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Pseudomonas syringae pv. *tomato* and severity of bacterial speck disease on tomato plants in Egypt

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Abstract

Tomato is one of the most widespread vegetable crops in Egypt, which has a wide variation of cultivars. Tomato bacterial speck caused by *Pseudomonas syringae* pv. *tomato* is one of the most important tomato bacterial diseases. This study aimed to isolating and identifying *P. syringe* pv. *tomato* from infected tomato plants in Egypt, and investigate the tomato plants reaction towards the Egyptian bacterial strain, which the tomato cultivars or hybrids have low sensitivity to it. Currently, the Egyptian tomato plants exhibited typical symptoms of tomato bacterial speck disease. The isolation and identification experiments resulted in the recovery of typical isolates of *P. syringae* pv. *tomato*. The pathogenicity of the bacterial isolate was confirmed by molecular identification through detection of coronatine production and 16S rDNA analysis. The obtained nucleotide sequence was deposited at GenBank with accession no. OQ117369.1 and designed as *P. syringae* pv. *tomato* strain Pst1-MAS. The tomato plants only produced the typical bacterial speck symptoms among the tested solanaceous and cruciferous plants. The tested tomato plants exhibited variation in disease severity against *P. syringae* pv. *tomato* strain Pst1-MAS using a lot of disease severity scales. The tomato cultivars VFN-8 and Peto 86 were sensitive to infection with severe symptoms, while the tomato cultivars Super Marmande and Edkawy expressed relatively low symptoms. According to the results of disease severity; depending on the mean of bacterial speck symptoms, the tested plants were divided into high; moderate, low sensitive, and resistant. The related defense enzyme activity was correlated inversely with the disease severity for some of the tested tomato plants.

Keywords: *Pseudomonas syringae* pv. *tomato*, Bacterial speck, Tomato cultivars, Disease severity, Coronatine production

1. Introduction

Tomato is a member of the family *Solanaceae* and is one of the most important vegetables growing in Egypt throughout the year. The world production of tomato reached 189133955.04 tons from 5167388 hectares. The Egyptian production of tomato reached 6245787.13 tons from 150109 hectares. Egypt is considered as the 5th country in tomato production and is the 6th cultivated areas worldwide. In Africa, Egypt is the 1st in tomato producer and is 2nd the second in the tomato cultivated areas (FAO. 2021). The plants are surrounded by a wide variety of microorganisms including several plant pathogens (Ghosh *et al*., 2019). The pests and the diseases infecting microorganisms of tomato are among the major problems facing tomato production worldwide (Gatahi, 2020). The tomato bacterial speck disease that is caused by *P. syringae* pv. *tomato* is one of the serious bacterial diseases in the different tomato-growing locations around the world (Bastas, 2015; Alfaro-Quezada *et al*., 2023). The *Pseudomonas* taxa include several strains such as *P. syringae* pv. *tomato* that is a pathogen of tomato plant, and *P. avellanae* 6CH2 that has various advantageous traits for the plants and acts as a biocontrol agent (Chetverikov *et al*., 2021). On the other hand, some of these *Pseudomonas* strains are used for crude oil and diesel degradation (Chaida *et al*., 2022). *P. syringae* pv. *tomato* has extracellular vesicles (EVs), which exit out of the bacterial cell membrane during infection of the plant; in addition, they can alter the host plant defenses and acquire nutrients from the host plant (Janda *et al*., 2023).

Tomato bacterial speck disease decreases the tomato yield and causes loss in the marketing of these tomato fruits (Ekici and Bastas, 2014; Abd El-Fatah *et al*., 2023). Seeds of tomato contaminated by *P. syringae* pv. *tomato* are one of the most important tools for spreading this disease (Bastas, 2015). These seeds contaminated by *P. syringae* pv. *tomato* have arisen from the infected tomato fruits (Randhawa *et al*., 2017). Both of the seedling and the mature tomato plants can be infected by the bacterial pathogen (Psallidas, 1988), and the pathogen can infect all aerial parts of this plants, and finally the infected plant may die (Skliros *et al*., 2023). The tomato bacterial speck disease symptoms seemed as dark necrotic spots with a yellowish halo on the tomato leaves and without a halo on the stem; pedicels, and the peduncles (Valenzuela *et al*., 2022). *P. syringae* pv. *tomato* produces coronatine toxin and the chlorotic halo observed around the spots are attributed to this coronatine toxin. Coronatine is necessary for colonization of the tomato plants by the pathogen and is responsible for expressing symptoms of the disease (Uppalapati *et al*., 2008; Kottayi and Abhyankar 2022). Tomato cultivars are divided into two races; mainly race 0 that is resistant and race 1 that is susceptible to infection by the pathogenic *P. syringae* pv. *tomato* (Milijašević et al., 2009). *P. syringae* strains have many mechanisms to collapse the host plant, including reproduction and survival on the plant surface (Xin *et al*., 2018). The contaminated tomato seeds; tomato seedlings, and the plant residues are considered as inoculum sources for *P. syringae* such as *P. syringae* pv. *tomato* and *P. syringae* pv. *populus* (Gitaitis and Walcott, 2007; Saint-Vincent *et al*., 2020).

In this context and with the wide range of cultivated cultivars and hybrids of the tomato plant in Egypt, the objectives of this research were to isolate and identify the bacterial pathogen responsible for the tomato bacterial speck disease, and determine which tomato cultivars or hybrids had low sensitivity to infection by this Egyptian strain of *P. syringae* pv. *tomato*.

2. Materials and methods

2.1. Isolation of *Pseudomonas syringae* pv. *tomato*

Samples of tomato (*Solanum lycopersicum* L.) plants exhibiting small spots surrounded by yellowish

hall and were suspected to be infected by the tomato bacterial speck disease were collected from Giza governorate in Egypt. The samples were kept in bags and transferred immediately to the plant pathology laboratory for bacterial isolation. The samples were washed with sterile dist. H_2O several times; small pieces of the infected parts were cut using a sterile scalpel, and crushed within a few drops of sterile dist. water in a sterile mortar (Bibi *et al*., 2018). After filtration, the filtrate was streaked on the surface of King's medium B (KB) petri plates (20 g of proteose peptone, 1.5 g of dipotassium hydrogen phosphate, 1.5 g of magnesium sulfate heptahydrate, 15 ml of glycerol, 15 g of bacteriological agar, and then completed to 1 l using dist. water, pH 7.2± 0.2) (King *et al*., 1954), and then incubated for 5 d at 26 ±2 °C (Klement *et al*., 1990). The cultures were checked daily and the suspected bacterial colonies were streaked on KB medium plates to obtain pure cultures. The isolates were maintained in KB slants at 4 °C till further use.

