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Original article

Biochemical and molecular diagnosis of different tomato cultivars susceptible and resistant to *Tuta absoluta* (Meyrick) infestation

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ABSTRACT

Resistant plant cultivars which used in breeding programs are considered one of the modern integrated management programs to reduce the usage of synthetic insecticides and environmental contamination the present study aimed to characterize the resistant and susceptible tomato cultivars to *Tuta absoluta* based on biochemical and molecular levels, in Egypt. The biochemical characters of the tested tomato cultivars (tomato- 86, tomato- Alissa, tomato- Fayarouz, tomato- Omniya, tomato- 036, tomato- GS) were determined colorimetrically and characterized by using native- polyacrylamide gel electrophoresis (PAGE) and agarose gel. Our results showed that there were variations highly significant in all biochemical constituents of the resistant tomato cultivar (tomato- 86) compared with the susceptible one (tomato- GS). Also, native-(PAGE) for peroxidase (POD) isoenzymes techniques of the tested tomato cultivars showed variations in protein band numbers and densities in tomato-86 resistant compared with tomato-GS susceptible to *Tuta absoluta* infestation. The correlation coefficient between total phenols and peroxidases in infested tomato leaves and percentages of damaged leaves with the tested insect pest was negative and highly significant, while in case of total proteins and reducing sugars in infested tomato leaves as well as lycopene contents in infested tomato fruits was positive, highly significant and significant, respectively. The correlation coefficient between tomato yield means and the infested fruit percentage with *T. absoluta* larvae was negative and highly significant. Respecting molecular diagnosis random amplified polymorphism DNA- polymerase chain reaction (RAPD- PCR), the results demonstrated that the presence of polymorphism in the resistant tomato cultivar (tomato- 86) compared with (tomato-GS), the most susceptible to the tested insect pest infestation.

1. Introduction

Tomatoes, *Lycopersicon esculentum* Mill. are the most economical crops all over the world (Ingegno et al., 2017a; Ingegno et al., 2017b; EPPO, 2019). It's infested with numerous insect pests and the destructive tomato leafminer, *T. absoluta*, was the major threats to tomato cultivations worldwide (Desneux et al., 2011; Biondi et al., 2018; Mansour et al., 2018; Verheggen & Fontus, 2019). While breeding tomato cultivars to get resistant cultivars is a valuable solution (Hassanien et al., 2020). *T. absoluta* larvae feed on the mesophyll of aerial parts of host plants, mining the leaves, stems, apics, flowers and fruits (Miranda et al., 1998), leading to heavy losses, the traditional management strategies are not efficiently developed (Caparros Megido et al., 2013). Because the presence of *T. absoluta* with high populations in Egypt, where the appropriate climate, it controlled with different insecticides with high dosages (Mohammed, 2015). As a result, it was found a development of resistance in the tested insect pest to these synthetic insecticides. Because of stated above, screening for tolerant/ resistant tomato cultivars to *T. absoluta* infestation and using in plant

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breeding programs. Resistance levels of some tomato cultivars to the tested insect pest have been previously characterized by some authors (Gharekhani and Salek- Ebrahimi, 2014 a & b; Ghaderi et al., 2017).

So, the present study aimed to characterize the susceptible and resistant tomato cultivars using biochemical and molecular determination. These diagnoses will help us in developing susceptible commercial tomato cultivars by transferring specific resistant factors from resistant tomato cultivars and this will be useful in pest management programs against *T. absoluta*.

2. Materials and methods

The biochemical and molecular characterizations of both resistant and susceptible tomato cultivars to *T. absoluta* infestation were conducted in Agriculture Faculty Laboratories, Zagazig University, Egypt.

2.1. Tomato host plant

Six tested tomato cultivars, *L. esculentum* Mill. were cultivated under field conditions in Agriculture Faculty, Zagazig University, Egypt. These cultivars namely; tomato- 86, tomato- Alissa, tomato- Fayarouz, tomato- Omniya, tomato- 036 and tomato- GS were obtained from El- Zahraa Farm, El- Salhia City, Sharkia governorate, Egypt. All agronomic practices as fertilization and irrigation were carried out according to Agricultural Ministry recommendations for each plot area (150 m^2). The leaves samples of the tested tomato cultivars were collected when the tomato leafminer incidence level was at peak for biochemical and molecular diagnoses.

