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Research article

Evaluation of tomato (*Solanum lycopersicum* L.) genotypes sensitivity to *Fusarium solani* infection under protected cultivation

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ABSTRACT

This study evaluated the susceptibility of ten tomato (*Solanum lycopersicum* L.) genotypes to *Fusarium solani* and the biochemical defense responses in the greenhouse. One *F. solani* isolate (F4) was found to be most virulent and its identity was molecularly confirmed as *F. solani* based on ITS region sequencing (Accession No. PZ146854). The incidence and severity of the disease were assessed sixty days after inoculation. Genetic variability was observed among the genotypes, with a substantial resistance response. In particular, 'Mercur' and 'Fairuz F1' were highly resistant (0.00% incidence and severity), whereas 'Balkis F1' was the most susceptible (100% incidence and 85% severity). To better understand the defense mechanism, the activities of defense enzymes - polyphenol oxidase (PPO); peroxidase (POD); and catalase (CAT) - were measured. In resistant cultivars, all defense enzymes were rapidly and highly activated after infection. In 'Mercur', PPO increased from 1.67 to 2.50 U/g FW, POD from 1.57 to 2.34 U/g FW, and CAT from 3.35 to 4.36 U/g FW. In contrast, the susceptible 'Balkis F1' exhibited a relatively mild increase in all defense enzymes (PPO: 1.09 to 1.78 U/g FW; POD: 0.96 to 1.38 U/g FW; CAT: 2.84 to 2.85 U/g FW). This suggests that the high genetic resistance in 'Mercur' and 'Fairuz F1' is intimately associated with the antioxidant enzyme system. Therefore, these genotypes are suggested as potential breeding lines for resistance to soil-borne fungal pathogens in tomato.

1. Introduction

The tomato crop (*Solanum lycopersicum*) is a member of the Solanaceae family, contributing significantly to the agricultural economy and food security (FAO, 2021). However, the cultivation of tomato is seriously impacted by biotic stresses, especially soil-borne pathogens that flourish at different stages of growth and under different environmental conditions (Shaji and Hemalatha,

2025). One of the most destructive fungi is *Fusarium solani*, the main contributor to root rot and seedling wilt (Severo et al., 2024). The pathogen causes typical symptoms such as yellow leaves, root growth arrest and dark discoloration of vascular tissues, resulting in significant yield loss. *F. solani* is

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a major pathogen in protected cultivation (greenhouses) due to the high humidity and temperature that can promote fungal growth(Mamaghani et al.,2024). Moreover, the long-term survival of this pathogen in the soil, in plant residues or organic matter, makes it extremely difficult to control(Severo et al., 2024). Although fungicides are widely used for disease management, extensive and long-term use of fungicides carries several major problems, such as environmental and human health risks, and induction of fungicide-resistant pathogens(Garcia-Marti and Simal-Gandara,2023). As a result, the use of disease-resistant genetic variants is the most environmentally sound, efficient, and sustainable approach. While several studies have extensively investigated resistance genes (e.g., I-1 to I-7) against *Fusarium oxysporum* (Fusarium wilt) in tomato, little information exists about the systematic assessment of the susceptibility of commercially grown and newly released tomato genotypes to *F. solani*, particularly under the environmental conditions of Iraqi greenhouses. In addition, the biochemical basis and enzymatic responses (such as induction of polyphenol oxidase, peroxidase and catalase) that distinguish resistant from susceptible genetic variants to this particular pathogen need to be further clarified (Van Loon et al., 2006). Thus, the current research sought to isolate and identify a highly aggressive strain of *F. solani*, and assess the sensitivity of ten tomato genotypes under controlled greenhouse conditions. Moreover, the study aimed to explore the biochemical basis of resistance by determining the induction of key plant defense enzymes, and to identify potential genetic resources that can be used for breeding purposes.

2. Materials and Methods

2.1 Isolation and identification of *F. solani* from infected tomato roots

Sampling was conducted on diseased tomato plants during the 2023-2024 growing season in Karbala (Al-Hurr and desert areas) and Najaf (Khan Al-Nus) Governorates. Root samples were immediately collected and transferred to the Postgraduate Laboratory in the Department of Plant Protection, the College of Agriculture, University of Kerbala, where fungal isolation was done. The roots were rinsed under running tap water to remove attached soil and surface debris for 30 minutes upon arrival . After that, they were sliced into 0.5 to 1 cm cm pieces and surface-sterilized with a 1% sodium hypochlorite solution. (NaClO) 2 minutes. This was done followed by three washes using sterile distilled water and then the segments were dried on sterile filter papers on top of Potato Dextrose Agar (PDA) medium with the addition of 125 mg/L amoxicillin to inhibit bacterial contamination. The root segments were prepared and then incubated for three days at 25 ± 2C in petri dishes. When the colonies of the fungi appeared, the hyphal ends were carefully transferred and placed on new PDA plates which had the same antibiotic as used before. These inoculated plates were then subjected to the best incubation to ensure that the resulting cultures were pure. An initial step to identifying the fungi was through the study of morphological features such as the colour of colonies, shape and borders of the colonies using classification keys that were readied by Leslie and Summerell (2006).