2.2. Characterization of the bacterial isolates

2.2.1. Pathogenicity test

The bacterial isolates were grown on KB medium at 26± 2 ºC for 24 h, suspended in 10 ml of sterile dist. H_2O, and the suspension was spectrophotometry adjusted to 10^8 cfu/ ml at OD_{600} nm. Leaves of 8 weeks old tomato seedlings (*S. lycopersicum* cv. Super strain B) were gently wiped with a cotton pad moistened with carborandum powder. Immature tomato fruits growing on fruiting plants were treated similarly. About 100 ml of a bacterial suspension (10^8) cfu/ ml was sprayed using a hand-held sprayer to treat the tomato leaves and fruits until runoff. The plants were covered for one day with plastic bags to increase the humidity (Wreikat *et al*., 2006), while some of the fruiting plants continued at these humidity conditions for 4 d. All plants were incubated under greenhouse conditions. The plants were observed daily for 11 d. The bacteria were re-isolated from the plants exhibiting the bacterial speck disease symptoms (Schaad *et al*., 2001).

2.2.2. Bacteriological features

Bacteriological identification of the bacterial isolates was carried out using several characteristics, including microscopical; biochemical, and physiological, according to Lelliott and Stead, (1987); Schaad *et al*., (2001). The bacterial cultures (24 h old) were tested for their ability to produce levan, oxidase activity, potato soft rot, and arginine dihydrolase. In addition to Gram stain; the bacterial isolates were tested for determination of cell shape; motility, sporulation, 3 % KOH reaction, catalase activity, and gelatin hydrolysis (Schaad *et al*., 2001). A hypersensitive reaction against tobacco leaves (LOPAT) was performed, in reference to Element *et al*., (1964). Approximately, one month old *Nicotiana tabacum* leaves were used to detect this hypersensitive reaction. About 1 ml bacterial suspension (10^8 cfu/ ml) was gently infiltrated into the *N. tabacum* leaves using a hypodermic medical syringe. Sterile water was used as a negative control. The plants were maintained in the greenhouse at 28± 2 ºC with a 16 h photoperiod for 5 d with daily follow.

2.2.3. Molecular identification

Some of the pathogenic isolates that induced bacterial speck symptoms on the upper parts of the tomato plants were selected, and identified molecularly through detection of coronatine gene production and sequencing of the 16S rDNA. The bacterial isolates were streaked on KB medium and incubated at 26 ºC ±2 for 24 h. After incubation, the growing colonies were suspended in sterilized dist. water. The DNA was extracted from the bacterial suspension using the boiling and cooling method, according to Yahiaoui-Zaidi *et al*., (2003); Kawaguchi *et al*., (2005). In these assays, the bacterial suspension was heated at 95 ºC for 10 min.; followed by direct cooling using ice for 5 min., centrifuged using a cooling centrifuge (Eppendorf, centrifuge 5417R) at 12000 for 3 min., and then the resulting supernatant containing the bacterial DNA was transferred to a sterile Eppendorf tube. The DNA was estimated

spectrophotometrically at OD_{260} nm and then separated through running on 0.6 % agarose gel.

2.2.3.1. Detection of coronatine-production

According to Bereswill *et al*., (1994), two pairs of specific primers were used for coronatine gene production; primer 1 (5'- GGCGCTCCCTCGCACTT-3') and primer 2 (5'- GGTATTGGCGGGGGTGC- 3'). According to the manufacturer's instructions, the polymerase chain reaction (PCR) analysis was performed in a total volume of 25 µl; containing 2 µl of template DNA and 1µl of each primer, which were mixed with 12.5 µl Master Mix 2x and reached to the total volume by water (de-ionized H_2O). The thermal cycles were performed according to Wreikat *et al*., (2006) using 37 cycles with denaturation at 93 ºC (2 min. at the first cycle and then 1 min. for the next cycle); annealing at 57.2 ºC for 2 min., polymerization at 72 ºC for 2 min., and finally extraction at 72 ºC for 10 min. A non-pathogenic isolate was used as a negative control for coronatine production. Approximately 5 µl of the PCR product was separated by electrophoresis on 1.2 % agarose gel with a 100 bp ladder marker. The agarose gel electrophoresis was run for 30 min. at 100V and the separated bands on the gel were photographed using a UV unit.

2.2.3.2. Sequencing of the 16S rDNA gene

A single representative pathogenic isolate was selected for sequencing of its 16S rDNA gene. The DNA extracted previously from the tested bacterial isolate was used to confirm the identification through the 16S rRNA gene sequence analysis. PCR reaction set-up was carried out using 27F primer (5'-AGAGTTTGATCCTGGCTCAG –'3) and 1429R primer (5'- GGTTACCTTGTTACGACTT -'3), as universal primers for identification of the bacterial isolate based on 16S rRNA sequencing (Weisburg *et al*., 1991). A PCR cocktail tube contained 25 µl Taq Master Mix 2x (dye plus); 8 µl of DNA template, 1 µl (20 pmol) from the forward primer, 1µl (20 pmol) from reverse primer, 15 µl of Nuclease-free water, and then the total volume was reached to 50 µl using de-ionized H_2O. The setup thermal cycles were performed as follows, initial denaturation at 94 ºC for 6 min., denaturation at 94 ºC for 45s, annealing for 35 cycles at 56 ºC for 45s, the extension was done at 72 ºC for 1 min., and a final extension was carried out at 72 ºC for 5 min. The PCR product was used for identification of the nucleotide sequences of the 16S rRNA gene, according to GATC Biotech company, Germany. The nucleotide sequence was aligned and compared using the BLASTn tool to search through the deposit database in NCBI GenBank (https://blast.ncbi.nlm.nih.gov).

