 0.0 _{bc}	18.3 $\pm$ 1.2 _b	12.3 $\pm$ 3.3 _c	3.7 $\pm$ 0.3 _b	33.3 $\pm$ 1.2 _{cd}	16.7 $\pm$ 2.3 _d	5.3 $\pm$ 0.7 _b	0.9 $\pm$ 0 _{bcd}
TZA5562	9 $\pm$ 0.6 _{ab}	9 $\pm$ 0.6 _{ab}	0.3 $\pm$ 0.3 _a	15 $\pm$ 1.2 _{ab}	15.3 $\pm$ 0.7 _{bcd}	0.3 $\pm$ 0.3 _a	1.2 $\pm$ 0.08 _d	7.3 $\pm$ 0.9 _{ab}	9 $\pm$ 0.6 _{abc}	0.3 $\pm$ 0.3 _a	14.3 $\pm$ 1.8 _b	15.3 $\pm$ 0.7 _{bc}	0.3 $\pm$ 0.3 _a	1 $\pm$ 0.1 _{bcd}
TZA5552	12.3 $\pm$ 0.7 _{abc}	18 $\pm$ 0.6 _{bc}	4.7 $\pm$ 0.3 _{cd}	31.3 $\pm$ 1.5 _c	17.7 $\pm$ 1.8 _{cde}	9 $\pm$ 1 _{cd}	0.9 $\pm$ 0.0 _b	13 $\pm$ 0.6 _{ab}	18 $\pm$ 0.6 _c	5 $\pm$ 0.6 _{bc}	14.7 $\pm$ 0.7 _b	14.7 $\pm$ 0.7 _d	9 $\pm$ 1 _{cd}	0.8 $\pm$ 0.1 _{bc}
TZA3177	15.7 $\pm$ 0.7 _{bc}	21.7 $\pm$ 1.5 _c	6.3 $\pm$ 0.7 _{cd}	36 $\pm$ 1.2 _c	21.7 $\pm$ 3.8 _{de}	8.7 $\pm$ 0.3 _{bcd}	0.8 $\pm$ 0.0 _b	16 $\pm$ 0.6 _{eab}	21.7 $\pm$ 1.5 _{bc}	6 $\pm$ 0.6 _{bc}	19.3 $\pm$ 1.5 _c	19.3 $\pm$ 1.5 _e	0 _{bcd}	0.8 $\pm$ 0 _b
TZA5157	16.7 $\pm$ 3.1 _{bc}	9.3 $\pm$ 0.9 _{ab}	1 $\pm$ 0.6 _{ab}	18 $\pm$ 0.6 _{cb}	9.3 $\pm$ 0.9 _{abc}	1 _a	1.2 $\pm$ 0.0 _{cd}	7.7 $\pm$ 0.3 _{cab}	9.3 $\pm$ 0.8 _{bc}	0 $\pm$ 0.00 _a	9 $\pm$ 1.15 _b	9 $\pm$ 1.2 _{bc}	1 $\pm$ 0 _a	1.0 $\pm$ 0.1 _{bcd}
V1030462	6. $\pm$ 1.2 _a	1 $\pm$ 0.0 _a	0.6 $\pm$ 0.3 _a	9 $\pm$ 1.7 _a	3 $\pm$ 0.6 _a	0 _a	1.4 $\pm$ 0.0 _{bcd}	1.7 $\pm$ 0.3 _a	1 $\pm$ 0.00 _a	0 $\pm$ 0.00 _a	3 $\pm$ 0.58 _a	3 $\pm$ 0.58 _a	0 $\pm$ _a	1.1 $\pm$ 0.11 _{cd}
TZA5496	70.3 $\pm$ 0 _f	62.3 $\pm$ 4.1 _d	10.7 $\pm$ 1.5 _e	84 $\pm$ 3.5 _e	72.3 $\pm$ 4.3 _f	11 $\pm$ 1.2 _d	0.1 $\pm$ 0.07 _a	73 $\pm$ 7.3 _c	62.3 $\pm$ 4.0 _d	7.3 $\pm$ 0.3 _c	57 $\pm$ 4.7 _e	48.7 $\pm$ 3.2 _f	10.67 $\pm$ 0.9 _d	0.1 $\pm$ 0.1 _a
F-S	138.4	82.1	41.1	176.3	71.2	36.2	68.2	56.1	71	29.4	169.9	47	42.9	33.7
<i>p</i>	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001

AW/L—Adult Whitefly per leaflet, leaflet E/NY/L—Egg/Nymph per, HD—Honey Deposition per leaflet, Y/P = Yield per Plant and F-S = F-Statistics.