2.2 Molecular Identification of the Pathogen

Polymerase chain reaction (PCR) was performed using the Cat(i-Tag) Maxime PCR PreMix kit manufactured by the Korean company Intron for the purpose of identifying the fungi isolated in this study. The PCR reaction was performed in a total volume of 20 µL, containing 1 µL of each primer (forward: TCCGTAGGAACCTGCGG; reverse: TCCTCCGCTTATTGATATGC; (Cheng et al., 2016).

Additionally, 1 µL of extracted DNA was added. All the above components were placed in a tube provided by the manufacturer, and the volume was brought up to 20 µL with nuclease-free water.

Subsequently, DNA amplification of the pathogenic fungus was performed following the steps and conditions of the PCR reaction.

2.3 Pathogenicity assessment of the isolated fungi

1. Evaluation of pathogenicity against tomato seed germination on water agar medium

The pathogenicity of the isolated fungi was assessed based on their effect on tomato seed germination using water agar (WA) medium. The experiment followed the methodology described by Bolkan and Butler.(1974)

The water agar medium (W.A.) was made by dissolving 17 grams of agar in a liter of distilled water. The solution was then distributed into the glass flasks and was sterilized using an autoclave at a temperature of 121 ° C and 15 pounds per square inch for 20 minutes. After pouring the medium into petri dishes and allowing it to solidify, the middle of each plate was inoculated with a disc (0.5 cm in diameter) taken from the edge of a five-day-old fungal colony. The culture Petri-dishes were placed in an incubator at 25 ± 2°C for three days. Variant treatment involved surface- sterilized tomato local seeds with 1% sodium hypochlorite (NaClO) and sown at 10 seeds per Petri dish in a circular manner. A control was carried out in which all such steps were followed but the plates were not inoculated with any of the fungi that were tested. All Petri dishes were incubated at 25 ± 2 °C until all seeds of the control treatment germinated. The ratio of germinated seed was then measured. The germination rate was calculated using the formula:

Germination Percentage (%) = Number of germinated seeds/(Total number of seeds) x 100

The percentage of seedling rot was also calculated as in the following equation.

Percentage of seedling rot (%) = (Number of rotten seedlings)/(Number of healthy seedlings) x 100

2. Testing the pathogenicity of fungal isolates against tomato seed germination in plastic Pots

The experiment was carried out in the greenhouse of the college of Agriculture, University of Kerbala by using a well-mixed soil mixture with animal manure (1: 2). Loam soil :Peat moss The soil was sterilized in an autoclave at 121°C and 15 psi pressure for 60 min twice, with one day interval. Fungal inoculum was prepared on millet seeds (1%v/v) and mixed with the soil in plastic bags and then transferred to a plastic pot (500 g per pot). Pots were randomly arranged in the experimental area and watered gently. The same methodology was used with a control treatment, except that soil was not added a fungus isolate .

Three days later, locally obtained Tomato seeds (10 seeds per pot) were surface-sterilized with a 1% sodium hypochlorite (NaClO) solution and then planted in the pots. The pots were also well watered and additional water was applied as needed. The germination rate was computed after 14 days of planting and the formula used was the same as the one previously discussed. Based on the results of this experiment, the most pathogenic fungus (isolate F4) was selected for morphological identification and used in subsequent experiments. The infection percentage was calculated using the following formula:

Infection Percentage(%)= Number of infected seedlings/(Total number of seedlings) x 100

2.4 Evaluation of the response of selected tomato cultivars to *F. solani*

2.4 .1. Tomato seed sowing

Ten tomato cultivars (Table 1), not previously grown or tested under Iraqi conditions, were obtained from the Al-Rayahin Company to assess their response to infection by the fungus *F. solani*. Seeds of these cultivars were sown separately in seedling trays, each containing 50 cells, with two seeds placed in each cell. The trays contained only peat moss as a growing medium. Twenty days after germination, when the seedlings reached a suitable stage for transplanting, they were moved into 8 x 13 cm plastic pots for use in the experiment.

Table 1. Tomato cultivars used in this study to evaluate their response to *F.solani*

ID	Cultivar	Origin	Producing company
1	Oscar F1	Netherlands	Delta Seeds
2	Balkis F1	France	Elise Seeds S.A.S
3	Reto-77	Netherlands	Rain Seeds
4	Zaha F1	UK	Wave Seeds
5	Mahavis F1	India	Nunhems Netherlands BV
6	Tala F1	India	SEMILLAS Fito
7	Nermin 66	Netherlands	Enza Zaden
8	Bahja F1	Netherlands	Gold Seeds
9	Fairuz F1	France	Elise Seeds S.A.S
10	Mercur	India	Dyngsta Seeds

2.4 .2. Transplanting of seedlings into plastic pots

Peat moss loam soil was mixed in a ratio of 2:1 (v/v). This mixture was sterilized in an autoclave at 121 C and pressure of 15 psi in 60 minutes. On the subsequent day, the sterilization procedure was reiterated, and the complete eradication of microorganisms was confirmed. After sterilization, the soil was inoculated with 1% (w/w) *F. solani* previously prepared on millet seeds inoculum. The inoculum was mixed thoroughly with the soil in plastic bags to obtain an even distribution and then 1 kg of the prepared mixture was put into every plastic pot. After an initial watering, the pots were covered with perforated polyethylene bags for two days to maintain moisture and encourage fungal activity in the soil.A control treatment was also established following all the same procedures, except that the soil was not inoculated with the fungus. After preparation, three seedlings from the trays were transplanted into each pot. The experimental design consisted of four replicates for each genotype (Four pots, each pot contains three seedlings), as well as four replicates for the control treatment of each cultivar.