2.3. Bacterial pathogenicity to the Solanaceae and *Brassicaceae* plants

Ability of the pathogenic *P. syringae* pv. *tomato* to induce the bacterial speck symptoms was tested on various plants from the *Solanaceae* family and *Brassicaceae* family (Cuppels and Ainsworth, 1995), including tomato (*S. lycopersicum* L.); pepper (*Capsicum annuum* L.), eggplant (*S. melongena* L.), potato (*S. tuberosum* L.), datura (*Datura stramonium* L.), black nightshade (*S. nigrum* L.), ground cherry (*Physalis pubescens* L.), and petunia (*Petunia hybrida*) as Solanaceous members, and the cauliflower (*Brassica oleracea*) as a member of the *Brassicaceae* family. The plants were grown in 20 cm plastic pots containing 3 kg clay and sand (2:1). The old leaves (6-10) on the plant were inoculated by spraying 50 ml (10^8 cfu/ ml) of the bacterial suspension using a hand-held sprayer until runoff on the leaves (Soliman, 2022). Another set of plants was sprayed with sterile dist. H_2O and served as a control. The plants were covered for one day with plastic bags to increase the humidity, observed daily for 12 d with regular irrigation and balanced fertilization.

2.4. Evaluation of disease severity of the pathogenic bacterium

Seven commercial cultivars and hybrids of tomato plants were used to test their sensitivity to *P. syringae* pv. *tomato*; through evaluating the bacterial speck disease severity. The tested experimented cultivars

included; tomato cv. Edkawy; cv. Floradade, cv. Peto 86, cv. Super Marmande, cv. VFN-8, cv. T-186 hybrid, and cv. 023F1. The tomato seeds used in these experiments were purchased from public marketplaces for seeds in Cairo, Egypt. The seeds were sown in a divided tray to 2×2×4 cm cells containing sterilized peat moss with sandy (2:1) with regular irrigation. The growing seedlings (4 weeks old) were transplanted into 15 cm pots containing 1.5 kg of clay and sand soil (2:1), and then maintained in the greenhouse. After 10 d of transplanting, the plants were inoculated with 20 ml/ plant of the bacterial suspension (10^8 cfu/ ml) using a hand-held sprayer until run-off on the leaves. Plants of the same cultivars or hybrids were sprayed with sterile H_2O and served as controls. The plants were covered with transparent plastic bags for one day to increase the humidity, and were grown for 3 weeks at 21± 2 ºC with high humidity and 12 h photoperiod in the greenhouse. Six replicates were used for each treatment with balanced irrigation. The disease severity was calculated using four scales; 1st according to Yunis *et al*., (1980) as follow: (0= no symptoms, 1= 2:5 specks together are spread on the leaf, 2= 6:10 specks and 3= more than 11 specks on the leaf), 2nd according to Chambers and Merriman, (1975) as follow: (0 = no symptoms, 1= 1:10 specks on the plant, 2= 11: 20 specks on the plant, 3= 21: 40 specks on the plant and 4= more than 40 specks on the plant), 3rd according to Bastas, (2015) as a modification to the Chambers and Merriman, (1975) scale as follow: (0= no disease symptoms, 1= 1:5 specks, 2= 6:12 specks, 3= 13: 20 specks and 4= 21:30 specks on the tomato leaves), and the 4th scale was calculated as means of the specks appearing on the surface of the inoculated leaves.

2.5. Defense-related enzymes activity

Leaves of the different tomato plants were used to measure the defense-related enzyme activity at the second stage of disease severity evaluation. The defense-related enzymes included peroxidase (POD) and polyphenol oxidase (PPO). The POD and PPO activity was measured in the infected and healthy plants. The crude enzyme was extracted according to the previous method conducted by Elshahawy *et al*., (2022). Approximately 0.2 g of the fresh leaves was homogenized with 4 ml of potassium phosphate buffer 0.05 M (pH 7.8) and then centrifuged under cooling (Eppendorf, centrifuge 5417R) at 14000 rpm for 10 min. A spectrophotometer (JENWAY 6305 UV/Vis. spectrophotometer) was used to measure the absorbance for 40s with the different samples.

Peroxidase enzyme was measured at 420 nm using a crystal cuvette. According to Maehly and Chance, (1954), the cuvette cocktail contained 60 µl of the crude enzyme added to 1.7 ml of 0.1 M phosphate buffer pH 6, 300 µl of pyrogallol solution (5 %), 100 µl of H_2O_2 (5 %), and 700 µl of dist. water. The blank was measured with the same previous contents without the enzyme.

Polyphenol oxidase activity was measured spectrophotometrically at 420 nm using a crystal cuvette. According to Duckworth and Coleman, (1970), the cuvette cocktail contained 60 µl of the crude enzyme and 1.7 ml of 0.02 M catechol solution (solute in 0.05 M phosphate buffer pH 6.8 at 25 ºC). The blank was measured with the previous contents without the enzyme. The activity of the PPO enzyme was measured according to the following equation of Elshahawy *et al*., (2022):

$$U\ (\mu mol)/\ ml) = (\Delta A \times Vt \times 106) / (\Delta t \times I \times \varepsilon \times Vs) \times 1000$$

Where: ΔA= change in absorbance, Vt= total volume for measurement, Δt= incubation period by moment, I= cuvette diameter (1 cm), ε= extraction coefficient by $M^{-1}cm^{-1}$ (H_2O_2 at 420 nm = 43.6, pyrogallol at 420 nm = 2640, and catechol at 420 nm = 2450), and Vs= enzyme volume in the measured sample in ml.

2.6. Statistical analysis

Data were analyzed using the mean with standard error involving six replicates at most of the experiments and through three replicates at others. CoStat software version 6.311 and one-way analysis of variance (ANOVA) were used to calculate the least significant differences (LSD) at 0.01 for the laboratory

experiments and at 0.05 for the plant experiments. The correlation coefficients between the disease severity and the defense-related enzymes activity were used (Anon, 2006).

3. Results

3.1. Documentation of the natural disease symptoms and isolation of the bacterial pathogen

The natural bacterial speck symptoms on tomato plants were used for the pathogen isolation experiment. Symptoms on the leaves were almost 1:2 mm diameter necrotic dark specks surrounded by a chlorotic or yellowish halo, and some of them were expanded and fused together (Fig. 1a), while on the mature fruits the lesions were bigger than on the leaves and were surrounded by a wide yellowish halo (Fig. 1b). The symptoms on the petioles, the flower clusters, and the immature fruits seemed as necrotic dark lesions; however, the chlorotic halo was not clearly observed (Fig. 1c, d and e). Seven isolates suspected to be the pathogens were obtained in the isolation plates. After incubation at 26± 2 ºC for 48 h, the obtained pure cultures on KB medium were raised; circular, smooth, and glistening with fluorescence (Fig. 1f).

