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INDUCTION OF PR-PROTEINS AND OXIDATIVE ISOZYMES IN TOMATO GENOTYPES RESISTANT AND SUSCEPTIBLE TO *TOMATO MOSAIC VIRUS* AND *TOMATO SPOTTED WILT VIRUS*

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ABSTRACT

Tomato spotted wilt virus (TSWV) and *Tomato mosaic virus* (ToMV) are two of the most common viruses that threaten tomato crops in Egypt and worldwide. The purpose of this study was to investigate the role of PRs, and oxidative isozymes in the protection of tomato plants from oxidative damage induced by viral infection in 16 tomato genotypes. A total of 16 tomato genotypes were evaluated against TSWV and ToMV separately. Changes in the content of protein and defense enzymes were studied in tomato genotypes resistant and susceptible to TSWV or ToMV. The results of the investigation showed that 16 tomato lines gave different responses to infection with TSWV or ToMV [highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S)]. In this study, the total soluble protein profiles, polyphenol oxidase (PPO), and peroxidase (POX) isozymes of the healthy tomato plants and the TSWV or ToMV infected ones were estimated by electrophoresis in Polyacrylamide gel electrophoresis (PAGE). The results showed quantitative and qualitative differences in the number of bands among the 16 tomato genotypes. Thus, the protein content and isozyme activities were increased or decreased or not changed in inoculated tomato plants with TSWV or ToMV, compared with the un-inoculated plants, depending on the genotype, virus, and degree of resistance. On the other hand, it was found a negative or low-positive correlation between disease incidence and (protein content and isozyme activities). Therefore, it is important to understand the defense strategy of plants against viruses and how tomato plants defend themselves from virus invasion. Therefore, tomato genotypes resistant to TSWV or ToMV could be used in the tomato breeding programs to prevent viral infection.

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INTRODUCTION

Tomato (*Lycopersicon esculentum*) is the second most important vegetable crop next to potato. It is grown on a surface area of 5.0 Mha and has an export value of 14.1 Billion dollars (FAO, 2021). *Tomato spotted wilt virus* (TSWV) and *Tomato mosaic virus* (ToMV) are two of the most devastating viral infections threatening tomato (*Solanum lycopersicum* L.) crops around the world (Saidi and Warade, 2008; Mahfouze *et al.*, 2022). The best strategy for virus control appears to be to use host resistance. Virus resistance is linked to both preformed and induced processes (Hammond-Kosack and Jones, 1996). The preformed mechanisms involve physical barriers like trichomes and cuticles, which prevent the

microorganism invasion into the host tissue, and secondary metabolites, e.g., terpenoids, phenolics, and alkaloids, which can hinder the multiplication of the microorganism. On the other hand, induced resistance is activated by plants after viral infection. It depends on the efficiency of plants in recognizing the presence of virus and transduction of signal (Van Loon *et al.*, 2006). Induced resistance is recognized as a hypersensitivity reaction, i.e., the death of plant cells through hours after infection with the virus. Other reactions involve synthesis of secondary metabolites, accumulation of reactive oxygen species (ROS), structural changes, and the generation of pathogenesis-related proteins (PRPs) (Van Loon *et al.*, 2006; Elvira *et al.*, 2008). PRPs were indicated in *Nicotiana tabacum* that expressed hypersensitivity reaction after invasion by TMV. Later, it was characterized in 13 plant families during a pathogen attack. These proteins are divided into families based on sequences of amino acids and isozyme activities (Van Loon *et al.*, 2006). Van Loon and Van Strien (1999) identified 14 kinds of PRs. The first five classes were discovered, e.g., PR-1, PR-2 (β-1,3-glucanase), PR-3, PR-4 (chitinase), and PR-5 (thaumatin-like) in tobacco plants infected with *Tobacco mosaic virus* (TMV). However, other 17 groups of PRs were discovered later, involving PR-6 (proteinase inhibitor), PR-7 (endoproteinase), PR-8 and PR-11 (chitinase), PR-9 (peroxidase), PR-10 (ribonuclease-like), PR-12 (defensin), PR-13 (thionin), PR-14 (lipid carrier protein), PR-15 (oxalate oxidase), PR-16 (oxalate oxidase-like), and PR-17 (unknown) (Van Loon *et al.*, 2006).

During the defense response against the virus, peroxidase (POX) isozymes share in the synthesis of cell wall such as suberization, phenol oxidation, and lignification of plant host cells (Chittoor *et al.*, 1999). In plants infected by pathogen, increased POX activity has been linked to resistance, which can utilize H_2O_2 as a substrate for cell wall cross-linking. In susceptible plants, POX activity was delayed or stayed unaltered during the compatible reaction (Montalbini *et al.*, 1995; Mohammadi and Kazemi, 2002). Several investigations have found that ROS are important mediators of programmed cell death during the hypersensitive reaction in incompatible reactions (Vranová *et al.*, 2002). In contrast to incompatible interactions, little data is known about the role of ROS in development of symptoms and pathogenesis in compatible interactions.

Polyphenol oxidase (PPO) is a class of enzymes that containing copper, it oxidizes of phenols to o-quinones (Oliveira *et al.*, 2011). On the other hand, o-quinones are extremely reactive compounds that can undergo non-enzymatic secondary reactions to generate melanins, brown complex polymers with protein functional groups (Rolff *et al.*, 2011). PPO is found in chloroplasts. The role of PPOs in plant physiology is less clear. Many studies have found a positive relationship between pathogen tolerance or resistance and PPO expression. For example, potato cultivars showing improved PPO isozyme activity also displayed higher tolerance to *Erwinia carotovora* pathogen caused by soft rot disease (Ngadze *et al.*, 2012). Furthermore, the expression of PPO could be employed as a biochemical marker to predict the result of the reaction between various tomato varieties and the pathogens *Xanthomonas axonopodis* pv. vesicatoria and *Ralstonia solanacearum* which cause bacterial leaf spot and bacterial wilt disease, respectively (Kavitha and Umesha, 2008; Vanitha *et al.*, 2009).

The present study aimed to investigate the role of PRs, and oxidative isozymes in protection of tomato plants from oxidative damage induced by viral infection in 16 tomato genotypes.

MATERIALS AND METHODS

Plant Materials

A total of 16 tomato genotypes, involving accessions and commercial cultivars, were used in this study. The names and sources of these genotypes were mentioned in Table 1. Fifteen tomato seeds from each genotype were sown in a greenhouse at 27 °C:16 °C (Light:Dark), photoperiod of L16:D8 h and relative humidity of 68-75%. Seedlings were grown in peat moss: vermiculite: sand (1:1:1) in pots (Mahfouze and Mahfouze, 2019).