After 60 days, disease incidence was calculated using the following equation:

Infection Percentage (%) = Number of infected seedlings/(Total number of seedlings) x 100

Disease severity of root rot was assessed using the following rating scale:

Table 2. Severity scale for *F. solani* infection in tomato plants.

Infection severity (%)	Severity description	Severity score
0	Healthy plant (no symptoms)	0
>1–10%	Very mild brown discoloration(rot) in secondary roots	1
>11–25%	Coloring in secondary roots and part of the main root.	2
>26–50%	The main root is colored without rotting the base of the stem.	3
>51–75%	Root discoloration and stem base rot	4
>75–100%	Plant death	5

The percentage of disease severity was calculated using the formula of McKinney (1923), as cited in (Dakhil ,2021), as follows:

Infection Severity (%) = (Number of plants at each score x Score value)/ (Total no. of examined plants×Highest score) x 100

2.5 Measurement of plant defense enzymes

2.5 .1. Polyphenol oxidase enzyme (PPO)

The activity of polyphenol oxidase (PPO) was identified based on the procedure presented by Zhang (2023). Approximately 1 g of leaf and stem tissue of 50 days old plant was homogenized with 20 mL of 0.1 M sodium phosphate buffer (pH 6.5). The mixture was then centrifuged at 16,000 rpm for 15 min at 4°C and the resulting clear supernatant was employed as crude enzyme extract Assay reaction mixture consisted of 1 5 mL of 0.1 M sodium phosphate buffer (pH7.0) and 200 µL of catechol (0.01 M). 200 µL of the enzyme extract was placed in the assay to start the reaction. Finally, the reaction velocity was observed, at 495 nm of wavelength, every 30 seconds during three minutes.The enzyme was then quantified by measuring the change in absorbance per minute per gram of fresh weight, calculated using the following formula:

Enzymatic activity*= (Spectrophotometric Reading)/(Sample weight (g)/Extraction volume (mL)×Volume used in assay (mL) x 100

*Enzymatic activity absorption unit g /wet weight.

2.5 .2. Peroxidase enzyme (POD)

Peroxidase enzyme (POD) activity was measured according to the procedure described by Hammerschmidt and Kuć (1982). About one gram of tomato leaf and stem tissue was ground with 20 mL of 0.1 M sodium phosphate buffer (pH 7.0). The obtained homogenate was filtered through two layers of muslin cloth and centrifuged at 16,000 rpm for 15 minutes at 4°C. The transparent supernatant was gathered and used as the crude enzyme extract in subsequent analyses. The 0.5 mL of this extract was combined with 0.5 mL of 0.05 M pyrogallol solution and 1.5 mL of 1% distilled water to the reaction mixture. The absorbance was recorded at 420 nm at 30-second intervals over a period of three minutes to measure the reaction rate. The enzyme activity was expressed as the change in absorption per minute per gram of fresh tissue weight, which was calculated according to the following equation:

Enzymatic activity*= (Spectrophotometric Reading)/(Sample weight (g)/Extraction volume (mL)×Volume used in assay (mL) x 100

*Enzymatic activity absorption unit g /wet weight.

2.5 .3. Catalase enzyme (CAT)

1-Aebi (1983) method was used to measure catalase (CAT) activity as cited in the research of Al-Shammari (2023). The required reagent solutions were then prepared as follows:

2-20 mMol of potassium phosphate solution (pH 7.0): Prepared by mixing two buffered solvents as shown below:

A. Solution A (K_2HPO_4): 0.346 g of dipotassium phosphate was dissolved in a small volume of distilled water, and the solution was brought to a final volume of 100 mL.

B. 10 mM Hydrogen Peroxide (H_2O_2) Solution: Prepared by diluting 1.030 mL of a 30% H_2O_2 stock solution in a small volume of potassium phosphate buffer, and the final volume was then adjusted to 100 mL with distilled water.

Assay Procedure:

1. One gram of leaf tissue was taken from the upper part of the plant and thoroughly homogenized using a ceramic mortar and pestle. The homogenate was passed through two layers of muslin cloth and centrifuged at 10,000 rpm for 10 minutes at 4°C.
2. To start the reaction, 40 µL of the enzyme extract was added to 2 mL of a freshly prepared 10 mM hydrogen peroxide solution. The mixture was incubated for one minute at room temperature, and the decrease in absorbance was recorded at 240 nm using a spectrophotometer.
3. Enzyme activity was calculated according to the following equation:

$$\text{Catalase Activity (Units)} = \frac{\text{Abs} \times \text{Reaction Volume} \times \text{Reaction Time} \times 2}{0.01} \; x \; 100$$

Abs = difference between absorbances (first absorbance - second absorbance)

Reaction volume = 2.04 mL

0.01 = constant

2.6 Computational Tools and Analytical Framework

All bioinformatics, phylogenetic and statistical analyses have been conducted with MEGA11. All tomato cultivars experiments were conducted according to a completely randomized design (C.R.D.) and the results were statistically analyzed using the GenStat program. and significant differences among treatment means were determined by the Least Significant Difference (L.S.D.) test at the $P \leq 0.05$ level, as described by Al-Rawi and Abdulaziz (2000).