3.2. Characterization of the bacterial isolates

3.2.1. Pathogenicity test

Among the 7 bacterial isolates isolated from the symptomatic tomato tissues, 3 isolates exhibited typical bacterial speck symptoms on tomato plants cv. Super strain B. On the leaves, the symptoms started as small specks (1:2 mm almost), which became slightly widen and turned to a dark center with a yellowish halo around the specks (Fig. 2a and b). On the petioles, the lesions seemed water-soaked after 6 d of inoculation and then turned to necrotic with dark color and without a halo after 9 d (Fig. 2c and d). On the fruiting plants, the symptoms started as small specks, then they became wide, and some of them had expanded and fused together without a yellow halo on the immature fruits. Meanwhile, on the mature fruits the yellow halo became obvious (Fig. 2e and f). With continued humidity on the immature tomato fruits, the lesions expanded and fused together with a tan shape (Fig. 2g). The plants sprayed with sterile H_2O as negative controls didn't exhibit any symptoms. The bacteria were re-isolated from the symptomatic tissues to confirm their identity.

3.2.2. Bacteriological features

The results demonstrated that four pathogenic isolates were Gram (-); rod shape cells, motile, no spore formation, and levan producers. They produced negative results for oxidase activity; potato soft rot, and arginine dihydrolase test. However, these isolates displayed positive results with 3 % KOH solubility; catalase activity, and hydrolysis of gelatine (Table 1). In the hypersensitive reaction assay, necrotic areas were produced on the tobacco leaves at the infiltration sites after 24 h of incubation (Fig. 2h); however, these areas were not produced on the control leaves infiltrated with sterile H_2O (Table 1).

3.2.3. Molecular characterization

3.2.3.1. Detection of the coronatine-producing bacteria

Confirmation of identity of the four pathogenic bacterial isolates was based on using the coronatine gene primers (*i.e.*, primer 1 and primer 2) with the DNA extracted from these isolates. On the agarose gel, the PCR product displayed an obvious bands at 650 bp (Fig. 3) and their size were expected to refer to *P. syringae* pv. *tomato*. This result indicated that the four studied isolates produced the coronatine toxin like *P. syringae* pv. *tomato*, while the control isolate did not show any bands.

3.2.3.2. Sequencing of the selected pathogenic isolate by 16S rDNA

The sequence of 16S rDNA gene for the selected pathogenic isolate was searched by BLASTn at GenBank, which showed that the partial sequence

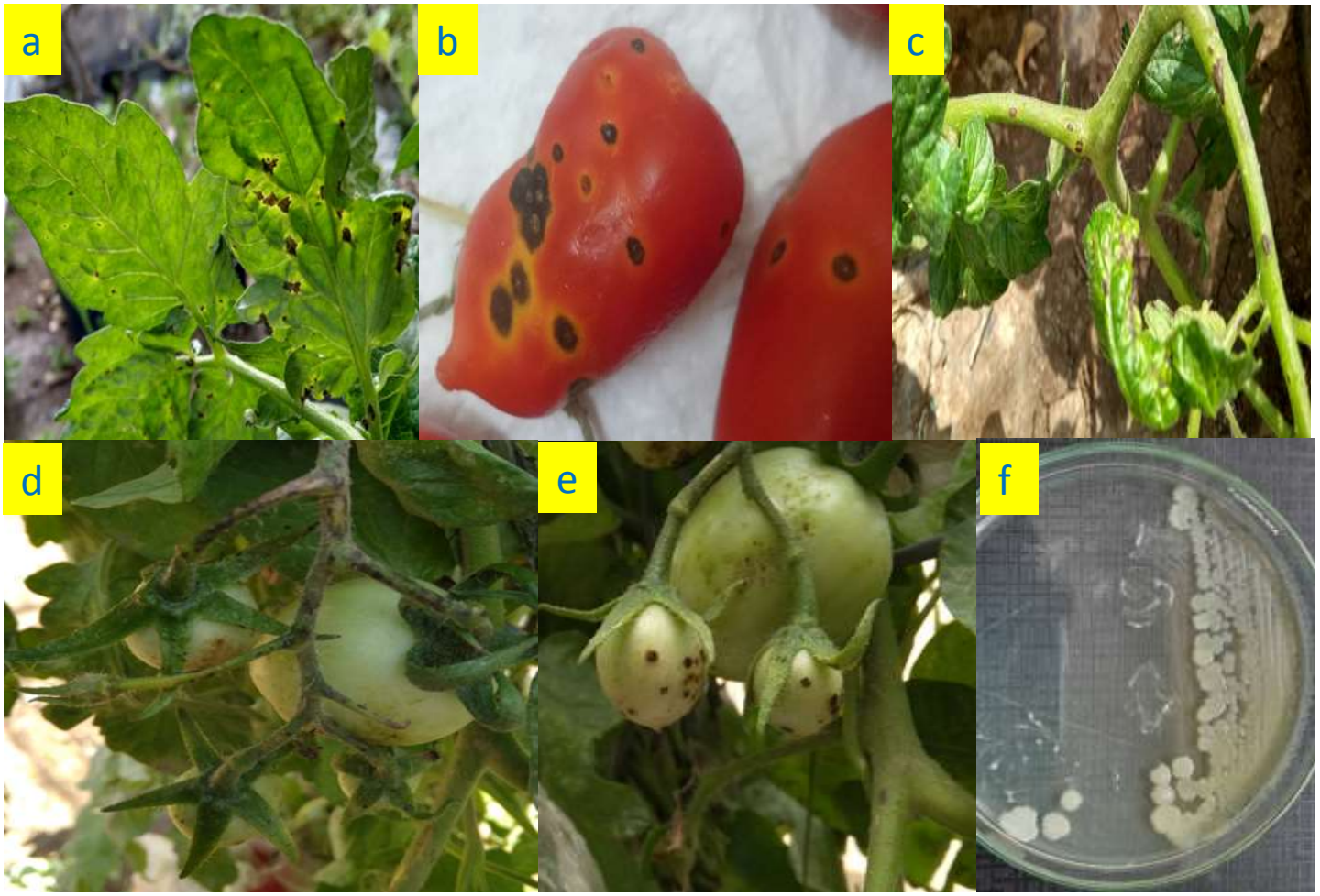

Fig. 1. Bacterial speck symptoms on (a): The tomato leaf seemed a dark center surrounding a yellowish halo while some of them were expanded and fused together, (b): Tomato mature fruits infected by bacterial speck seemed as dark center surrounding wide yellowish halo and some of them were expanded and fused together, (c): The petioles of tomato leaves seemed having small necrotic dark lesions, (d): Flower clusters of tomato plant at the fruiting stage showing small necrotic dark lesions, (e): Immature tomato fruits, where the chlorotic halo was not clearly noted, and (f): Bacterial colonies were isolated from symptomatic tomato leaves and fruits on KB medium