To calculate the infestation percentage with *T. absoluta* larvae on leaves of the different six tomato cultivars, especially during the peak of the tested insect. Leaves samples were taken after 15 days from transplanting and were transferred to the laboratory in paper bags for inspection. Thirty infested leaflets each of the tested six tomato cultivars were recorded carefully using binocular microscope weekly/ plot for 16 weeks. While, in case of calculating the infestation percentage with *T. absoluta* on fruits of the tomato cultivars, ten tomato fruits were transferred to the laboratory in paper bags and cut with clean knife for inspection *T. absoluta* larvae weekly/ plot for seven weeks. The leaf and fruit damages percentage = Number of infested leaves or fruits/ Total number of leaves or fruit × 100.

For determination the tomato yield, fruits were weekly collected from each plot and weighted immediately in the field. The yield of each treatment expressed as Kg of fruits per plot was calculated.

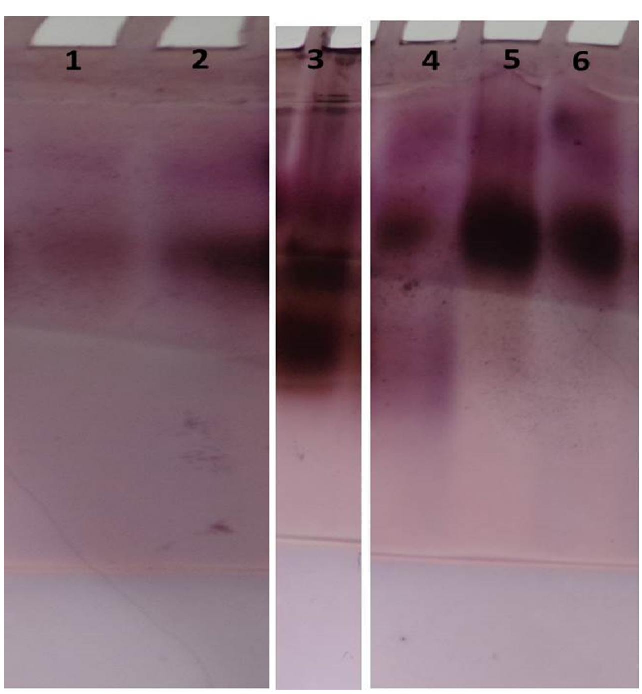

Fig. 1. POD isomers profile in the tested tomato cultivars, L_1: T-GS, L_2: T- 036, L_3: T-86, L_4: T- Omniya, L_5: T- Alissa and L_6: T- Fayrouz.

2.2. Biochemical characterization

Total proteins contents (Saad et al., 2016), peroxidases activities in mature and juvenile tomato leaves, leaves total phenols, leaves reduced sugars, lycopene in fruits and native- PAGE for peroxidases isoenzymes were characterized.

2.2.1. Total proteins

Total proteins concentrations colorimetrically estimated in all tomato leaves samples using a spectrophotometer (6705 UV/Vis. Spectrophotometer, JENWAY) at 595 nm according to Bradford (1976); Saad et al, (2016).

2.2.2. Peroxidase activities

Peroxidase activities were determined colorimetrically in mature and juvenile leaves of the six tested tomato cultivars according to Trevisan et al., (1997).

2.2.3. Total phenols

Determination of total phenols content in leaves of the tested tomato cultivars was done according to the method developed by Singleton et al., (1999); Malick and Singh, (1980); Saad et al, (2021a).

2.2.4. Reducing sugars

Reducing sugars content like glucose, galactose, lactose and maltose in leaves of the six tested tomato cultivars was estimated as the method developed by Benedict, (2015) and Somogyi, (1952); Saad et al, (2021b).

2.2.5. Lycopene pigment

The total lycopene pigment content in fruits of six tomato cultivars was estimated as per the method developed by (Ranganna, 1976).

Lycopene (μg/g fresh weight) = 3.121x OD value at 503 nm × volume of sample × dilution factor/ fresh weight of sample (g) × 100.

*The OD value at 503 nm was taken into consideration. From the standard curve, concentrations of total lycopene content expressed in terms of μg per gram fresh weight for six different tomato cultivars.

2.2.6. Native- PAGE for peroxidase isozymes

Peroxidase isozymes were analyzed using the native- PAGE (10%) according to (Vallejos, 1983; Mahfouze et al., 2012).

2.3. Molecular diagnosis (RAPD- PCR)

The randomly amplified polymorphic DNA (RAPD) technique includes the amplification, by polymerase chain reaction (PCR) of random segment of genomic DNA using a single short primer of arbitrary sequence (Callejas and Ochando, 1998).