3. Results and Discussion

3.1 Isolation and morphological identification of *F. solani*.

The fungi that were isolated were 14 fungi from the roots of tomato plants showing symptoms of root rot, wilting and discolouration of vascular tissues. According to Leslie and Summerell's (2006)

keys, all the isolates were first identified as *Fusarium*. Colonies on PDA media ranged in colour from white to creamy-white, with some producing pinkish to violet pigments. Isolate F4, which exhibited the greatest virulence in later testing, exhibited fast-growing mycelia. Hyphae were septate and two types of conidia were present: macroconidia and microconidia. The macroconidia were fusiform, slightly curved, rounded at the tip, thick-walled and 3-5 (seldom 6) septate. The microconidia were mostly oval, reniform or fusiform and 0-1 septate. Also, globose, thick-walled chlamydospores were found in chains. This description matches that of *F. solani* as described by Qiu et al. (2024).

3.2 Pathogenicity test of the isolated fungi

3.2 .1. Pathogenicity test on tomato seed germination in water agar medium

Pathogenicity tests showed a clear difference in the effect of the fourteen fungal isolates on tomato seed germination, among these isolates, isolate F4 was classified as the most virulent, with a seed germination rate of 6.70%, followed by isolate F8 with a germination rate of 10.00%. Isolate F9 was the least virulent, recording a germination rate of 56.70% (Table 3, Figure 1) .

Furthermore, isolate F4 recorded the highest seedling drop rate at 50.0%, while the lowest rate was observed with isolate F10 at 7.90% (Table 3). The significant difference in the pathogenicity of these isolates is explained by their genetic differences and the variety of the substances that they produce, including toxins and degradation enzymes, which help the pathogen to enter the host and lead to the infection (Stępień and Chełkowski, 2010).

Several studies have indicated that pathogenic fungal species are characterized by their high capacity to produce enzymes such as proteases, degrading enzymes, cotinases, and cellulose enzymes.This could result in differences in the makeup of harmful metabolites and enzymes that break down cellulose and pectin, which could work alone or in concert to prevent seed germination (Haggag and El-Gamal, 2012; Bhattacharya et al., 2013). Indeed, besides the production of enzymes that digest pectin, *Fusarium* species are also known to produce compounds that inhibit the germination of the seeds (Idris et al., 2003).

Table 3. Pathogenicity of fungal isolates in this study on tomato seed germination in water.

ISOLATE SYMBOL	GERMINATION %	SEEDLING ROT%
Control	100.00	000.0
F1	040.0	040.0
F2	033.3	46.70
F3	040.0	0.004
F4	06.7	09.50
F5	033.3	10.00
F6	023.3	09.10
F7	023.3	13.30
F8	010.0	036.7
F9	056.7	043.3
F10	030.0	09.7
F11	026.7	043.3
F12	023.3	043.3
F13	033.3	040.0
F14	023.3	020.0
$LSD_{0.05}$	**18.43**	**27.13**

3.2 .2. Pathogenicity of fungal isolates on tomato seed germination in plastic pots.

Table (4) and Figure (1) showed that the isolates of *Fusarium* spp. had a significant impact on decreasing seed germination with the percent ranging between 20.0% and 75.0 as compared to the 100.00% germination rate in the control treatment. It was revealed that the isolate F4 was the most virulent which led to the highest percentage of reduction of seed germination with a germination rate of 20 only. On the other hand, the isolate F9 was least virulent, with the germination rate being 75, F11 and F5 germination rates being 73.3 and 70 respectively. Isolate F4 is more pathogenic than the other strains of *Fusarium*. The present findings are in line with those by Al-Tamimi (2019), Al-Asadi (2020), and Mutab and Al-Abidi (2025). In this regard, all subsequent experiments in this study were done using isolate F4, which was chosen as the most virulent of all *F. solani* isolates.

Table 4. Pathogenicity of fungal Isolates in this study on tomato seed germination in plastic pots.

ISOLATE NUMBER	GERMINATION	ISOLATE NUMBER	GERMINATION
Control	100.00	F8	063.3
F1	063.3	F9	075.0
F2	063.3	F10	053.3
F3	050.0	F11	073.3
F4	020.0	F12	060.0
F5	070.0	F13	060.0
F6	050.0	F14	043.3
F7	066.3	-	-
$LSD_{0.05}$ = 21.38			