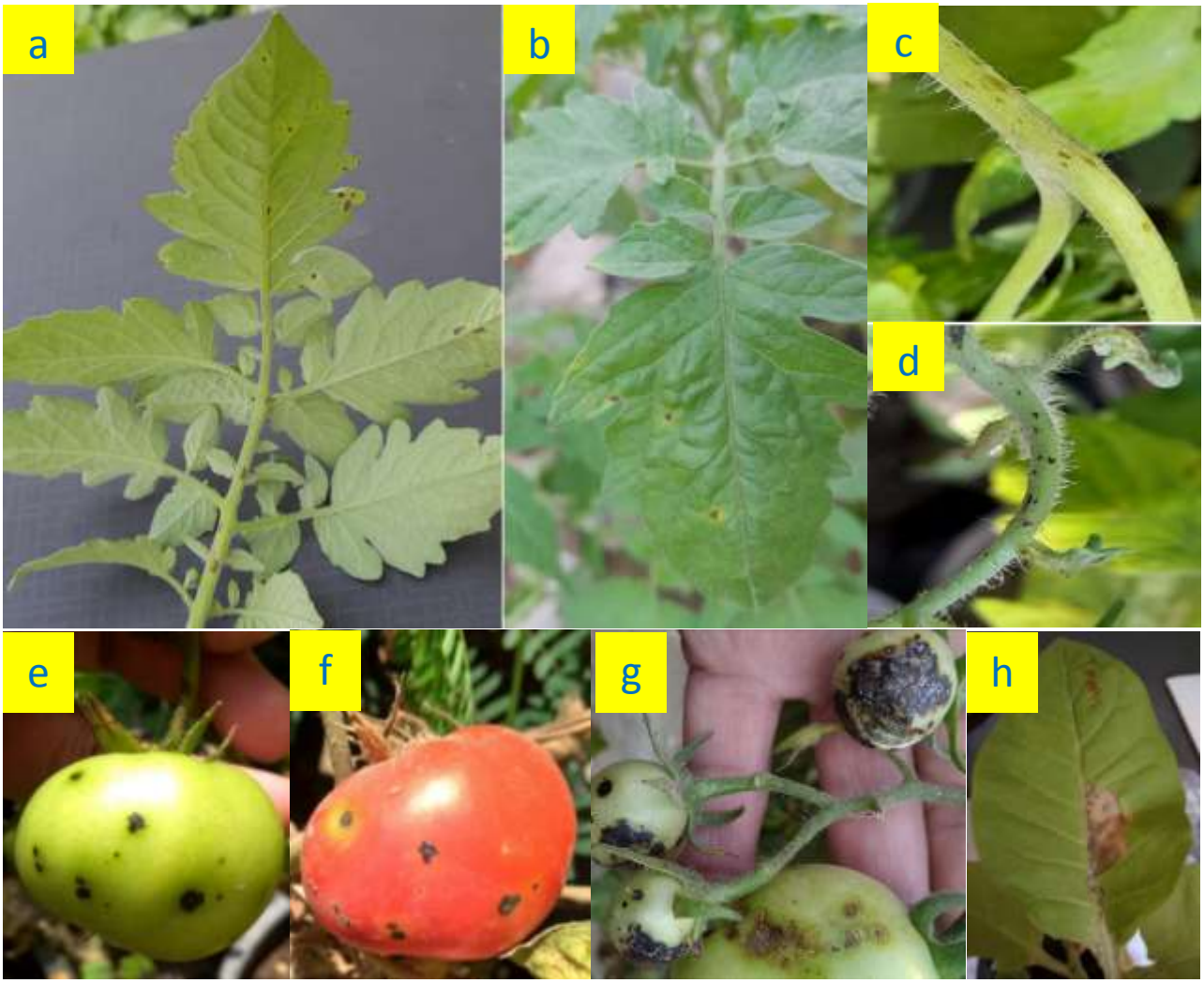

Fig. 2. The positive results for the pathogenicity assay on the inoculated tomato shoots; (a): The lower surface of the tomato leaf, (b): The upper surface of the tomato leaf, (c): Close-up for the small specks observed as water-soaked lesion symptoms on the tomato leaf petiole after 6 d of inoculation, (d): Necrotic lesions appearing after 9 d of inoculation on the leaf petiole, (e): On the immature fruit showing necrotic lesions without a yellow halo, (f): Lesions with an obvious yellow halo on the mature fruit, and (g): On continued humidity, the lesions expanded and fused together with a tan shape, and (h): Necrotic area on tobacco leaf as a hypersensitive reaction against the tested pathogenic bacterium

Table 1. Results of the microscopical, biochemical, and hypersensitive assay characterization of the 4 pathogenic bacterial isolates

Test	Result
Levan production	+
Oxidase activity	-
Potato soft rot	-
Arginine dihydrolase	-
Hypersensitive reaction with tobacco leaves	+
Gram stain	-
Cell shape	rod
Motility	+
Sporulation	-
3 % KOH solubility test	+
Catalase activity	+
Gelatin hydrolysis	+