Virus Resistance Tests

Source of Virus Isolates

The TSWV and ToMV isolates were observed from the Virology Laboratory, Department of Agricultural Microbiology, Faculty of Agriculture, University of Ain Shams, which were previously isolated and identified from systemically infected tomato plants. The TSWV and ToMV were maintained on *Nicotiana tabacum* cv. White Barley and *Datura metel* L. plants, respectively (Mahfouze *et al.*, 2022). Systemically infected leaves were used as sources of inoculum in all experiments.

Virus inoculation

One-month age tomato genotypes grown in the greenhouse were mechanically inoculated using TSWV or ToMV infected tomato sap according to Green (1991).

Inoculated plants were observed for the development of virus symptoms for four weeks post-inoculation. All the TSWV or ToMV inoculated tomato plants were tested for the presence of virus by Agristripe using the virus specific polyclonal. These materials were evaluated in two successive seasons, 2019/20 and 2020/21, in the greenhouse.

Evaluation of TSWV and ToMV Infection under Greenhouse Conditions

A disease rating scale of 0 to 4 was adopted and used as described by Gill *et al.* (2019). On the rating scale, 0, 1, 2, 3, and 4 signified 0 = no symptoms [highly resistant (HR)], 1 = slight symptoms visible only on close inspection [resistant (R)], 2 = intermediate symptoms visible on part of the plant [moderately resistant (MR)], 3 = severe symptoms over the entire plant [moderately susceptible (MS)], and 4 = severe symptoms and stunting of the entire plant [susceptible (S)]. The plants that have been rated on the scale of 0, 1, and 2 were considered resistant, while those of 3 and 4 were susceptible.

Serological Assay using Immune Lateral Flow for Detection of TSWV and ToMV

The TSWV and ToMV AgriStrips are a one-step assay which was developed and manufactured by BIOREBA AG, Reinach, Switzerland. Bands start developing after 1-2 min and reach maximum intensity after 10-15 min.

Electrophoretic Analysis of Protein by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was done according to (Laemmli, 1970) as modified by (Studier, 1973) by using 12% SDS gel for total protein profiling. After, the electrophoresis gel was stained with Coomassie Brilliant Blue dye, and then destained for visualizing the protein bands.

Polyphenol Oxidase (PPO) and Peroxidase (POX) Isoforms

For the assay of antioxidant enzymes, POX and PPO were extracted based on the method described in (Stegemann *et al.*, 1985). PPO and POX isozymes were separated by Native-polyacrylamide gel electrophoresis (Native-PAGE). The activities of POX and PPO were determined according to (Baaziz *et al.*, 1994). Relative mobility (*Rf*) values were calculated for each band based on the migration of the band relative to the front or tracking dye (*Rf*). The gels were scored as presence (+) or absence (-) of isozyme bands.

Statistical Analysis

A correlation analysis was performed using Microsoft Excel 2010 with an evaluation of Pearson's correlation coefficients to the determine correlation coefficient (r) between the protein content and defense enzymes (POX and PPO) and disease severity.

RESULTS

Pathogenicity Test

Sixteen tomato lines were tested for resistance against TSWV and ToMV under greenhouse conditions (Table 1). Results of the study showed that two tomato accessions, *S. Peruvianum* 1333 and *S*. *chilense* 56139, were highly resistant (HR) to TSWV (scale value= 0), seven lines were resistant (R) (scale value= 1), e.g., *S. neoricki* 0247, *S. huaylasense* 1358, *S. habrochaites* 1352 and 1739, Super Marmande, *S*. *pennellii* 1942, and *S*. *corneliomulleri* 1274. Two accessions were moderately resistant (MR) (scale value=2) like, *S. pimpinellifolium* 1279 and 1342, four moderately susceptible (MS) (scale value= 3), such as *S. arcanum* 1346, Strain B F1, *S. pimpinellifolium* 1332, and *S*. *pennellii* 2963, and one is susceptible (S) to TSWV infection (scale value= 4), viz. *S. corneliomulleri* 1283. For ToMV, ten lines were highly resistant (HR) (scale value= 0), e.g., *S. neoricki* 0247, *S. huaylasense* 1358, Super Marmande, *S. corneliomulleri* 1283 and 1274, *S. habrochaites* 1739, *S. pimpinellifolium* 1279, *S*. *pennellii* 2963 and 1942, and *S. chilense* 56139. Three genotypes were resistant (R) (scale value= 1), i.e., *S. arcanum* 1346, *S. peruvianum* 1333, and Strain B F1, two accessions were moderately resistant (MR) (scale value= 2), viz., *S. pimpinellifolium* 1332 and 1342, and one moderately susceptible (MS) (scale value=3), such as *S. habrochaites* 1352 (Table 1).

Detection of TSWV and ToMV

Infection with TSWV or ToMV on the tomato leaves of 16 genotypes was confirmed by the ImmunoStrips kits containing polyclonal antibodies against protein specific to TSWV or ToMV. Thus, the presence of a red-colored band in the test line indicated positive results. In contrast, absence of red-colored band in the test line indicated negative results (Figure 1).

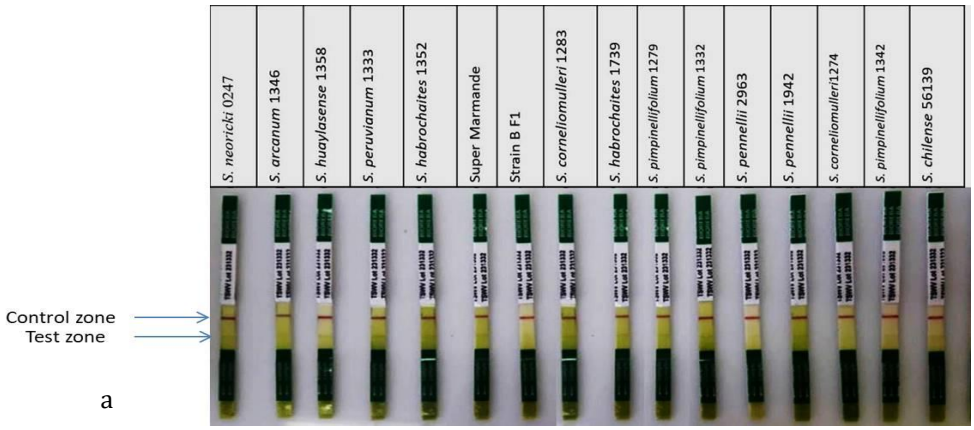
Protein expression of tomato genotypes infected with the Virus

Total soluble protein profiles from the leaf extracts of the 16 tomato genotypes inoculated with TSWV or ToMV and the healthy control were observed by SDS-PAGE. Banding patterns revealed clear differences in total number of bands among tomato lines resistant and susceptible to virus depending on the genotypes.