2.3.1. DNA extraction

DNA from the tested six tomato leaves was extracted according to Agrawal et al., (1992). A set of 15 primers was analyzed based on the accurate amplified bands profiles of DNA fingerprinting and only six different primers were selected because of their good RAPD profiles shown in Table 1.

2.3.2. DNA amplification cycles

The temperature cycling program used with a Perkin-Elmer Gene Amp PCR system (model 2400) was as follows: one cycle at 94 °C for 5 min followed by 30 cycles consisting of one step of denaturation (94 °C) for 40 sec, one step of annealing (37 °C) for 1 min, followed by one step of synthesis (72 °C) for 2 min and a final extension step 72 °C for 7 min and finally 4 °C infinitive (Williams et al., 1990).

2.3.3. Band analysis

Reaction products were analyzed by electrophoresis on 1.0 % agarose gels stained with ethidium bromide and photographed under UV light. The synthetic DNA, ladder 10000 bp (Pharmacia) was employed as molecular markers for bands molecular weight. Each amplified band profile was defined by the presence or absence of bands at particular positions on the gel. Profiles were considered different when at least one polymorphic band was identified. Fragments were scored based on standard marker using Gel Analyzer 3 (Egygene) software.

2.4. Statistical analysis

All biochemical constituent's data of the six tested tomato cultivars were submitted to one-way analysis of variance (ANOVA) and when significant differences among the averages were found, they were compared with the Duncun Test (Duncan, 1955) at 1% and 5%, using the statistical software SPSS 14.00 software (SPSS Inc. Chicago, Il, USA).

3. Results

3.1. Biochemical characterization

3.1.1. Total proteins

The protein content in the tested different tomato cultivars leaves varied from 0.84 to 6.35 mg/dL. The highest protein content (6.35 mg/dL) was recorded in Tomato-86. The protein content in other tomato cultivars, Alissa, Fayrouz, Omniya and Tomao- 036 were 5.18, 4.18, 3.48 and 2.90 mg/ dL, respectively. Whereas, the lowest protein content was recorded in Tomato- GS (0.84 mg/ dL) (Table 2).

3.1.2. Peroxidase activities

Data in (Table 2) show that there are highly significant differences in peroxidase activities (PODs) according to the tomato leaves ages. PODs activity values in the tested mature tomato leaves were high more than juvenile ones. Also, the obtained results indicated that the POD activity values were increased with highly significant in the infested mature leaves with *T. absoluta* compared with the healthy leaves. POD activities were the highest and descendingly as 13.32, 8.65; 11.38, 7.46; 10.60, 7.24; 9.31, 5.18; 7.03, 4.54 and 5.17, 2.91 mg^{-1} ml^{-1} protein min $^{-1}$ in the infested mature and healthy leaves of Tomato- 86, Tomato- Alissa, Tomato- Fayrouz, Tomato- Omniya, Tomato- 036 and Tomato- GS, respectively.

Respecting the POD activities in juvenile tomato leaves, the results indicated that the POD activities were increased with highly significant in the infested juvenile leaves with the same tested insect pest compared with the activity of the same enzyme in healthy juvenile leaves. The POD activities were the highest and descendingly ordered as 9.47, 5.27; 6.03, 3.22; 5.88, 2.93; 5.09, 2.44; 3.62, 2.55, and 3.03, 1.70 mg^{-1} ml^{-1} protein min $^{-1}$ in the infested and healthy juvenile leaves of Tomato- 86, Tomato- Alissa, Tomato- Fayrouz, Tomato- Omniya, Tomato- 036 and Tomato- GS, respectively.

3.1.3. Total phenols

The phenols content in all the tested tomato leaves ranged 2.24 to 5.05 %. The highest amount of phenol content was present in Tomato- 86 (5.05%) followed by tomato- Alissa (4.72%) and Tomato- Fayrouz (4.02%). Whereas, the lowest one was recorded in Tomato- GS (2.24%) followed by Tomato- 036 (2.82%) and Tomato- Omniya (3.37%) (Table 2).

3.1.4. Reducing sugars

Respecting the reducing sugars (%), the results in (Table 2) indicate that the tomato cultivar Tomato- GS contained the lowest amount of reducing sugars (1.80%) and it was followed by tomato- 036 (3.08%), Tomato- Omniya (3.63%) and Tomato- Fayrouz (5.21%), while the leaves of tomato- 86 had the highest amounts of reducing sugars (7.79%) followed by Tomato- Alissa (6.54%).