Where; += positive with reaction, - = negative with reaction

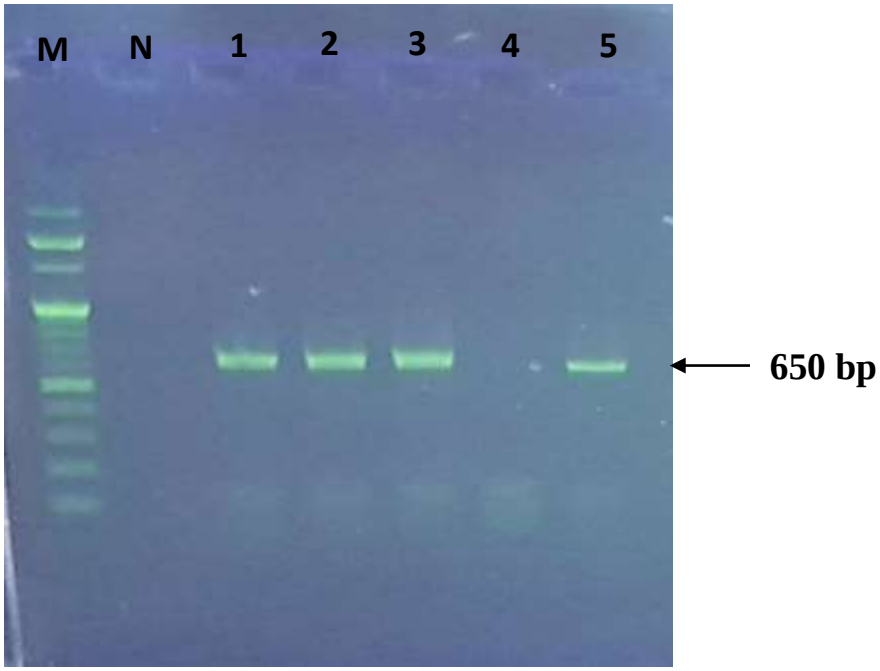

Fig. 3. Results of the PCR amplification. Where; Lane M: ladder marker (GeneRularTM 100 pb DNA ladder), Lane N: negative control, Lanes 1,2,3, and 5 represent the four selected pathogenic bacterial isolates, which exhibited an obvious band at 650 bp on the 1.2 % agarose gel, and Lane 4: the nonpathogenic control isolate

produced significant alignment and neighborhood to *P. syringae* pv. *tomato* (Fig. 4); with a similarity percentage of 99 %. The sequence for the studied isolate was deposited in GenBank as *P. syringae* pv. *tomato* strain Pst1-MAS and was assigned an accession no. of OQ117369.1. According to the obtained results of the pathogenicity test; the traditional biochemical assays, and the molecular characterization, the bacterial isolate was identified as *P. syringae* pv. *tomato*.

3.3. Sensitivity of the solanaceous and cruciferous plants to the Egyptian bacterial pathogen

Except for the tomato plants, all the tested plants didn't exhibit any pathogenic symptoms of bacterial speck throughout the observation period. The pathogenic symptoms observed on the treated tomato plants weren't different from those recorded previously in the pathogenicity test results as typical tomato bacterial speck symptoms. No symptoms were expressed on the control plants.

3.4. Disease severity

The tomato plants reaction was evaluated depending on the disease severity of the infected plants. The disease severity was evaluated at the first time after 8 d (first stage) and at the second time after 15 d (second stage) of inoculation by *P. syringae*. pv. *tomato* strain Pst1-MAS; while after more than 20 d of inoculation, the specks expanded and fused together and become difficult to calculate..

After 8 d of inoculation, the majority of tested tomato plants exhibited typical symptoms of bacterial speck disease on the leaves while the others plant parts expressed slight symptoms. After 15 d, the typical symptoms of bacterial speck became clearer on all the tested tomato cultivars and hybrids. Disease severity was estimated according to Yunis *et al*., (1980) scale, where the disease severity at the first stage exhibited deficiency of the scale degrees with small data (single speck on plan leaf) for the tomato cultivars cv. Edkawy and cv. Super Marmande. Meanwhile, the tomato cv. VFN-8 was higher in disease severity (2.5) among the different tested cultivars and hybrids; recording varying degrees of disease severity on the other tomato cultivars. In the second stage and according to the same scale, the tomato cultivar VFN-8 expressed high disease severity (3) followed by cv. Peto 86 (2.83). The tomato cv. Super Marmande was the lowest in expressing disease severity (0.66), while the scale displayed deficiency in estimating the severity with tomato cv. Edkawy, as the plant leaves contained a single speck (small data) (Table 2). The results of disease severity at the first stage according to the Chambers and Merriman, (1975) scale showed that tomato cultivars cv. Peto 86 and cv. VFN-8 displayed equal disease severity (1.42) and were the higher without significant differences among them. The lowest cultivar showing disease severity was tomato cultivars cv. Super Marmande (0.14). The other tested tomato cultivars and hybrids varied in their disease severity. In the second stage, the tomato cultivars VFN-8 and Peto 86 were equal in disease severity without differences among them (2.42). The tomato cv. Super Marmande was the lowest in terms of disease severity (0.42), while the other tested tomato cultivars and hybrids varied in their disease severity (Table 2). According to Bastas, (2015) scale as a modification to Chambers and Merriman, (1975) scale, the disease severity at the first stage of inoculation exhibited deficiency of the scale degrees with large numbers of specks on the leaf (large data) for tomato cv. Peto 86; while at the second stage, the deficiency included most of the studied cultivars (Table 2).

After 8 d of inoculation (at the first stage), the recorded disease severity that dependent on the mean of bacterial specks on the tested plants showed that tomato cultivar cv. Peto 86 was higher in disease severity (17), followed by cv. VFN-8 (10.5). The disease severity with tomato cv. Edkawy was (0.33), while the lowest disease severity was observed with tomato cv. Super Marmande (0.166). The disease severity on the other tested tomato cultivars varied

(Table 2). After 15 d of inoculation (the second stage), the results of detecting disease severity depending on the mean of bacterial specks on the tested plants showed that the tomato cultivar with the highest disease severity and sensitivity to bacterial infection was cv. Peto 86 (31), followed by cv. VFN-8 (30.5); without significant differences between these two cultivars. The disease severity for the tomato cv. Edkawy was (3.83), while the lowest disease severity was recorded in tomato cv. Super Marmande (2.33); with relatively light symptoms. The disease severity on the other tested tomato cultivars varied (Table 2). As presented in Table (3), depending on the mean of disease severity (mean number of specks/ leaves), the tested tomato cultivars and hybrids were divided into high sensitivity (severity = 25 and more); moderate sensitivity (severity = 24:11 specks/ leaves), low sensitivity (severity = 10:1 specks/ leaves), and resistant plants (severity = 0).