We conducted a correlation analysis to rule out the relationships between the study variables. From Table 2, a positive correlation existed between adult whiteflies and eggs/nymphs ($p \leq 0.001$) and between adult whiteflies and honeydew deposition ($p \leq 0.001$). Additionally, a negative relationship existed between adult whiteflies and the yield ($p \leq 0.001$), egg/nymph and the yield ($p \leq 0.001$) and between honeydew deposition and the yield ($p \leq 0.001$).

Table 2. Correlation matrix showing the relationships between the study variables.

		Adult Whiteflies	Egg/Nymph	Honeydew Deposition	Yield (Kg)
Adult Whiteflies	Pearson's r	-			
	<i>p</i> -value	-			
Egg/Nymph	Pearson's r	0.928	-		
	<i>p</i> -value	<0.001	-		
Honeydew Deposition	Pearson's r	0.826	0.662	-	
	<i>p</i> -value	<0.001	<0.001	-	
Yield (Kg)	Pearson's r	−0.921	−0.804	−0.849	-
	<i>p</i> -value	<0.001	<0.001	<0.001	-

The GC-MS results from the three screened accessions indicated the presence of some secondary metabolites that are reported to act against insect pests during crop production. All three local tomato accessions: TZA3729, TZA5562 and TZ5157, exhibited resistance against whiteflies in the first and second screen house experiments and showed an array of secondary metabolites responsible for plant growth self-defenses. Accession TZA3729 showed more resistance in the screen house experiment and had more secondary metabolites for self-defense than accessions TZA5562 and TZ5157, as indicated in Table 3. Such secondary metabolites included different terpenes such as diterpenes and tetraterpenes such as phorbol. Additionally, alkaloids, carotenoids and lipid esters such as palmitoleic acid and trilinolein were present. Some phenolic compounds, such as flavonoids, were also present in the studied resistance accessions.

Table 3. Major chemical compounds identified in methanolic extract of the local tomato accessions screened.

Chemical Information				Accessions	References
Name	Formula	Class	R/T (Min)		
1,2-15,16-Diepoxyhexadecane	C ₁₆ H ₃₀ O ₂		34.769	TZA5562	Unknown
3,7,11,15-Tetramethyl-2-hexadecan-ol	C ₂₀ H ₄₀ O	Phytol/Diterpene	15.034	V1030462, TZA5562, TZA5157	Insect repellant [24], Toxic to Insects and herbivore [25]
Demeclocycline/Declomycin/Clortetrin	C ₂₁ H ₂₁ ClN ₂ O ₈		50.968	TZA5562	Unknown
4aR*,6S*,7S*,7As*,8E,10R*,11R*,11As*	C ₃₂ H ₃₉ NO ₁₀	Alkaloid		V1030462, TZA3729, TZA5562, TZA5157	Affect insect nerve transmission [26]
2-(5-(5-[Cyano-(9,9-dimethyl-1,4-dioxo-7-aza-spiro [4.4] 3-Pyridinecarboxylic acid,2,7,10-tris(acetyloxy-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-oxo-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester,[1aR-(1aR*2R*,3S*.non-7en-8-yl)-methylene]-3,3-dimethylpyrrolidin-2-ylidenemethyl)-3,3-dimethyl- ^δ 1-pyrrolin-5-ylidenemethyl-4,4,4-trimethyl ^δ 1-pyrroline-5-carbonitrile]	C ₃₂ H ₄₂ N ₆ O ₂	Alkaloid		V1030462, TZA3729	Affect insect physiology and behaviour [27]
7,8-Epoxy lonostan-11-ol,3-acetoxy-	C ₃₂ H ₅₄ O ₄	Flavonoid/Phenol	49.417	TZA5562	Insecticidal activity [28] Insect pests feeding Deterrents [29] Antifeedant Regulates plant responses to environmental stress [30]
Hematoporphyrin ix	C ₃₄ H ₃₈ N ₄ O ₆	Alkaloid	25.418	V1030462, TZA3729	Affect insect nerve transmission [26]
Lycoxanthin	C ₄₀ H ₅₆ O	Tetraterpene	48.073	V1030462, TZA3729, TZA5562, TZA5157, TZA5496	toxins to herbivore, feeding deterrents, or oviposition deterrents [31]
4a-Phorbol12,13-didecanoate	C ₄₀ H ₆₄ O ₈	Tetraterpene	30.152	V1030462, TZA3729, TZA5562, TZA5157	Expression of genes responsible for plant defense upon herbivore attack [32]
Rhodopin	C ₄₀ H ₅₈ O	Tetraterpene	28.870	V1030462, TZA3729, TZA5562	Mediate manufacturing of compounds in response to stress factors and herbivore attack [33]
Rhodoxanthin	C ₄₀ H ₅₀ O ₂	Tetraterpene	48.462	V1030462, TZA3729, TZA5562	Act against pathogens, herbivores [34]
Lycopene/β carotene	C ₄₀ H ₅₆	Tetraterpene	28.870	V1030462, TZA3729, TZA5157	Repellant to herbivore arthropods [35]
B,,Psi,-Carotene,3',4,-didehydro-1',2'-dihydro-1',2',-dihydroxy-,(2'R)-	C ₄₀ H ₅₆ O ₂	Tetraterpene	31.485	V1030462, TZA3729, TZA5157	Act against pathogens, herbivores [34]