Table 1. SDS-PAGE and isozyme activities in tomato genotypes inoculated with TSWV or ToMV.

No	Genotype	Source	Scale value and resistance degree				Protein content (SDS-PAGE)		PPO isozymes (Native-PAGE)		POX isozymes (Native-PAGE)	
			TSWV[d]		ToMV[e]		TSWV	ToMV	TSWV	ToMV	TSWV	ToMV
1	*S. neoricki* 0247	TGRC[a]	1	R[g]	0	HR[f]	-	+	No	No	+	No
2	*S. arcanum* 1346	TGRC	3	MS[i]	1	R	No	-	No	No	-	+
3	*S. huaylasense* 1358	TGRC	1	R	0	HR	-	-	+	No	+	No
4	*S. peruvianum* 1333	TGRC	0	HR	1	R	No	No	+	-	+	-
5	*S. habrochaites* 1352	TGRC	1	R	3	MS	-	-	-	+	-	+
6	*S. lycopersicon* cv. Super Marmande	Egypt[b]	1	R	0	HR	+	+	No	+	-	+
7	*S. lycopersicon* cv. Strain B F1	Egypt[b]	3	MS	1	R	-	+	+	+	+	+
8	*S. corneliomulleri* 1283	TGRC	4	S[j]	1	HR	-	+	-	No	N0	No
9	*S. habrochaites* 1739	TGRC	1	R	0	HR	No	-	No	No	+	+
10	*S. pimpinellifolium* 1279	TGRC	2	MR[h]	0	HR	No	+	-	-	-	+
11	*S. pimpinellifolium* 1332	TGRC	3	MS	2	MR	+	+	-	-	No	No
12	*S. pennellii* 2963	TGRC	3	MS	0	HR	-	-	NO	No	+	+
13	*S. pennellii* 1942	TGRC	1	R	0	HR	No	+	-	No	-	No
14	*S. corneliomulleri* 1274	TGRC	1	R	0	HR	+	No	+	No	-	No
15	*S. pimpinellifolium* 1342	TGRC	2	MR	2	MR	-	No	No	+	No	+
16	*S. chilense* 56139	CGN[c]	0	HR	0	HR	-	+	+	+	No	+

[a]TGRC= Tomato Genetics Resource Center (TGRC), Department of Plant Sciences, University of California, Davis, CA 95616 (http://tgrc.ucdavis.edu), [b]Two commercial cultivars were purchased from Egyptian Company for Seeds, Oils and Chemicals, Egypt, [c]CGN= Centre for Genetic Resources, Netherlands (http://www.wur.nl); [d]TSWV= *Tomato spotted wilt virus*; [e]ToMV= *Tomato mosaic vius*; [f]HR= highly resistant; [g]R= resistant; [h]MR= moderately resistant; [i]MS= moderately susceptible; [j]S= susceptible;+ = an increase in bands number; - = a decrease in bands number, No = there were not changed in number of bands.



a

Figure 1 (a). Detection of TSWV in 16 tomato genotypes by Immune lateral flow assay, using the virus specific polyclonal.

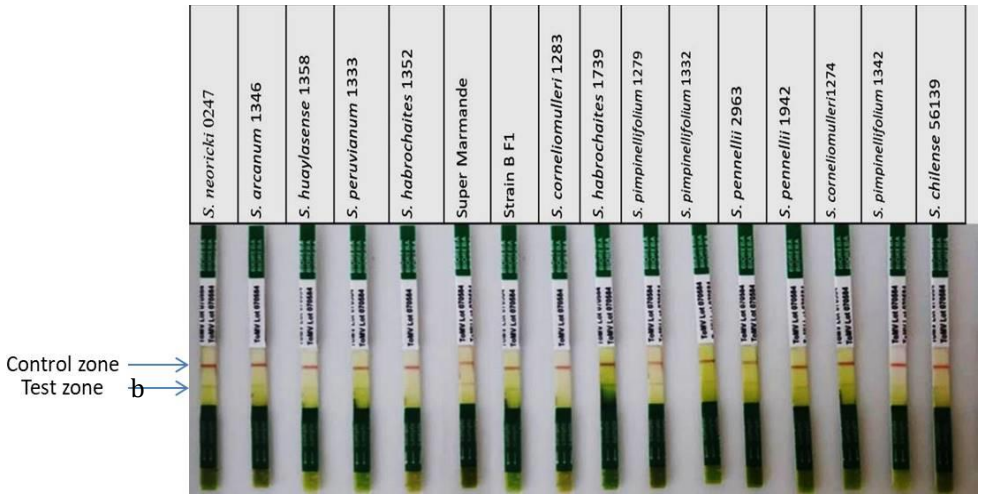


Figure 1 (b). Detection of TSWV in 16 tomato genotypes by Immune lateral flow assay, using the virus specific polyclonal.
Positive result = presence of red-colord band in the test line.
Negative result = absence of red-colored band in the test line.

Analysis results of TWSV showed that two tomato genotypes Super Marmande and *S. corneliomulleri*1274 resistant to TSWV scored three proteins with molecular weights (MWs) of (42, 44, and 122 kDa) and (25, 32, and 170 kDa), respectively. However, the tomato accession *S. peruvianum* 1333 (HR) recorded two PRs of 25 and 170 kDa. Besides, the tomato line, *S. habrochaites* 1352 (R), induced one PR with MW of 32 kDa. On the contrary, two moderately susceptible tomato accessions, e.g., *S. arcanum* 1346 and *S. pimpinellifolium* 1332, displayed one PR of 11 and 25 kDa, respectively (Figure 2 and 3). For ToMV, the highest number of PRs was showed in the highly resistant tomato plants, e.g., *S. corneliomulleri* 1283 (5 PRs of 30, 37, 44, 85, and 90 kDa), *S. pimpinellifolium* 1279 (4 PRs of 68, 85, 90, and 170 kDa), *S. neoricki* 0247 (2 PRs, 90 and 170 kDa), *S. pennellii* 1942 and *S. chilense* 56139 (2 PRs of 44 and 68 kDa). In contrast, the lowest number of PRs was found in the ToMV resistant tomato lines, such as *S. peruvianum* 1333 and Strain B F1 gave one PR with MW of 68 and 90 kDa, respectively (Figure 4 and 5).