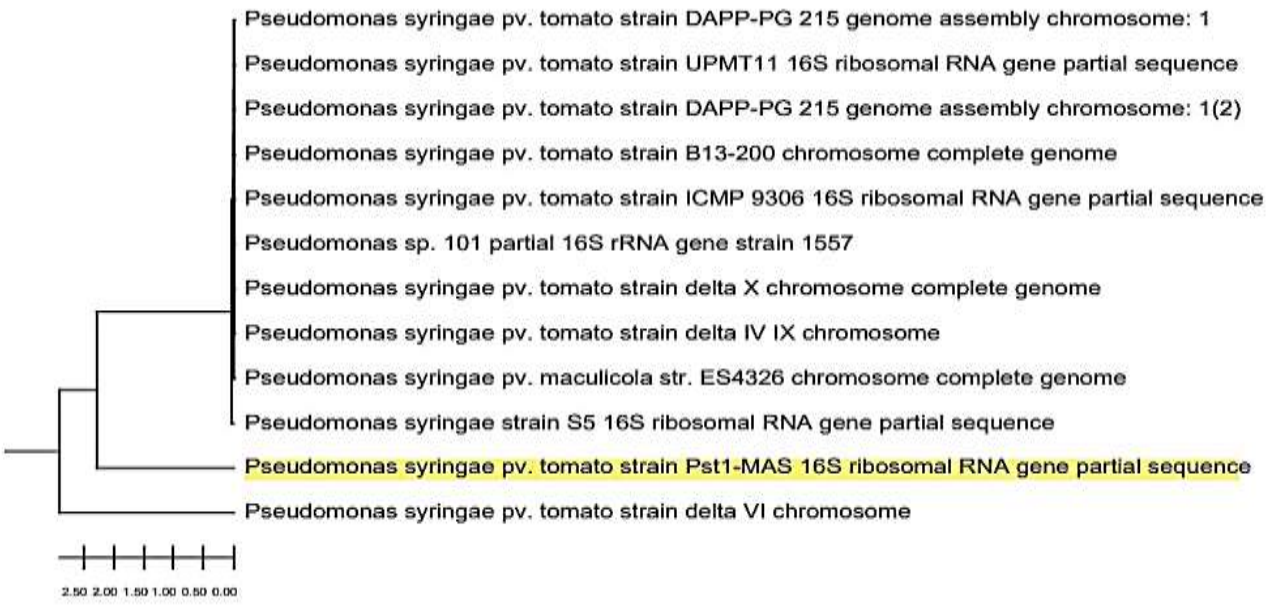

Fig. 4. The phylogenic tree showing the neighborhood between the studied *P. syringae* pv. *tomato* strain and the other strains deposited in GenBank

Table 2. Evaluation of the disease severity in treated tomato seedling, in comparisons among the different scales for estimating the disease severity

Cultivar/ hybrid	The bacterial treatment							Bacterial speck disease severity			
		Replicates									
	Data stage	1	2	3	4	5	6	***Mean number of specks/leaf ± SE	Yunis *et al.*, (1980) scale	Chambers and Merriman, (1975) scale	Bastas, (2015) scale
VFN-8	1st	11	5	18	11	12	6	10.5 ±1.9 ab	2.5	1.42	2
	2nd	51*	20	50*	20	29	13	30.5 ±6.6 a	3	2.42	*
Peto 86	1st	31*	7	9	5	50*	0	17 ±7.9 a	1.83	1.42	*
	2nd	44*	22	40*	10	55*	15	31 ±7.3 a	2.83	2.42	*
Super Marmande	1st	1**	0	0	0	0	0	0.166± 0.1 c	**	0.14	0.16
	2nd	7	4	3	0	0	0	2.33 ±1.1 d	0.66	0.42	0.66
Floradade	1st	21	0	0	7	11	5	7.33± 3.2 b c	1.5	1.00	1.5
	2nd	60*	7	3	17	10	17	19 ±8.5 a b c	2.33	1.57	*
Edkawy	1st	2	0	0	0	0	0	0.33 ±0.3 c	0.16	0.28	0.16
	2nd	12	1**	1**	3	2	4	3.83 ±1.7 c d	**	1.00	1.16
023F1	1st	3	6	1**	1**	3	6	3.33 ±0.9 b c	**	0.85	1.33
	2nd	27	33*	9	11**	19	51*	25 ±6.4 a b	**	2.14	*
T-186 hybrid	1st	4	2	5	3	3	3	3.33 ±0.4 b c	1	0.85	1
	2nd	13	5	33*	12	5	10	13 ±4.2 b c d	2.16	1.42	*

Where; LSD among the plants with the first-time severity at 0.05= 9.5; LSD among the plants with the second-time severity at 0.05= 16.5

*= Data not included at Bastas, (2015) scale as a modification to Chambers and Merriman, (1975) scale.

**= Data not included at Yunis *et al.*, (1980) scale.

***= Total number of specks/ leaf. Values followed by the same superscript letter were not significant.

Table 3. Assessment of different tomato seedling depending on the mean of bacterial speck disease severity

Cultivar/ hybrid	Bacterial speck disease sensitivity
VFN-8	High
Peto 86	High
Super Marmande	Low
Floradade	Moderate
Edkawy	Low
023F1	High
T-186 hybrid	Moderate

Where; High: severity = 25 and more, Moderate: severity = 24:11, and Low: severity = 10:1

3.5. Defense-related enzymes activity

The infected tomato plants varied in their enzymatic activity of POD and PPO as defense-related enzymes. Compared with the control (healthy) plants, PPO enzyme activity rate was higher with the infected tomato cv. Super Marmande by more than five times; followed by tomato 023F1 by more than three times, and tomato cv. T-186 hybrid by more than two times. The tomato cv. VFN-8; cv. Edkawy, and cv. Peto 86 showed moderate activity of PPO enzyme, while the tomato cv. Floradade was low, compared with the control plants. On the other hand, the tomato cv. Floradade and cv. VFN-8 displayed moderate activity with POD enzyme activity, compared with the control plants. Meanwhile, POD enzyme activity with the other tomato cultivars was low compared with the control plants. The PPO enzyme activity rate was higher with infected tomato cv. 023F1 by more than five times, followed by tomato cv. T-186 by more than four times, and the tomato cv. Super Marmande by more than three times, compared with the value of the control plants (Table 4).

The correlation coefficient between disease severity of the tested tomato plants and PPO activity was (r = -0.190), while with POD activity it was (r = -0.467), which means an inverse or a negative correlation.