3.1.5. Lycopene

The quantity of lycopene content in fruits of different tomato cultivars varied from 72.82 to 390.13 μg/g fr.wt. The cultivar tomato- 86 possessed the highest amount of lycopene (390.13 μg/g fr.wt) followed by tomato- Alissa (332.91 μg/g fr. wt), tomato- Fayrouz (306.90 μg/g fr.wt), tomato- Omniya (265.29 μg/g fr.wt) and tomato- 036 cultivar (114.44 μg/g fr.wt). However, the tomato- GS cultivar (72.82 μg/g fr.wt) showed the lowest amount of lycopene content (Table 2).

3.1.6. Native- PAGE for POD isozymes

The electrophoretic results (native- PAGE) revealed that the presence of three POD isozymes relatively more intensive and thickness in tomato- 86 cultivar, the most resistant tomato cultivar to *T. absoluta* infestation. Our results are accordance with the colormetric determination of total proteins in the tested tomato leaves. Whereas, the two POD isozymes bands in both Tomato- 036 and Tomato- GS were the least intensive and thickness other regarding POD isozymes bands in tomato- Omniya, Tomato- Alissa and Tomato- Fayrouz. It was noticed that these isozymes may play a role in resistant tomato cultivar, particulary T- 86, the most resistant tomato cultivar to *T. absoluta* infestation Fig. 1.

3.2. Correlation between some biochemical constituents in leaves and fruits tomato and T. Absoluta infestation.

The correlation coefficient between proteins in the six tested tomato cultivars and damaged leaves percentage were positively and highly significant (0.719), which indicated that increase in

Table 1
RAPD-PCR primers with their nucleotide sequences in the tested tomato cultivars.

Primer	Nucleotide Sequence (5′- 3′)
1	GTA GCA CTC C
2	GTT GCC AGC C
3	TGG ACC GGT G
4	GTT TCG CTC C
5	GAT GAC CGC C
6	GTG ATC GCA G

Table 2
Some biochemical constituents in leaves (total proteins, peroxidase activities, total phenols and reducing sugars) and fruits (lycopene) of different tested tomato cultivars.

Tomato Cultivar	Total protein (mg/ dL) ± SE	Peroxidase activities mg/ mL Protein/ min ± SE				Total phenol (%) ± SE	Reducing sugar (%) ± SE	Lycopeneμg/g fr.wt ± SE
		Mature leaf		Juvenile leaf				
		infested	Healthy	infested	Healthy			
Tomato- 86	6.35 ± 0.15^{a}	13.32 ± 0.59^{a}	8.65 ± 0.30^{a}	9.47 ± 0.41^{a}	5.27 ± 0.62^{a}	5.05 ± 0.16^{a}	7.79 ± 1.09^{a}	390.13 ± 68.02^{a}
Tomato-Alissa	5.18 ± 0.15^{ab}	11.38 ± 0.40^{b}	7.46 ± 0.41^{b}	6.03 ± 0.06^{b}	3.22 ± 0.15^{b}	4.72 ± 0.43^{a}	6.54 ± 0.31^{ab}	332.91 ± 50.16^{a}
Tomato-036	2.90 ± 1.06^{c}	7.03 ± 0.55^{d}	4.54 ± 0.49^{c}	3.62 ± 0.46^{d}	2.55 ± 0.51^{bc}	2.82 ± 0.30^{ed}	3.08 ± 0.19 ^{cd}	114.44 ± 73.75^{b}
Tomato- Fayrouz	4.18 ± 0.16^{bc}	10.60 ± 1.40^{bc}	7.24 ± 0.52^{b}	5.88 ± 0.48^{bc}	2.93 ± 0.19^{b}	4.02 ± 0.28^{b}	5.21 ± 0.38^{b}	306.90 ± 64.96^{a}
Tomato- GS	0.84 ± 0.55^{d}	5.17 ± 0.33^{e}	2.91 ± 0.44^{d}	3.03 ± 0.05^{d}	1.70 ± 0.19^{c}	2.24 ± 0.25^{d}	1.80 ± 0.73^{d}	72.82 ± 23.84^{b}
Tomato- Omniya	3.48 ± 0.47^{e}	9.31 ± 0.59^{c}	5.18 ± 0.17^{c}	5.09 ± 0.21^{c}	2.44 ± 0.5^{bc}	3.37 ± 0.13^{bc}	3.63 ± 0.13^{c}	265.29 ± 54.06^{a}
F. test	**38.246	**77.645	**84.741	**142.336	**26.538	**47.608	**45.189	**14.138

-** indicates that the differences between means are high significant at 0.01 level of probability.