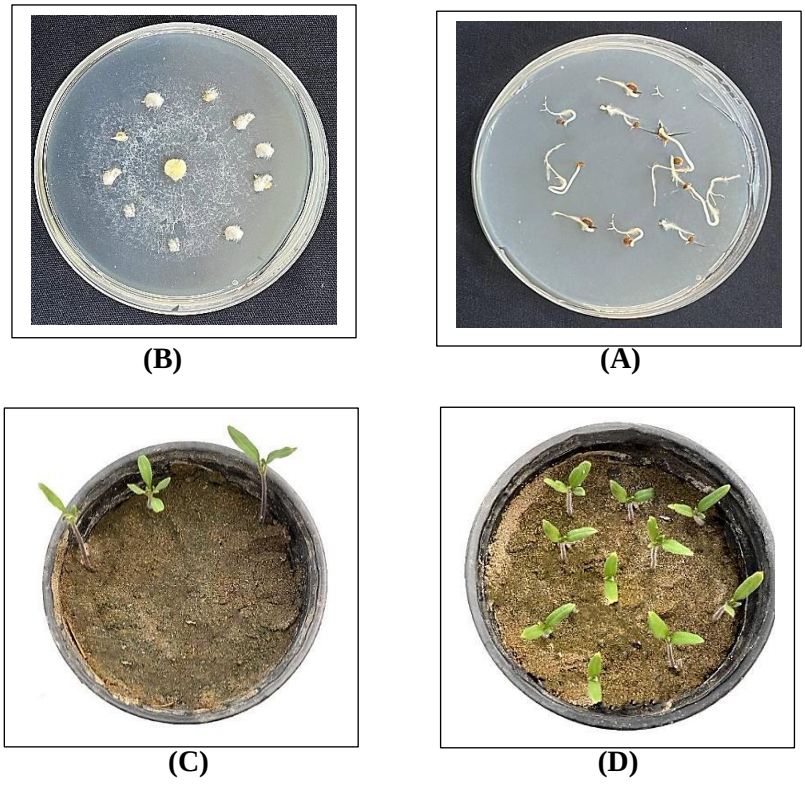
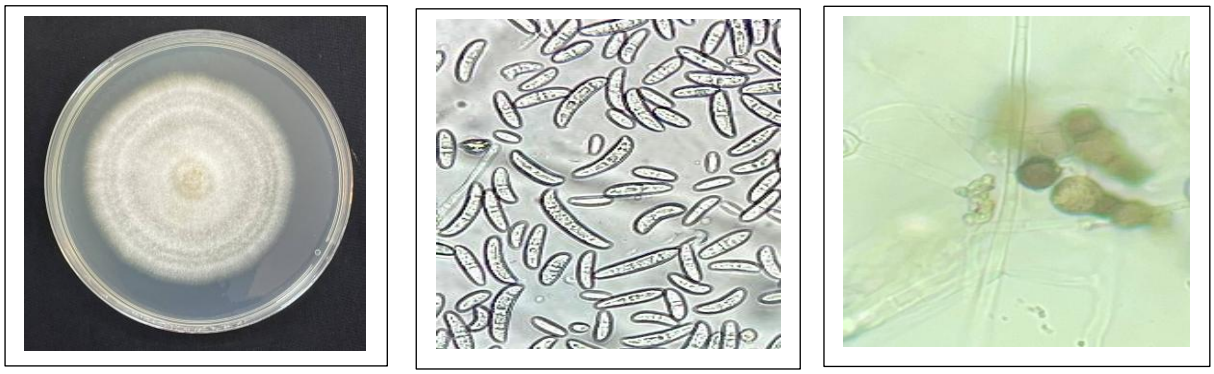


Figure 1. Pathogenicity of fungal isolates against tomato seed germination in Petri dishes and plastic pots. (A) A Petri dish inoculated with *F. solani*. isolate F4; (B) An uninoculated control dish; (C) Soil inoculated with *F. solani*. isolate F4; (D) Uninoculated control soil

3.3 Morphological characterization of the most virulent isolate, *F. solani* (F4), on tomato seeds and seedlings.

Isolate F4 that caused seed rot and damping-off in tomato was morphologically identified based on the taxonomic key According to Leslie and Summerell (2006), the growth of the isolate F4 on Potato Dextrose Agar (PDA) resulted in a rapid white mycelial growth, with colonies slowly changing to creamy-white as they grew.Microscopic observation revealed the presence of Septate mycelia and two types of conidia. The macroconidia were fairly broad, it was straight or slightly curved with rounded apices, thick-walled, and had 3-6 septa. Microconidia were globose, oval, reniform or fusiform, and most of them contained 1 to 2 septa. Chlamydospores were present in great abundance, and were generally globose to oval, and very often in chains. F9, as opposed to the other isolates, grew on PDA media forming a pinkish-red colony after seven days (Figure 2). Overall, the morphological and microscopic characteristics identified can be associated with the characteristics of *F. solani* reported by Qiu et al. (2024). *F. solani* have macroconidia that are straight or slightly curved with 3 to 5 (sometimes to 6) septa, broad central cells, an apical cell that is blunt and a notched or clearly foot-shaped base cell in their description. Its microconidia are usually oval or oblong, and globose and rough-walled chlamydospores are formed profusely.



(A) **(B)** **(C)**

Figure 2. Morphological and microscopic characteristics of *F. solani* isolate F4. (A) Colony of isolate F4 on Potato Dextrose Agar (PDA); (B) Macroconidia and microconidia; (C) Chlamydospores.

3.4 Molecular Identification of pathogenic fungal isolates using polymerase chain reaction (PCR)

The results of DNA extraction from isolates of *Fusarium solani*, followed by polymerase chain reaction (PCR), demonstrated the potential to amplify PCR-amplified DNA products with sizes (548 and 655 base pairs) using the ITS1 and ITS4 primers Figure (3).