4. Discussion

Tomato is one of the most important grown vegetable crops in Egypt, which has a large number of old and novel cultivars grown in open fields and under plastic greenhouses. The study sought to isolate and identify *P. syringae* pv. *tomato* as a causal agent of the bacterial speck disease of tomato plants in Egypt, and which the tomato cultivars or hybrids have low sensitivity to infection by it; especially with the wide tomato plant areas. The tomato bacterial speck disease incited by *P. syringae* pv. *tomato* can spread and turn into an epidemic disease under suitable environmental conditions such as high humidity and low temperatures; associated with the wrong farmer's practices (Gür *et al*., 2021). Depending on the conventional bacteriological tests, the results of isolation and identification of *P. syringae* pv. *tomato* are consistent with several previous studies conducted

Table 4. Enzymatic activity of peroxidase and polyphenol oxidase as defense-related enzymes for the tested tomato plant cultivars

Cultivar/ hybrid	Enzymes activity (U(µmol)/ ml)*					
	Peroxidase			Polyphenol oxidase		
	Healthy	Infected	%	Healthy	Infected	%
VFN-8	1.87 [a]	2.38 [a]	126.9 [e]	0.56 [a]	1.11 [a]	196 [d]
Peto 86	1.88 [a]	1.60 [d]	85.2 [g]	0.65 [a]	0.88 [b]	134.4 [f]
Super Marmande	0.42 [d]	2.17 [b]	515 [a]	0.20 [bc]	0.72 [c]	355.5 [c]
Floradade	1.05 [b]	1.98 [c]	188 [d]	0.56 [a]	0.50 [e]	88 [g]
Edkawy	0.88 [c]	0.84 [f]	95.2 [f]	0.31 [b]	0.61 [cd]	192.8 [e]
023F1	0.38 [d]	1.20 [e]	316.6 [b]	0.11 [c]	0.56 [de]	625 [a]
T-186 hybrid	0.35 [d]	0.76 [f]	211.7 [c]	0.20 [c]	0.95 [ab]	466.6 [b]
LSD at 0.01=	0.11	0.29	11.013	0.080	0.12	11.013

Where; *= Enzyme activity (U (µmol)/ ml) = (ΔA × Vt ×10 6) / (Δt ×l × ε ×Vs × 1000).

ΔA is the change in absorbance, Δt is the incubation period (min.), ε is the extinction coefficient (M^{-1} cm^{-1}), l is the cuvette diameter (1 cm), Vt is the total assay volume, and Vs is the enzyme sample volume (ml). The ε240 nm of H_2O_2 is 43.6 M^{-1} cm^{-1}, ε420 nm of pyrogallol is 2640 M^{-1} cm^{-1}, and ε420 nm of catechol is 2450 M^{-1} cm^{-1}. Values followed by the same superscript letter were non-significant.

by Mensi *et al*., (2018); Akter *et al*., (2019); Ahmed, (2022); Valenzuela *et al*., (2022); Alfaro-Quezada *et al*., (2023). Moreover, the results of molecular identification of the tomato bacterial pathogen through 16S rDNA and using the coronatine production primers are in agreement with Wreikat *et al*., (2006); Milijašević *et al*., (2009); Mensi *et al*., (2018); Ahmed, (2022). Depending on the molecular features, Abd El-Fatah *et al*., (2023) found great variation in virulence among the different isolates of *P. syringae* pv. *tomato* that were isolated from different localities in Egypt.

This study revealed that the tested bacterial isolate was able to induce the pathogenic symptoms of bacterial speck disease on tomato plants only; however, it could not do the same on the other tested solanaceous and cruciferous plants. This result is in consistent with Kraus *et al*., (2017); Milijašević *et al*., (2009). On other hand, Cuppels and Ainsworth, (1995) reported that among the 50 tested bacterial isolates of *P. syringae* pv. *tomato*, two isolates only had the ability to infect cauliflower and cabbage as well as the tomato plants.

The disease severity among the tested tomato cultivars varied and sensitivity to the studied strain of *P. syringae* pv. *tomato* strain Pst1-MAS was different among them. This variation in disease severity among the tested tomato cultivars may be attributed to more than one tool, including plant defense mechanisms; the contents of phenolic compounds in the different cultivars, and/ or activation of the defense-related enzymes. In the current study, estimation of the tomato bacterial speck disease severity and susceptibility of the different tomato plants to infection by *P. syringae* pv. *tomato* showed that tomato plant cv. Pst1-MAS had several limitations concerning the various used severity scales. These include the development of a single speck on a tomato leaf, which did not find a severity degree in Yunis *et al*., (1980) scale; with large numbers of specks (more than 30 specks on a leaf) in Bastas, (2015) scale, and at the different stages of estimation of the different plants in Chambers and Merriman, (1975) scale. On the other hand, estimation of the tomato bacterial speck disease severity based on the mean of the number of specks has skipped these limitations at the different stages of estimating the disease severity, and it provided a high level of confidence at the second stage. These results agree with the previous study reported by Quaglia *et al*., (2021), which estimated the disease severity depending on counting the mean numbers of specks on the plant leaves. This study revealed that the defense-related enzymes activity was higher with the infected tomato plants; however, the correlation between the severity and the defense-related enzyme activity had a negative correlation coefficient. The tomato cv. Super Marmande had a lower sensitivity to infection and a higher POD activity, whereas the cv. Peto 86 had a higher disease severity and lower POD and PPO potential. POD enzyme is considered as one of the main enzymes used in protection and defense mechanisms in the plants against the microbial pathogens (Khlaif *et al*., 2020). PPO endogenous enzyme rate had increased after infection by *P. syringae* pv. *tomato* with a higher rate after 15 d of infection (Khlaif *et al*., 2020). Most of the infected tomato plants exhibited high or moderate enzymatic activity either for both of the tested POD and PPO or for one of them only. El-Argawy and Adass, (2016) reported that the rate of enzymatic activity for POD and PPO may be employed as biochemical indicators to determine the susceptibility or resistance of the plant cultivars to the microbial infection.

Conclusion

The current study demonstrated that *P. syringae* pv. *tomato* strain Pst1-MAS that was isolated from the Egyptian tomato plants displayed bacterial speck disease symptoms. The tomato plants cultivated under Egyptian conditions were infected by *P. syringae* pv. *tomato* strain Pst1-MAS with differences in their disease severity. The tomato cv. Edkawy and cv. Super Marmande manifested relatively light bacterial speck symptoms and low sensitivity to infection, while the tomato cv. VFN-8 and cv. Peto 86 were highly sensitive to the tested bacterial strain. The disease severity for some of the tested tomato plants was inversely correlated with activity of the defense related enzymes.

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Conflict of interest

The author declares that no conflicts of interest exist.

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Author Contributions

The author contributed to all parts of this manuscript.

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