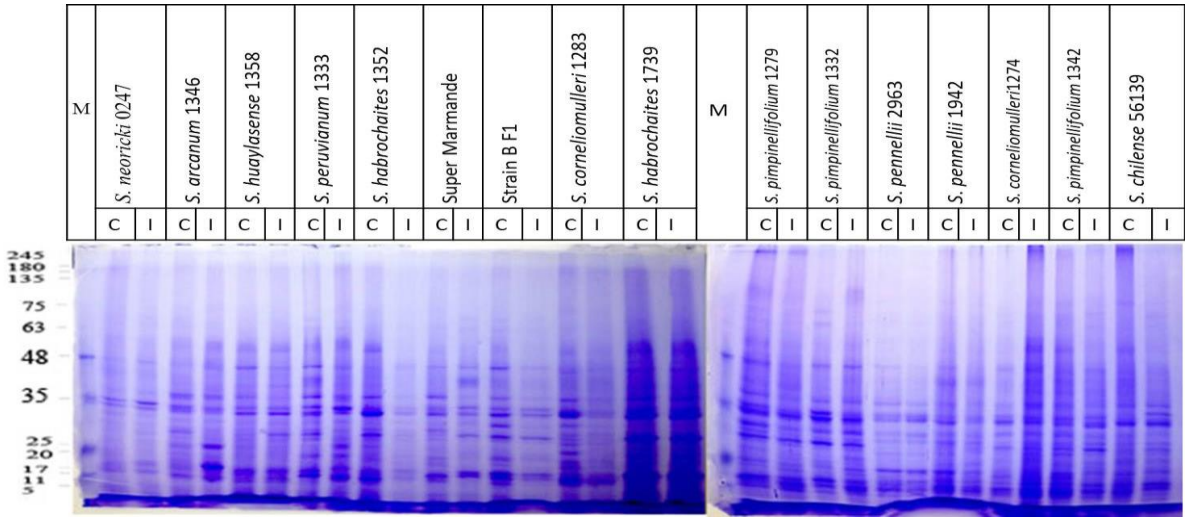


Figure 2. SDS-PAGE binding patterns of total protein extracted from tomato genotypes inoculated (I) with TSWV, compared with the control (C). Lane M: protein marker.

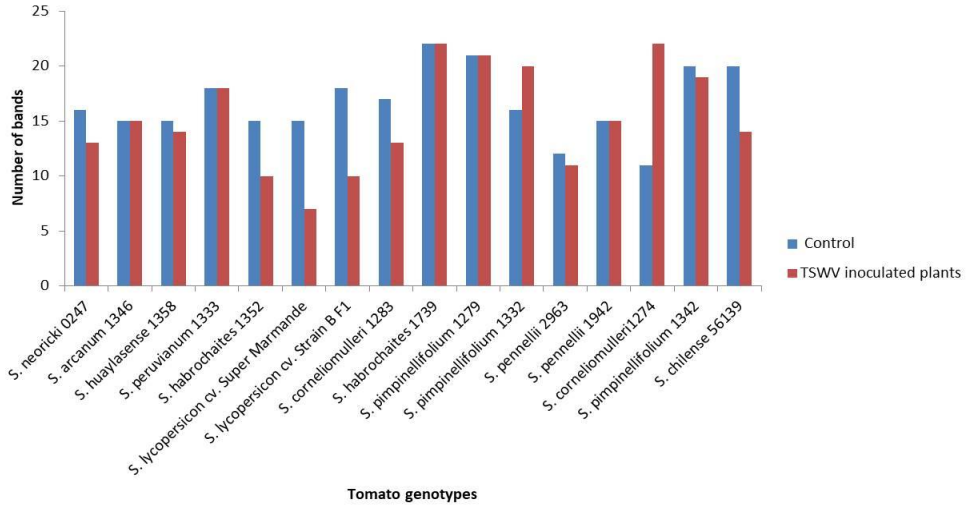


Figure 3. Bar chart illustrates changes in the protein content extracted from tomato leaves inoculated with TSWV, compared with the healthy control.

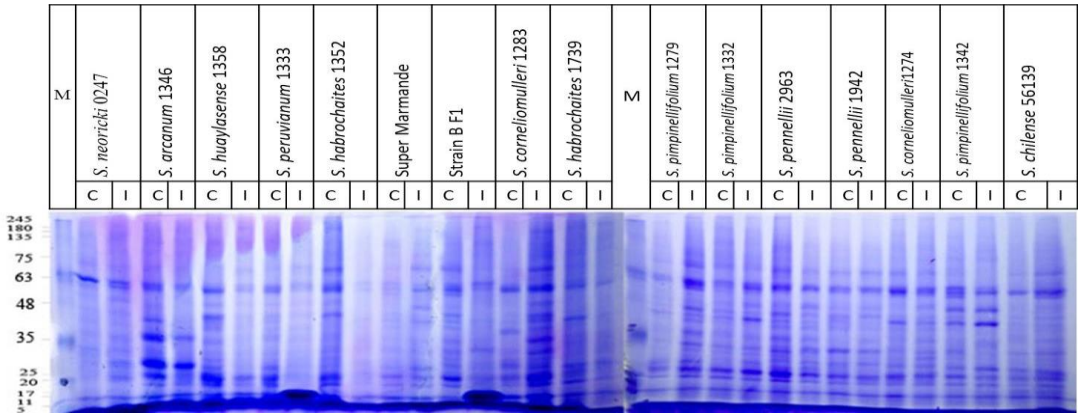


Figure 4. SDS-PAGE binding patterns of total protein extracted from tomato genotypes inoculated (I) with ToMV, compared with the control (C). Lane M: protein marker.

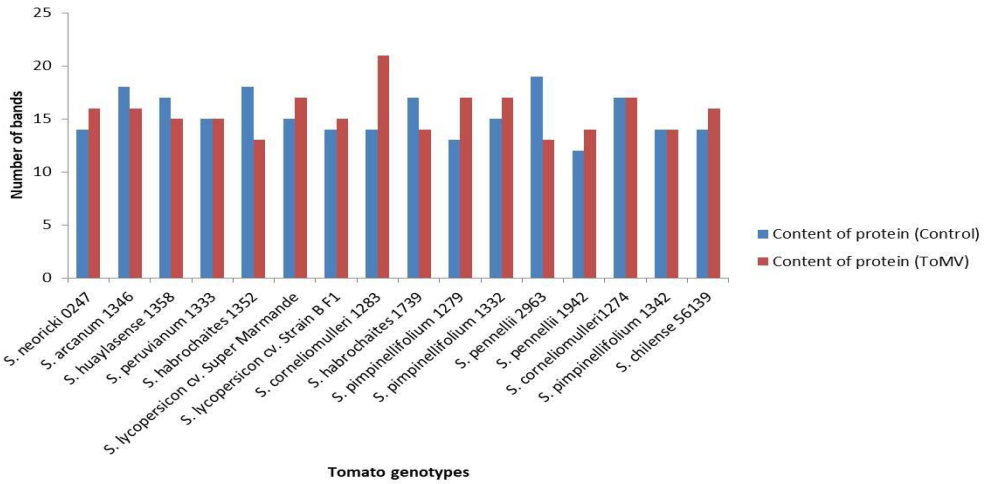


Figure 5. Bar chart illustrates changes in the protein content extracted from tomato leaves inoculated with ToMV, compared with the healthy control.