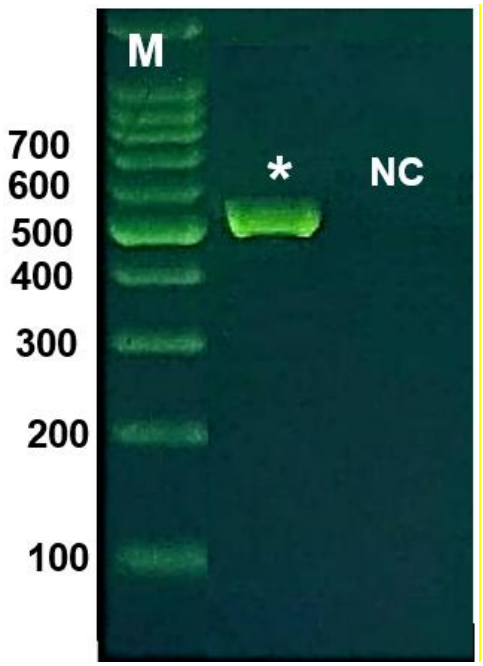


Figure 3. PCR amplification of the ITS region using ITS1 and ITS4 primers from the most pathogenic fungal isolate (indicated by), recovered from roots of diseased tomato plants in the present study. M = 100 bp DNA ladder; fragment sizes (bp) are indicated on the left side of the figure. NC = negative control (PCR reaction without DNA template).

The PCR results were sent to the Korean company Macrogen, where the nucleotide sequences were analyzed and processed using MGG6 and BioEdit software, and then compared with isolates in the U.S. National Center for Biotechnology Information (NCBI). The results showed that the *Fusarium solani* isolate were genetically distinct from other isolates previously registered in the NCBI, meaning they were isolate not previously registered in Iraq with the aforementioned center; therefore, they were registered under accession numbers (PZ146854) . Figure (4)Because some morphological diagnostic methods are often inaccurate, the polymerase chain reaction (PCR) technique has been widely adopted due to its high accuracy in fungal diagnosis. Studies have shown that the morphological diagnosis of *Fusarium* spp. may not be accurate due to the significant similarity in morphological characteristics among most of their species (Stoeva et al,2023). whereas differences in DNA sequences in the ITS (Internal Transcribed Spacer) region have facilitated the accurate diagnosis of many fungi belonging to the genera *Fusarium* spp. (El-Bebany et al, 2024).

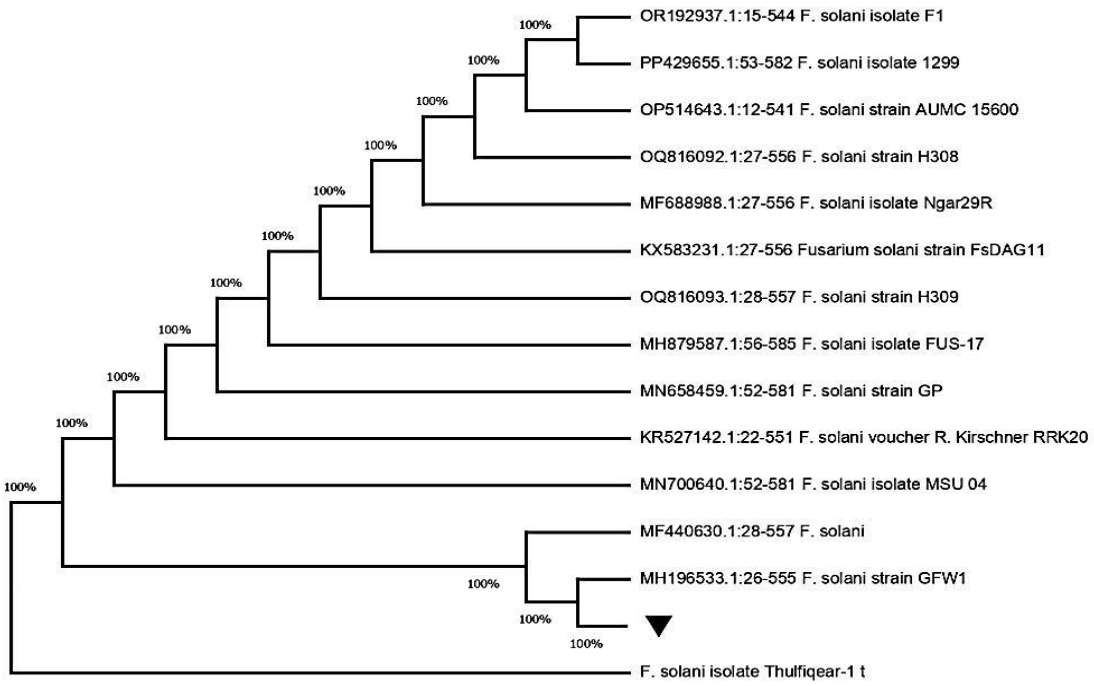


Figure 4. A Neighbor-Joining phylogenetic tree showing the phylogenetic relationship between the *F. solani* Thulfiqear-1 isolate identified in this study and other isolates of the same fungus previously registered with the National Center for Biotechnology Information (NCBI).