Table 3. Cont.

Chemical Information				Accessions	References
Name	Formula	Class	R/T (Min)		
Psi,psi,-Carotene,1,1',2,2',-tetrahydro-1,1',-dimethoxy-	C ₄₂ H ₆₄ O ₂	Carotenoid	43.564	V1030462, TZA3729, TZA5562, TZA5157	Unknown
L-Lysine,N6-acetyl-N2-[N-[N(N2-acetyl-N,N,N2-trimethyl-Lasparoginyl)-N-methyl-L-phenylalanui]-N-methyl-L-phenylalanyl]-N,1-dimethyl-L-tryptophyl]-2N,N6-dimethyl-methyl ester.	C ₅₃ H ₇₂ N ₈ O ₉	Alkaloid	42.17428.870	V1030462, TZA3729, TZA5562	Insect toxicity [31] Affect insect cytoskeleton, cell membrane and result into cell leakage and collapse [36]

Over time, plants have evolved a self-defense mechanism against invaders, as they live in a diverse environment [37]. Such mechanisms help plants to deal with stresses, both biotic and abiotic. Upon attack, plants activate defense-related signaling pathways, which trigger the expression of the defense-related phytochemicals and genes [38]. Additionally, plants can distinguish between a physical injury and an herbivore attack, which helps them activate a specific defense response to target stress. Herbivore oviposition fluid or their oral secretion contains active substances called elicitors. Upon herbivore attacks, the plant recognizes such substances and activates its defense by making a series of defensive-related signals, such as the release of some secondary metabolites that may deter feeding or egg laying by the herbivore pest [39].

The current study was built from this basis, where the level of defensive secondary metabolites of local tomato accessions showing resistance against whiteflies upon whitefly inoculation to these tomatoes was conducted. The screening experiment results showed some accessions to resist whitefly attacks. Significant differences were recorded in the mean number of adult whiteflies, the eggs/nymphs and the leaves deposited with honeydew per plant in both seasons. Information from these two seasons of screening gave credit to accession TZA3729, which is more resistant to whiteflies being adults or eggs/nymphs as compared to the performance of the positive control (V1030462), where it performs almost the same as that of the positive control followed by accessions TZA5562 and TZA5157. Differences in yields among these accessions are due to the varying ability of the accessions to resist whiteflies. These findings align with a study that reported tomato production to decrease with the whitefly population, with the production decrease differing among cultivars [40]. The three identified local tomato accessions: TZA3729, TZA5562 and TZA5157, were hypothesized to bear specialized plant structures responsible for plant self-defense, the trichomes.

Trichomes are hair-like, extending from the plant epidermis, and are single-celled or multicellular glandular or non-glandular, which is the distinctive feature among them [41]. They are found on the plant surface and are responsible for plant resistance to herbivores [40]. The glandular trichomes are reported to secrete, store and release natural plant products, also called secondary metabolites accountable for plant self-defenses that are released upon biotic or abiotic plant stress and deposited at the attacked site [30]. For example, the analysis of European corn borer feeding tunnels in maize stems revealed some diterpenoids reported to deter the pest [24]. Phorbol was also present in the identified resistant local tomato accessions. This natural compound and its derivatives were very toxic to insects and herbivores, which concurs with the current study [25].