Enzyme activities of Tomato Genotypes Infected with Virus

-PPO

The constitutive levels of PPO isozymes in both the tomato plants infected with TSWV and the healthy control scored 11 loci with *Rf* values ranging from (0.104 to 0.899). The maximum PPO activities were induced in *S. peruvianum* 1333 (HR) (three isoforms; *Rf* 0.313, 0.829, and 0.899), followed by *S. huaylasense* 1358 (R) gave two loci with *Rf* 0.185 and 0.313. On the contrary, the minimum PPO isoforms were scored in *S. corneliomulleri*1274 (R) and Strain B (MS) (one band; *Rf* 0.313, and 0.104), respectively (Figure 6 and 7). However, there were no any changes recorded in PPO isoform activities in the other tomato lines. For ToMV, the activities of PPO enzymes in the tomato plants inoculated with ToMV and non-inoculated ones displayed 11 isoforms with *Rf* values ranging from 0.104 to 0.899. The highest PPO isozyme activities were found in *S. habrochaites* 1352 (MS) (four isoforms; *Rf* 0.104, 0.185, 0.829, and 0.899), followed by *S. habrochaites* 1739 (HR) and *S. chilense* 56139 (HR), which displayed two loci with *Rf* (0.104 and 0.185). Also, the *S. arcanum* 1346 (R) showed two bands with *Rf* (0.829 and 0.899). On the contrary, the lowest activities of PPO enzymes were scored in the Super Marmande (HR) (one isoform; *Rf* 0.899) and *S. pimpinellifolium* 1342 (MS) (one locus; *Rf* 0.104). However, the other tomato lines either showed a decrease in the PPO enzyme activities or no change occurred, in comparison to the uninfected control (Figure 8 and 9).

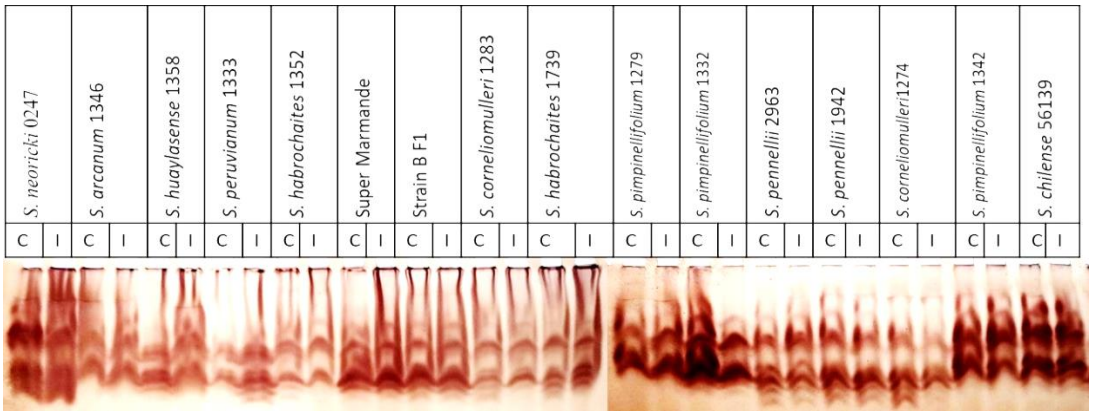


Figure 6. PPO isozyme activities of tomato genotypes inoculated (I) with TSWV, compared with the control (C).

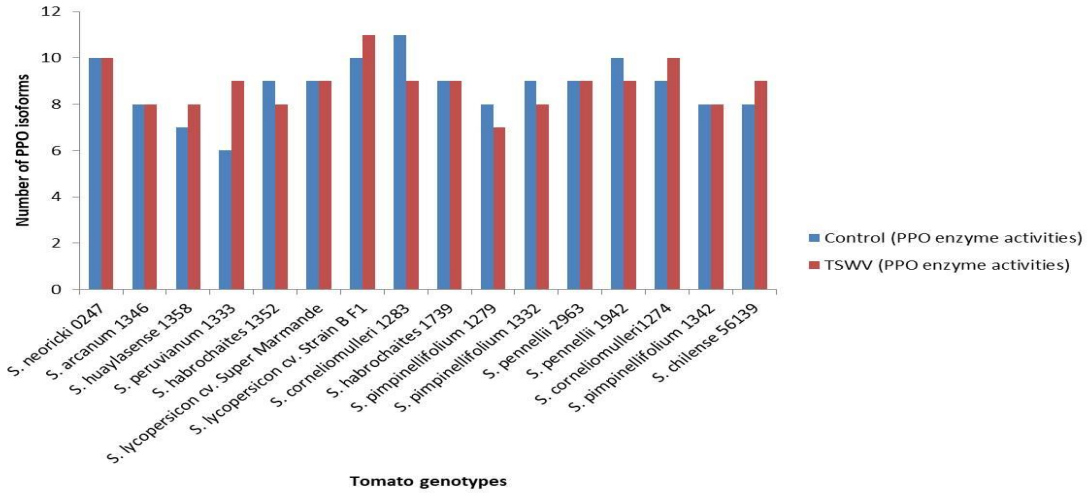


Figure 7. Bar chart illustrates changes in the PPO isozyme activities in tomato genotypes inoculated with TSWV, compared with the healthy control.

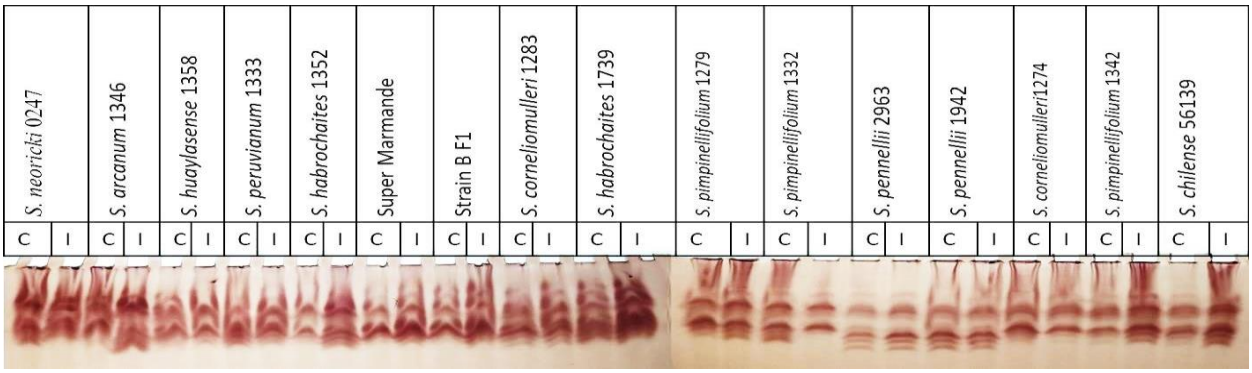


Figure 8. PPO isozyme activities of tomato genotypes inoculated (I) with ToMV, compared with the control (C).