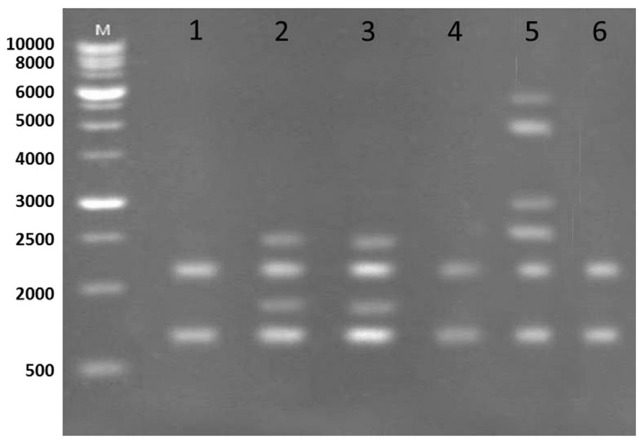

Fig. 2. RAPD- PCR DNA with primer (1) for Lane M: Marker, L_1: T- Fayrouz, L_2: T- Alissa, L_3: T- Omniya, L_4: T- 036, L_5: T- 86 and L_6: T- GS.

Table 3
Simple correlation coefficient between some biochemical constituents in both leaves and fruits of the tested tomato cultivars and percentages of damaged leaves and fruits by larvae of *T. absoluta*.

Tomato Cultivar	Damaged Leaf (%)	Damaged fruit (%)	Tomato yield mean/ cultivar (kg/ 150 m^2)
Tomato- 86	3.25 ± 0.07^{e}	1.32 ± 0.02^{e}	1350.00 ± 522.02^{a}
Tomato- Alissa	3.75 ± 0.05^{d}	4.03 ± 0.07^{c}	526.67 ± 25.17^{c}
Tomato- Fayrouz	4.68 ± 0.02^{b}	4.89 ± 0.01^{a}	606.67 ± 11.55^{c}
Tomato- Omniya	4.93 ± 0.01^{a}	3.79 ± 0.02^{d}	656.67 ± 81.45^{bc}
Tomato- 036	4.50 ± 0.01^{c}	3.96 ± 0.06^{c}	758.50 ± 73.12^{bc}
Tomato- GS	4.72 ± 0.01^{b}	4.69 ± 0.02^{b}	1216.67 ± 76.38^{ab}
r_1	0.719**	–	–
r_2	−0.637**	–	–
r_3	−0.514*	–	–
r_4	−0.562**	–	–
r_5	0.649**	–	–
r_6	–	0.586*	–
r_7	–	–	−0.779**

-r_1, r_2, r_3, r_4, r_5, r_6 and r_7 indicate the correlation coefficient values between protein contents, peroxidase activities in infested mature, infested juvenile leaves, phenols, reduced sugars, lycopene contents in damaged fruits as well tomato yield mean and the infestation percentage in both leaves and fruits with *T. absoluta*, respectively.
-r followed by * or ** mean that the correlation coefficient is statistically significant at 0.05 and 0.01 levels of probability.

tomato proteins and increased infestation with *T. absoluta* (Table 3).

Statistical analysis of the results clearly indicated that the infestation percentages on leaves negatively correlated with peroxidases in both infested mature and juvenile leaves. The correlations were statistically high significant and significant (-0.637& −0.514), respectively. The results showed that the correlations between phenols in the tested tomato leaves and the infestation percentage of *T. absoluta* were negative and high significant (-0.562).

Regarding to reducing sugars in the tested tomato leaves and the *T. absoluta* infestation. the correlations between the infestation percentage on tomato leaves were positive and highly significant (0.649).

The lycopene content in the six tested tomato fruits and the infested fruit percentage with the tested insect pest was positive and significant (0.586).

The correlation relationship between tomato yields and the percentage of tomato fruits infestation with *T. absoluta* was negative and highly significant (-0.779).