3.5 Evaluation of the susceptibility of ten tomato genotypes to *F. solani* in a greenhouse pot experiment

As it can be seen in Table (5), there was a considerable disparity in the infection and severity of the disease in the tomato genotypes, which is an indicator that there is genetic variation in the genotype in the way they respond to the disease in question. Mercur and Fairuz F1 genotype exhibited complete resistance to the infection with a rate of incidence and severity of 0.00 which could be explained by the efficiency of the natural defense mechanism toward *F. solani*. Conversely, Balqis F1 genotype was the most prone, having an incidence rate of 100 and a severity rate of 85, indicating that increased incidence is usually followed by an escalation of the severity of the disease. Besides, genotype (6) which had an incidence rate of 80 and a severity rate of 83.33 indicates that the severity of the disease generally increases with an increase in incidence.

This correlates with Agrios (2005) who demonstrated that the severity of the disease is critical in establishing the usefulness of resistance and how the defensive response is manifested in the infected tissues. Notably, certain genetic patterns with relatively low incidences of the disease recorded moderate severity including those of Zaha F1 and Reto-77. This can either indicate the existence of partial resistance mechanisms or that there is temporary defense mechanism that is limiting the spread of the disease despite the successful penetration of the pathogen.

This characteristic is very crucial in genetic improvement programs since Singh et al. (2019) reported that a partial resistance can be beneficial in delaying the disease process and minimizing losses in production in the long term. Considering these results, breeding programs should consider using genotypes having high or partial resistance so as to transfer the resistance characteristics to

generations ahead e.g. Mercur, Retto-77 and Oscar F1. More research should be conducted in order to have a better insight into the immunological and genetic causes of such resistance. On the other hand, there was a need to eliminate some of the most vulnerable genotypes, including Tala F1 and Balqis F1, in the production schemes or review their agricultural feasibility to guarantee crop activity.

Table 5. Evaluation of the susceptibility of ten tomato genotypes to *F. solani* .

ID	Cultivar	Disease Incidence (%)	Disease Severity (%)
1	Control	0.00	0.00
2	Mercur	0.00	0.00
3	Oscar F1	25.00	14.40
4	Reto-77	66.66	58.75
5	Zaha F1	50.00	31.80
6	Mahavis F1	58.33	.0050
7	Tala F1	83.33	80.00
8	Nermin 66	66.66	77.00
9	Bahja F1	75.00	65.00
10	Balkis F1	100.00	85.00
11	Fairuz F1	0.00	0.00
	LSD $_{0.05}$	**6.754**	**8.285**

To continue exploring the correlation between the pathological parameters, Pearson correlation analysis was carried out. The findings revealed the existence of a very significant positive correlation between disease incidence and disease severity.)r = 0.97 , P ≤ 0.05; Fig. 3). Moreover, the linear regression analysis (R 2 = 0.95 had the effect that the incidence rate was able to explain 95% of the variation in the severity of the disease across the genotypes. The strong association (Fig. 5) confirms that the rapid and severe tissue colonization by *F. solani* is consistently correlated with high frequency of infection in susceptible genotypes such as, but not restricted to, the following: Balkis F1 and Tala F1.

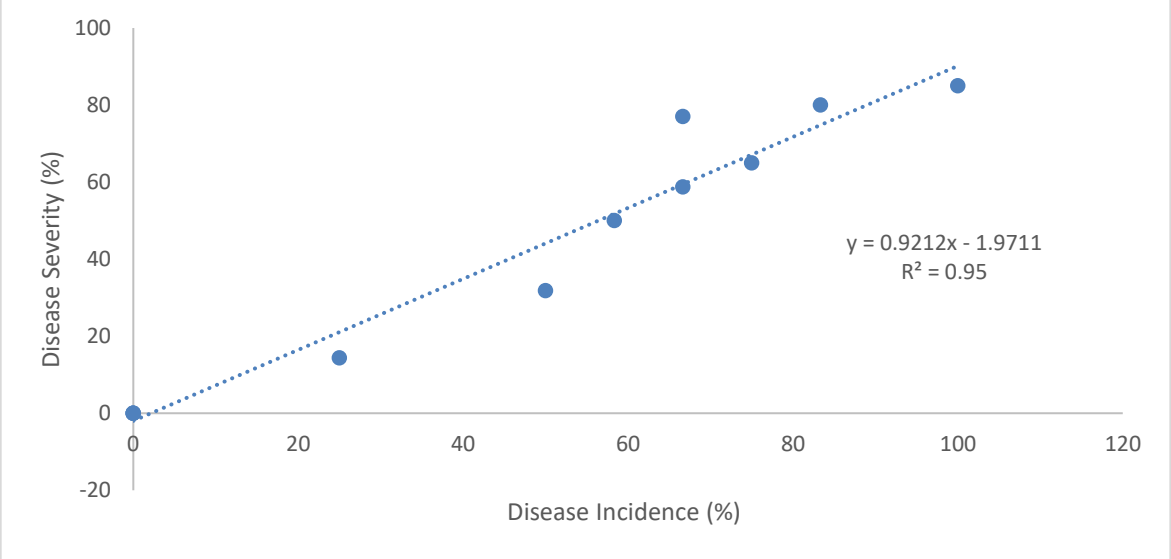


Figure 5. Linear regression analysis illustrating the relationship between disease incidence (%) and disease severity (%) across the ten tomato genotypes infected with *F. solani*. The dashed line represents the regression trendline (R2=0.95).