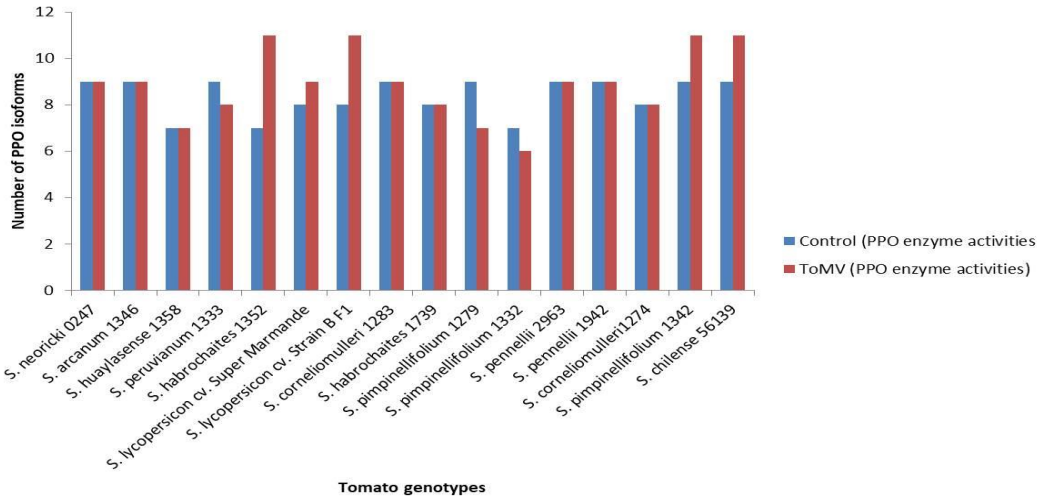


Figure 9. Bar chart illustrates changes in the PPO isozyme activities in tomato genotypes inoculated with ToMV, compared with the healthy control.

-POX

POX isozyme activities in the tomato plants infected with TSWV gave 10 isoforms with *Rf* values ranging from 0.207 to 0.765. It was observed that POX enzyme activities were higher in *S. peruvianum* 1333 (HR) than the control. It gave the greatest number of isoforms (5 loci; *Rf* 0.207, 0.276, 0.379, 0.705, and 0.765), followed by Strain B (MS) and *S. habrochaites* 1739 (R), which scored the same three isoforms with *Rf* 0.207, 0.276, and 0.379. Also, the *S. neoricki* 0247 (R) displayed two bands with *Rf* 0.207 and 0.765. Meanwhile, the tomato genotypes *S. huaylasense* 1358 (R) and *S. habrochaites* 1352 (R) and *S. pennellii* 2963 (MS) recorded one isoform with *Rf* 0.765, 0.705, and 0.705, respectively (Figs. 10 and 11). For ToMV, it is clear that, the *S. pimpinellifolium* 1342 (MR) showed the highest number of POX enzyme activities (5 bands; *Rf* 0.207, 0.276, 0.379, 0.652, and 0.705), followed by *S. pimpinellifolium* 1279 (HR) gave four isoforms with *Rf* 0.207, 0.276, 0.379, and 0.652, while *S. habrochaites* 1739 (HR) scored three bands with *Rf* 0.207, 0.379, and 0.705. Finally, *S. pennellii* 2963 (HR) and *S. habrochaites* 1352 (MS) gave two loci with *Rf* (0.652 and 0.705) and (0.276 and 0.379), respectively. In contrast, some tomato lines scored one band, such as the highly resistant Super Marmande (*Rf* 0.207), *S. chilense* 56139 (*Rf* 0.705), and *S. pimpinellifolium* 1332 (*Rf* 0.276) and the resistant *S. arcanum* 1346 (*Rf* 0.705), and Strain B (*Rf* 0.207) (Figs. 12 and 13). However, the remaining tomato lines have not recorded any marked differences in POX isozyme activities.

Correlation between Disease Severity and Protein Content and Isozyme Activities

The data represented in Table (2) revealed that a negative correlation (r) was detected between the content of protein and the disease severity of TSWV (r =

-0.105) and ToMV (r = -0.137). Similarly, it was found a negative correlation between disease degree of TSWV and PPO (r = -0.053) and POX (r = -0.207) isozyme activities. On the other hand, a low positive correlation was detected between the disease incidence of ToMV and PPO (r = 0.291) and POX (r = 0.003) enzyme activities (Table 2).

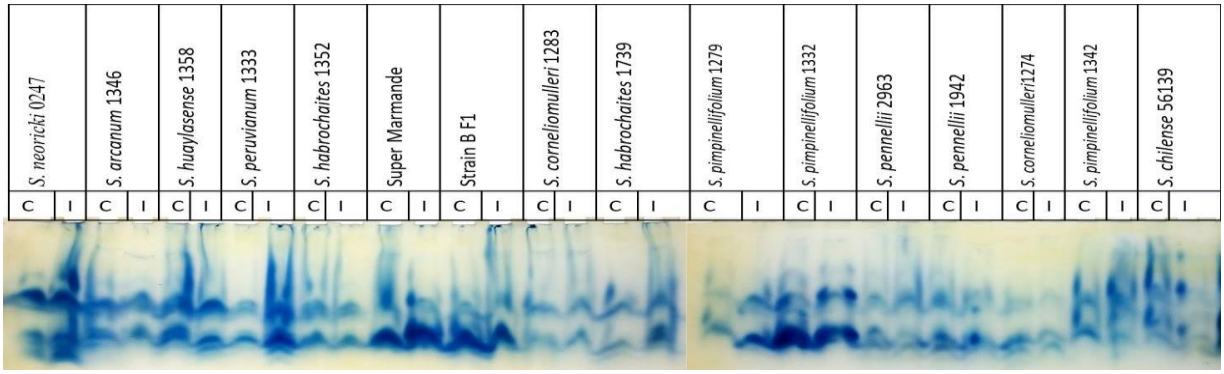


Figure 10. POX isozyme activities of tomato genotypes inoculated (I) with TSWV, compared with the control (C).