3.3. Moecular diagnosis (RAPD- PCR)

RAPD analysis method is a simple and very sensitive method and appears effective in detecting genetic variations among the six tomato cultivars tolerant/ resistant to *T. absoluta* infestation. The molecular weight of fragments after RAPD-PCR reaction with the six primers in the six tomato cultivars were separated, range of fragment size, the total number of fragments, number of monomorphic fragments, number of polymorphic fragments and percentage of polymorphism obtained per RAPD shown in Table 4 and Figs. 2, 3, 4, 5, 6 and 7. A total number of amplified fragments were 45 with average 7.50 fragments per primer which ranged from 400 to 10000 bp approximately. The total polymorphism percentage was 79.21%, where Primer 4 revealed the highest percentage of polymorphism (100 %), while primer 3 exhibited the lowest polymorphism (62.50%). The total polymorphic fragments were 33 with average 5.50 fragments per primer.

Tomato- 86 cultivar recorded the highest value of amplified fragments (31) ranged from 400 to 10000 bp across all the six primers, followed by Tomato- Alissa, Tomato- Fayrouz, Tomato- Omniya and Tomato- 036 tomato cultivars recorded values of amplified fragment (19, 18, 17 and 16) which ranged from 400 to 10000 bp, while Tomato- GS tomato cultivar revealed the lowest value of amplified fragments (15) which ranged from 400 to 10000 bp.

4. Discussions

Resistant cultivars are considered one of the modern integrated pest management methods (IPM) to reduce using insecticides, production costs reduction and finally environment without pollution. In Egypt, there are various tomato cultivars during season. The most destructive insect pest of tomato cultivars in both protected

Table 4
The Polymorphism in fragment size after RAPD-PCR reaction with the six primers in six tomato cultivars.

Primers	Range of fragment size	T-Fayrouz	T-Alissa	T-Omniya	T-036	T-86	T-GS	Total No. of Fragments	Monomorphic Fragments	Polymorphic Fragments	Polymorphism %
1	500–6000 bp	2	4	4	2	6	2	9	2	7	77.78
2	8000–10000 bp	2	1	1	1	4	2	5	1	4	80
3	1500–5000 bp	7	7	6	8	9	7	16	6	10	62.50
4	400–6000 bp	2	2	1	–	2	1	2	–	2	100
5	1500–4000 bp	2	2	2	2	6	2	8	2	6	75
6	500–8000 bp	3	3	3	3	4	1	5	1	4	80
Total	400–10000 bp	18	19	17	16	31	15	45	12	33	79.21
Average	–	3	3.17	2.83	2.67	5.17	2.50	7.50	2	5.50	-

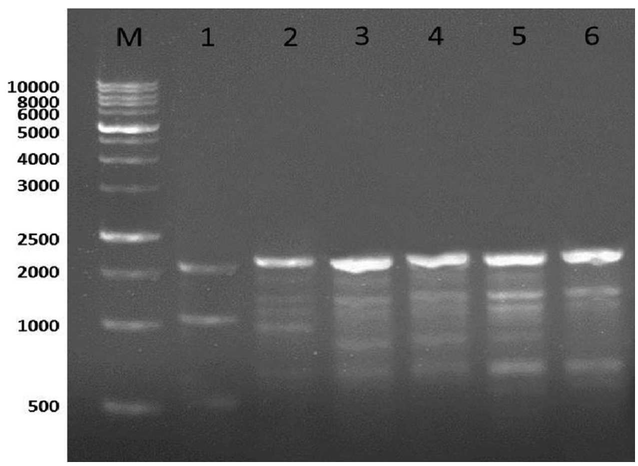

Fig. 3. RAPD- PCR DNA with primer (2) for Lane M: Marker, L_1: T- Fayrouz, L_2: T- Alissa, L_3: T- Omniya, L_4: T- 036, L_5: T- 86 and L_6: T- GS.

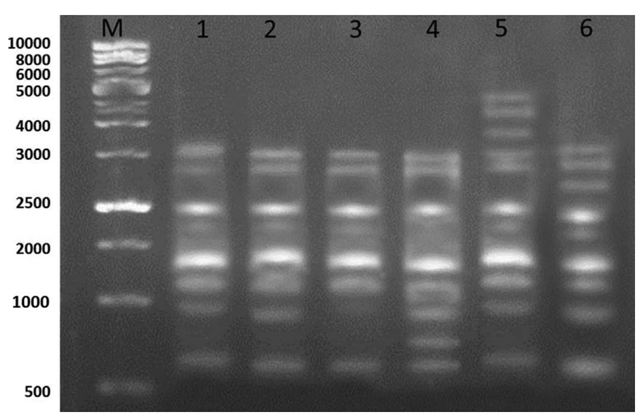

Fig. 4. RAPD- PCR DNA with primer (3) for Lane M: Marker, L_1: T- Fayrouz, L_2: T- Alissa, L_3: T- Omniya, L_4: T- 036, L_5: T- 86 and L_6: T- GS.