On the other hand, terpenes were the most abundant in the studied accessions, especially those that showed resistance to whiteflies. The resistant accessions had fewer adult whiteflies, eggs/nymphs, leaves deposited with honeydew and high yields. This indicated a negative correlation between terpenes and different stages of whitefly development and a positive correlation with the tomato yield. Terpenes are also reported to compose the largest group of secondary plant metabolites, where about 55,000 compounds belong to this group of natural products [27]. Members of this group are also reported to be poisonous and deter herbivore insects from egg laying and feeding [31], which is in line with the findings of this study. Terpenes are synthesized from five-carbon precursor isoprene units and are classified based on the same [42]. They are also reported to serve as allelochemicals, as well as defensive toxins and herbivore deterrents which is shown by the low mean number of adults and eggs/nymphs on accession TZA3729, as indicated in Figures 1 and 4 [43].

Terpenes are also reported to mediate inter- and intraspecies interactions, such as manufacturing compounds in response to stress factors and herbivore attacks and attracting beneficial insects such as mites that feed on the insect pests [33]. Additionally, volatile terpenoids penetrate the plasma membrane to increase their permeability due to their lipophilic nature and allow them to exert direct toxic and repellent effects on herbivores [35]. Terpenes have the added advantage of influencing the expression of the genes responsible for plant defense through acting as chemical messengers [32]. Such an ability makes

plants with terpenes resistant to herbivores and insect pests, as revealed in the present study findings.

Additionally, the study results revealed some alkaloids in the accessions that resisted whitefly attacks. This phenomenon is explained by the ability of alkaloid toxicity to affect the neural signal control of the insect pest, thereby changing their expression and concentration, which, in turn, affects the insect's physiology and behavior [31]. The study results also concur with another study that reported alkaloids to play roles in plant defenses against herbivores, as they are very toxic to insects [26]. Additionally, alkaloids are said to act as protective agents that discourage plants from animal and insect attacks and as detoxification agents [33]. Alkaloids are also reported to affect an insect's nerve transmission, cytoskeleton and cell membrane structure, leading to cell leakage and collapses [36]. They also interfere with protein synthesis, DNA replication and enzyme activities of the herbivore insect [44]. They are naturally present in the plant, but their production and accumulation increase upon herbivore attack on the plant. Their ability to react with DNA enzymes and membranes enables alkaloids to bear strong toxic effects on many organisms, including herbivores, as shown in this study [27].

Phenolic compounds, particularly flavonoids, were also present in the local tomato accessions that showed resistance to whiteflies, as per the GC-MS analysis results. These compounds are reported to be the most widely distributed, with about 10,000 compounds classified in the group differing in chemical structures [45]. Some of this group's compounds are flavonoids, tannins, lignin and stilbenes. The findings align with a study that reported flavonoids toxic to herbivore insects and, thus, protect plants against herbivores, as they are produced as a response to plant attack by herbivores or insect pests [45,46]. Phenol is also reported to reduce the population of eggplant fruit moths (*Leucinodes orbonalis*) on eggplants [29]. Additionally, the oxidation of flavonoids by peroxidases or polyphenol oxidases results in toxic metabolites that disturb insect growth physiology and development [28,45]. On top of that, upon the cell wall punctured by herbivore insects, phenolic compounds such as lignin accumulate at the attacked site to block the phloem flow. They are reported to deter herbivores from feeding and are also toxic [27]. For example, phenol, especially those with high concentrations of (Z)-isomers of C16, C18 and C20 coumarates, deterred sweet potato weevils from feeding [25], indicating the power of phenols in fighting insect pests.

Therefore, the study results found out that local tomato accessions that showed resistance to whiteflies had a number of secondary metabolites responsible for plant resistance against this devastating insect pest. Accessions TZA3729, which is the most resistant accession, has six terpenes and four alkaloids, followed by accessions TZA 5562 with two alkaloids, five terpenes and one phenolic compound and accession TZA5157 with one alkaloid and five terpenes. These accessions were compared to the commercially resistant positive control V1030462, which had four alkaloids and seven terpenes, whereas the negative control TZA5496 had only one terpene.

5. Conclusions

Local tomato accession TZA3729 showed a high resistance to whiteflies in the screen house by having a low number of adult and egg/nymph whiteflies and with fewer leaves deposited with honeydew, followed by accession TZA5562 and then TZA5157. Furthermore, the methanol leaf extract GC-MS results indicated that accession TZA3729 possessed several secondary plant metabolites responsible for a self-plant defense also revealed in the positive control/resistant accession used in this experiment compared to accessions TZA5562 and TZA5157. These metabolites had a higher concentration in TZA3729 compared to the other accessions used in this study and included several groups of terpenes, such as diterpenes and tetraterpenes. Others were alkaloids, carotenoids and fatty acid esters. Therefore, this study recommends accession TZA3729 as a source of tomato plant germplasm for breeding resistance against whiteflies in tomatoes.

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