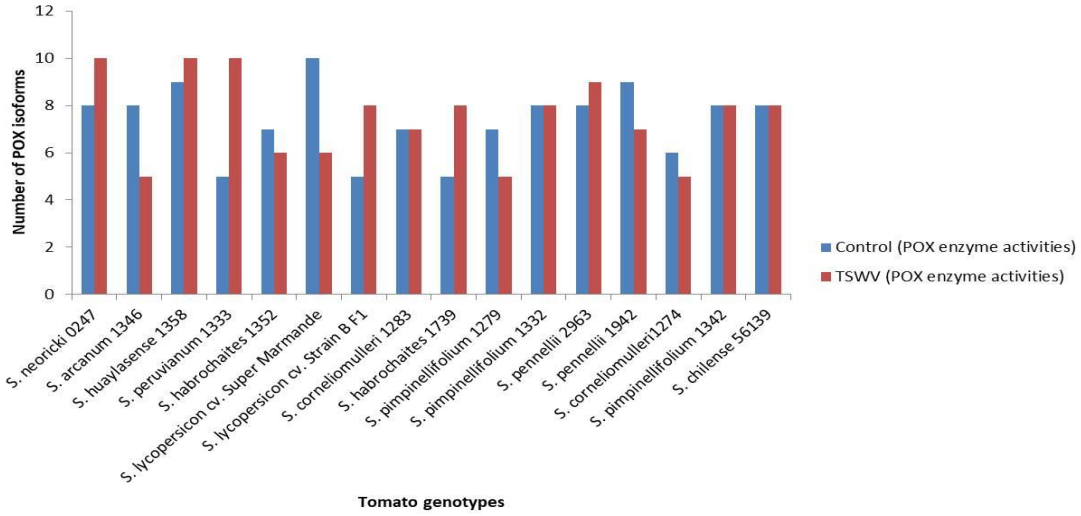


Figure 11. Bar chart illustrates changes in the POX isozyme activities in tomato genotypes inoculated with TSWV, compared with the healthy control.

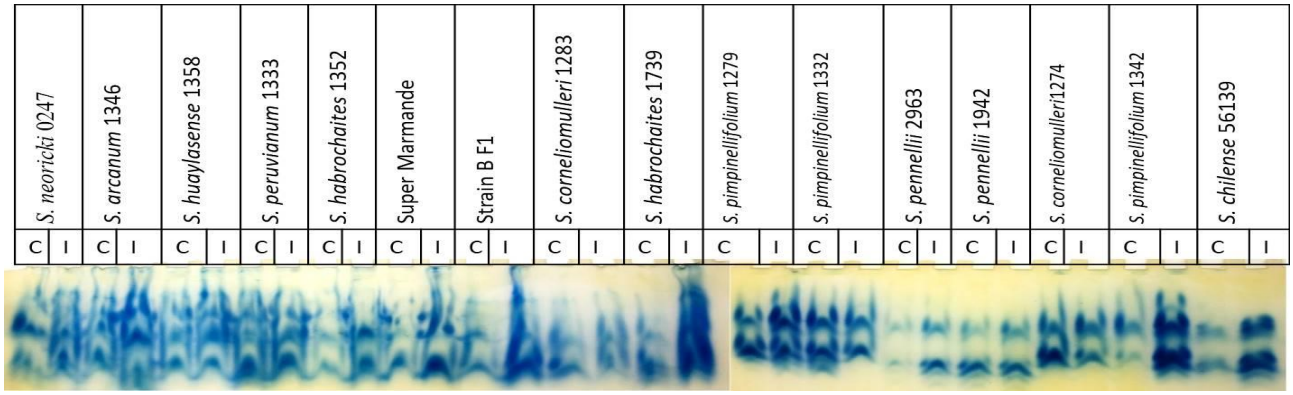


Figure 12. POX isozyme activities of tomato genotypes inoculated (I) with ToMV, compared with the control (C).

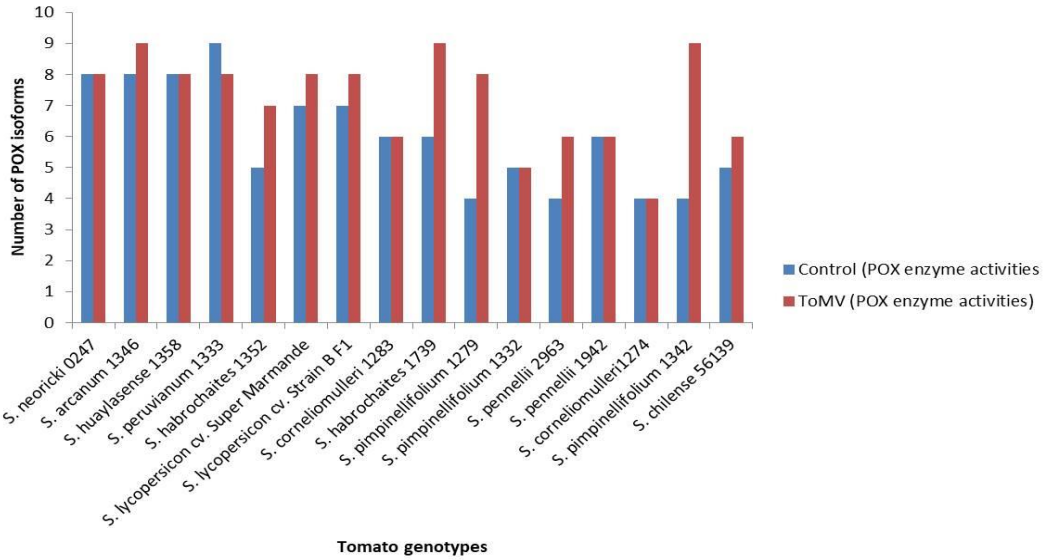


Figure 13. Bar chart illustrates changes in the POX isozyme activities in tomato genotypes inoculated with ToMV, compared with the healthy control.

Table 2. Correlation of protein content, PPO and POX with disease severity of tomato leaves.

Biochemical parameters	TSWV[a]	ToMV[b]
Protein content	-0.105	-0.137
PPO[c]	-0.053	0.291
POX[d]	-0.207	0.003

[a]TSWV= *Tomato spotted wilt virus*; [b]ToMV= *Tomato mosaic vius*; [c]PPO = Polyphenol oxidase; [d]POX=Peroxidase.