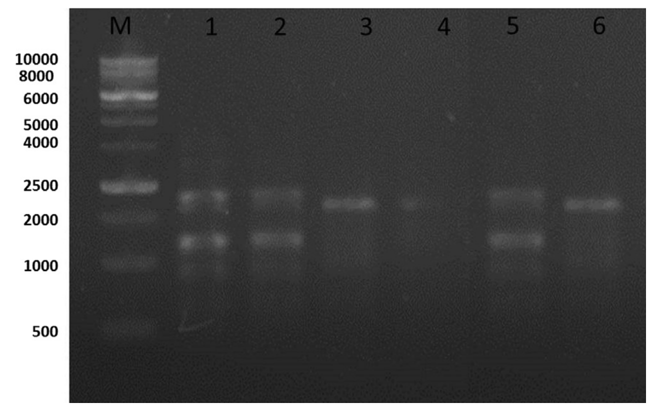

Fig. 5. RAPD- PCR DNA with primer (4) for Lane M: Marker, L_1: T- Fayrouz, L_2: T- Alissa, L_3: T- Omniya, L_4: T- 036, L_5: T- 86 and L_6: T- GS.

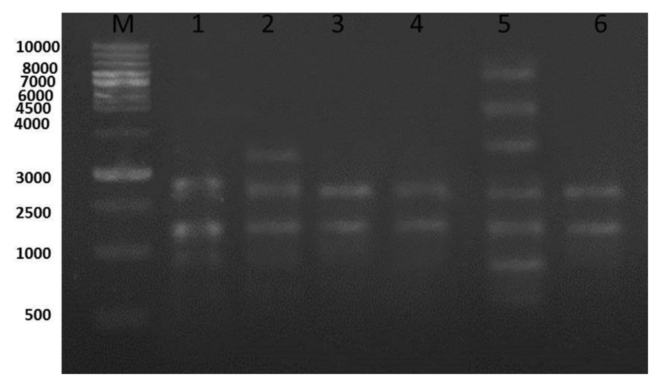

Fig. 6. RAPD- PCR DNA with primer (5) for Lane M: Marker, L_1: T- Fayrouz, L_2: T- Alissa, L_3: T- Omniya, L_4: T- 036, L_5: T- 86 and L_6: T- GS.

and open cultivations is the tomato leafminer, *T. absoluta.* Tomato plants are known to defend themselves against herbivores (Duffey & Stout, 1996; Romeo et al., 1996) and pathogens (McKenzie et al., 2002). The degrees of the defensive traits in host plants are dependent on the age of their leaves (Coley, 1980). In general, young leaves suffer significantly greater grazing damage from herbivorous insects than mature leaves (Reichle et al., 1973), which is presumably because they are nutritionally richer and are less tough than mature leaves (Coley et al., 2006). Insect feeding and pathogens are known to induce the expression of toxic proteins (McKenzie et al., 2002). In fact, the most important constituents of plant protective systems are proteomic defences (Fink & Scandalios, 2002; Rubio et al., 2002). This proteomic profile including total proteins colormetrically and native- PAGE. Peroxidases (PODs), which are known to have a number of functions, including roles in defence against infections by inducing the presence of defensive chemicals (Hammerschmidt et al., 1982; Trevisan et al., 2003) and insect infestation (Quecini et al., 2007). Plants have large number of peroxidase isoenzymes (Welinder, 1992; Quiroga et al., 2000), including tomato plants (Botella et al., 1993). Our results are in accordance with (Rath & Nayak, 2007; Abdul Rasheed et al., 2018), who reported that the higher proteins including POD isoenzymes in tomato cultivars, the lesser tomato infestation with *T. absoluta*. Conversely, Cevahir et al., (2004) assessed different physiological parameters of *Gazania splendens* leaves and found increased POD activity in juvenile as compared to adult leaves. Also, there are other biochemical constituents responsible for tomato resistant cultivars to *T. absoluta* infestation such as phenols, reduced sugars and acyl sugars. Our results are in accordance with (Benerjee & Kalloo, 1989; Selvanarayanan, 2000; Dhakshinamoorthy, 2002; Selvanarayanan & Narayanasamy, 2006), who demonstrated that the high concentrations of phenols and reduced sugars in tomato leaves caused resistance to the fruit