DISCUSSION

TSWV and ToMV are two of the most common the viral diseases which damage tomato crops leading to losses ranged from 59.77 to 100% (Roselló *et al.*, 1996; Ullah *et al.*, 2017; Mahfouze *et al.*, 2022). Resistance of TSWV and ToMV in tomato plants is attributed to induced or constitutive mechanisms (Golshani *et al.*, 2015; Soliman *et al.*, 2019). Induction of PRs-proteins is an important tool of an induced mechanism against the virus. In this study, 16 tomato genotypes were varied in resistance against TSWV or ToMV, two tomato accessions were highly resistant (HR) to TSWV, seven lines were resistant (R), two accessions moderately resistant (MR), four moderately susceptible (MS), and one susceptible (S) to TSWV infection. These results were confirmed by Canady *et al.* (2001) indicated several tomato lines resistant to TSWV, such as the Porter's strain of *S. pimpinellifolium*, a highly resistant Stevens variety from *S. peruvianum*. For ToMV, ten lines were highly resistant (HR), three genotypes resistant (R), two accessions moderately resistant (MR), and one moderately susceptible (MS). These results agree with Stevens *et al.* (1994); Li *et al.* (2019); Mahfouze *et al.* (2022). Many host-pathogen interactions have shown that protein components are involved in disease resistance (Carvalho *et al.*, 2006). In this work, the total soluble protein profile of the healthy tomato plants and the TSWV or ToMV infected ones was determined by SDS-PAGE. The results showed marked differences in number of bands among 16 studied tomato genotypes. Thus, the protein content in 16 tomato lines inoculated by the virus was increased or decreased or not changed in comparison with the control. Also, it was observed that accumulation of new proteins in TSWV highly resistant tomato accession *S. peruvianum* 1333 (25 and 170 kDa), the resistant tomato line, *S. habrochaites* 1352 (32 kDa), Super Marmande (42, 44, and 122 kDa), and *S. corneliomulleri* 1274 (25, 32, and 170 kDa), and two moderately susceptible accessions *S. arcanum* 1346 (11 kDa) and *S. pimpinellifolium* 1332 (25 kDa). For ToMV, the highly resistant *S. corneliomulleri* 1283 had the highest number of new proteins (30, 37, 44, 85, and 90 kDa), *S. pimpinellifolium* 1279 (68, 85, 90, and 170 kDa), *S. neoricki* 0247 (90 and 170 kDa), *S. pennellii* 1942, and

S. chilense 56139 (44 and 68 kDa). In contrast, the resistant *S. peruvianum* 1333 and Strain B F1 recorded the lowest number of PRs with MWs of 68 and 90 kDa, respectively. On the other hand, it has been observed that two PRs of 44 and 170 kDa were induced in the tomato lines resistant to TSWV and ToMV. These proteins may play a role in resistance of tomato plants to the virus. These results were in accordance with Gupta *et al.* (2020) used the SDS-PAGE profiles to isolate proteins from TSWV-inoculated tomato plants and un-inoculated ones. Clear variations in the protein content were found between TSWV-infected plants and the mock. Also, the authors used a tandem mass tags (TMT)-based quantitative proteome approach to examine the protein profiles of tomato cultivars susceptible and resistant to TSWV infection. The results of the study showed significant changes in response to TSWV. Thus, the proteome of resistant tomato variety remains unchanged, while the proteome of the susceptible tomato variety records distinct differences. Aseel *et al.* (2021) identified four different kinds of PRs in tomato cultivars infected with ToMV, e.g., 18 kDa (coat protein), 30 kDa (movement protein), and 130 and 180 kDa (replication gene). Agrios (1998) found that the protein content is higher in plants infected, which attributed to the pathogen attack mechanism and defense mechanism in the plant. However, the content of protein was decreased in banana plants infected with *Banana bunchy top virus* (BBTV). Siddique *et al.* (2014) found that cotton genotypes susceptible to *Cotton leaf curl burewala virus* (CLCuBuV) recorded a substantial decrease in content of protein. However, it was observed that change non-significantly in the resistant genotypes, as compared with the control. Christov *et al.* (2007) mentioned that the lower content of protein in plants diseased with the virus is due to a breakdown or denaturation of proteins or polypeptide chains and bound amino acids caused improved free amino acids content of the host. This decrease in protein in infected plants is attributed to damage chloroplast or protein synthesis inhibition (Pineda *et al.*, 2010).

In this investigation, the activities of PPO and POX isozymes and their isoforms were determined in the 16 tomato lines inoculated with the TSWV or ToMV and non-inoculated healthy ones by Native-PAGE. In the leaf samples infected with the virus, there was increasing or decreasing or no change in the PPO and POX activities depending on the genotype, virus, and degree of resistance. These results were agreed with Kumar *et al.* (2010) found that phenols play role in the defense response in the host against virus. Madhusudhan *et al.* (2009) recorded increase in POX activities in tomato plants inoculated with ToMV. This increment was higher in the resistant plants, compared with susceptible plants. The latter having greater POX levels than un-inoculated. Hammond-Kosack and Jones (1996) mentioned that POX isozymes play an important role in the suberification, lignification, regulation of cell wall elongation, wound healing, and resistance of the host against disease. Also, the activities of POX enzymes were increased significantly in tomato plants susceptible to ToMV, as compared to un-inoculated ones. Jockusch (1966); Mohamed *et al.* (2012) found that PPO isozymes play a critical role in the early stages of plant defense, when membrane disruption induces the production of phenols, such as chlorogenic acid. Li and Steffens (2002); Ngadze *et al.* (2012) indicated that total phenol and PPO isozymes play role in the plants resistance to viral diseases. Siddique *et al.* (2014) observed that POX enzyme activities were significantly higher in the control plants of cotton genotypes susceptible to CLCuBuV, as compared to resistant genotypes. Keen (1992) mentioned that host-pathogen interactions are supposed to produce signals that activate nuclear loci responsible for the defense response induction enzymes, expression of proteins, and accumulation of phenolic components, which in turn contribute in defense systems of the plant host. Chatterjee and Ghosh (2008) indicated the lower activity of POX enzymes in mesta plants infected with *yellow vein mosaic virus*, compared with healthy ones. In contrast, the activities of PPO enzymes in the diseased plants were higher than the control. According to Gupta *et al.* (2020), the best strategy for controlling plant viruses is to use host plant defence mechanisms. The identification of metabolites (amino acids, lipids, enzymes, and organic acids) that provide disease resistance in many crops. Therefore, the goal of disease breeding programs should be to identify the metabolite markers that contribute to resistance. Also, metabolic markers may be more useful than molecular markers. Consequently, in order to effectively manage plant diseases, it is imperative to investigate the natural metabolites in host plants.

In the current work, it was observed that a negative or low positive correlation between disease severity and (protein content and isozyme activities). Similar results

were confirmed by Biswas *et al.* (2012); Kumar *et al.* (2016) indicated the presence of a negative correlation between disease severity and total soluble protein and defense enzymes.

CONCLUSIONS

Expression of PRs and defense enzymes in tomato plants is considered an important mechanism of virus resistance. Our results showed that protein content and (PPO, and POX isozyme activities) differ significantly in 16 tomato genotypes susceptible and resistant to TSWV or ToMV. It was observed that TSWV or ToMV infection induces an increase or decrease or no change in the content of protein and defense enzymes depending on the genotype, virus and degree of resistance. On the other hand, there was a negative or low-positive correlation between the disease severity and (protein content and isozyme activities).

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.

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