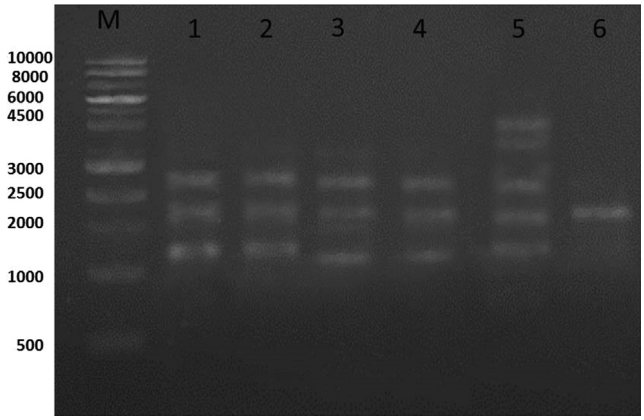

Fig. 7. RAPD- PCR DNA with primer (6) for Lane M: Marker, L_1: T- Fayrouz, L_2: T- Alissa, L_3: T- Omniya, L_4: T- 036, L_5: T- 86 and L_6: T- GS.

borer, *Heliothis armigera*. Abdul Rasheed et al., (2018), in India, revealed that tomato cultivars leaves contained more phenols and reduced sugars contents less infestation with *T. absoluta*. Also, de Falco et al., 2019 reported that *T. absoluta* feeding on tolerant/ resistant tomato cultivars led to increase the production of some compounds such as phenols, peroxidases and defensive proteins.

Nanoparticles exhibited various activates where silver nanoparticles (AgNPs) have insecticidal activity (Saad et al., 2021c). Other nanoparticles such as selenium nanoparticles (SeNPs) (El-Saadony et al, 2021c) can be used to improve the resistance of tomato cultivars because their antioxidant activity.

Nowadays, biochemical and molecular markers are widely used to screen tomato cultivars tolerance/ resistance to infestation with *T. absoluta*. New fragments can be amplified because some sites become accessible to the primer after structural changes in the DNA taking place (Bushra et al., 2002; Enan, 2006). This could be due to point mutations and/or large rearrangements of the DNA. A single point mutation within the primer site can generate significant changes in RAPD patterns (Walsh and McClelland, 1990). There were amplified polymorphism in the most resistant tomato cultivars (T- 86) compared with the other tested tomato cultivars. The results of polymorphisms may overexpression in peroxidase isoenzymes through gene amplifications. These results were indicated by the electrophoretic results (native- PAGE). These results are agreement with (Devonshire et al., 1993; Hemingway, 2000; Saad et al., 2021d), who reported that overexpression of PODs enzymes through gene amplification in *Myzus persicae*. Various natural compounds can be used to enhance tomato resistance against *Tuta absoluta*, these compounds such as bioactive peptides which El-Saadony et al., (2021a, b) reported their activities, additionally, essential oils (El-Tarabily et al., 2021; Abd El-Hack et al., 2021a, b; Alagawany et al., 2021). Erb and Reymond, (2019) indicated that the presence of different genetic variations in tolerant/ resistant tomatoes to *T. absoluta* and these extensive expressed genes are responsibe for the formation of some complicated compounds from secondary metabolism leading to a systemic plant defense response. These compounds resulted from gene amplification in tolerant tomatoes were phenylpropanoids, flavonoids, anthocyanins, alkaloids, terpenoids, glucosinolates), and anti-nutritive enzymes and proteins (e.g., proteinase inhibitors (PIs), amino acid catabolizing enzymes, polyphenol oxidases, and peroxidases).

5. Conclusions

The biochemical and molecular screening of some tomato cultivars tolerant/ resistant to *T. absoluta* at Sharkia governorate. Briefly, the obtained results indicated the presence of higher contents of some biochemical constituents in both resistant T- 86 tomato cultivar leaves and fruits. Also, the gathered data showed the different genetic variations between the susceptible (T- GS) and resistant (T- 86) tomato cultivars to *T. absoluta* infestation. So, we recommend using T-86 cultivar in breeding programs and in integrated pest management against *T. absoluta*, as it has biochemical and molecular factors and to diminish using synthetic insecticides for keeping environment clean and healthy for microorganisms, plants, animals, and humans.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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