3.6 Activity of defense enzymes in contrasting tomato genotypes in response to *F. solani* infection

Table 6 and Figure 6 shows that the resistant Mercur genotype displayed a clear increase in the activity of defense-related enzymes after inoculation with *F. solani*. In plants grown in the inoculated soil, the activities of polyphenol oxidase (PPO), peroxidase (POD), and catalase (CAT) were 2.5, 2.34, and 4.36 U/g fresh weight, respectively. These values differed significantly from those recorded in the control treatment (uninoculated soil), which were 1.67, 1.57, and 3.35 U/g fresh weight, respectively. In contrast, the sensitive genotype (Balkis F1) showed lower levels of the same enzymes when cultured in soil inoculated with *F. solani*, with activities of 1.78, 1.38, and 2.85 U/g FW, respectively, compared to the control treatment. values of 1.09, 0.96, and 2.84 U/g FW weight, respectively.

The above findings indicate that the high activity of the defense-related enzyme could be contributing to the resistance of the Mercur variety to fungal infection, such as PPO, POD, and CAT. The finding proves this hypothesis that the defense mechanism of the plant against fungal diseases is enhanced by the activation of these enzymes. Besides the enzymatic analysis, the geographical background of the most resistant cultivar (Mercur of India) and the most vulnerable (Balkis F1 of France) cultivar provided some insight on a valuable point which could be used to help justify the scientific interpretation of the results. It is logical to assume that this marked difference in resistance is a consequence of different environmental and evolutionary forces that each cultivar was undergoing as it formed .

The extreme climatic conditions experienced in the Indian subcontinent are bound to enhance the occurrence and the intensity of pathogens which develop in soil including *F. solani*. It is natural in such a situation that breeders put their effort on breeding varieties that have strong multi-layered defense mechanisms, as in the case of the Mercure variety. Bred and selected by the French, the cultivar known as Balkis F1 was probably the one bred and selected based on the main criteria of yield and the quality of fruits. These breeding priorities could be stressed in the environment when the selection pressure of the given pathogen was not significant .

This would explain its low defense mechanisms against the fungus. . This hypothesis is not just important in the fact that it provides a reasonable explanation to the results obtained, but also in the fact that it highlights the importance of taking advantage of genetic materials of the pathogen-infested tropical areas in subsequent breeding programs aimed at enhancing resistance to diseases. The interaction between plants and pathogens is a multifaceted dynamic process within which both parties interact by a set of mutually defensive and offensive mechanisms .

The most common of them is the secretion of proteins, such as extracellular enzymes, by pathogens, which is a complex process of breaking the plant defense system (Wang et al., 2022) .

Yamaguchi et al. (1995) and Shahbazi et al. (2009) found that the levels and the activity of the peroxidase enzyme rises in plants when they are subjected to different stresses as a way of protecting the cells against any form of injury.

These results coincide with the report by Gujjar et al. (2025) about catalase enzymes activity in the defense mechanism of sugarcane. They showed that the red rot resistant cultivar (BO91) has more catalase isoenzymes (19 isoenzymes: Catalase-1 to Catalase-10 and Catalase-11 to Catalase-19) than that of the susceptible cultivar (COJ64) which has 12 isoenzymes. Indeed, as examples, Gill and Tuteja

(2010), as well as Muteab and Al-Abedy (2025) reported that oxidative stress is a common feature of interactions between plants and pathogens, and essential antioxidants of the plant-pathogen system, such as catalase (CAT) and superoxide dismutase (SOD) have an essential role in the resistance regulation. Similarly, Hernandez et al. (2001) in another study, was able to state that pea plants that are resistant to severe environmental conditions always have increased antioxidants enzyme activity. Moreover, Apel and Hirt (2004) clarified that the fine balance between the synthesis and the neutralization of the reactive oxygen species (ROS) is the decisive element in defining whether these molecules are protective signaling molecules or harmful substances leading to cell damages. Accordingly, it is possible to conclude that not only a coincidental result of infection is the activity of defense-related enzymes such as PPO, POD, and CAT.

Instead, it is an active component and a specific biomarker, which correctly reflects the degree of resistance of tomato plants to the pathogen *F. solani*.

The characteristic feature of advanced defense mechanisms is the inherent genetic ability to maintain high levels of enzymatic activity at baseline levels and a high and rapid induction when infected. This therefore makes it a useful device that can be used in breeding and genetic enhancement initiatives with the view of coming up with disease-resistant cultivars.

Table 6. Activity of defense enzymes in the most and least susceptible tomato genotypes following inoculation with *F. solani*.

Genotype	Treatment	PPO (U/g FW)	POD (U/g FW)	CAT (U/g FW)
Mercur	Non-infected	1.67	1.57	3.35
	Infected	2.50	2.34	4.36
Balkis F1	Non-infected	1.09	0.96	2.84
	nfected	1.78	1.38	2.85
LSD (0.05)	**0.2797**			

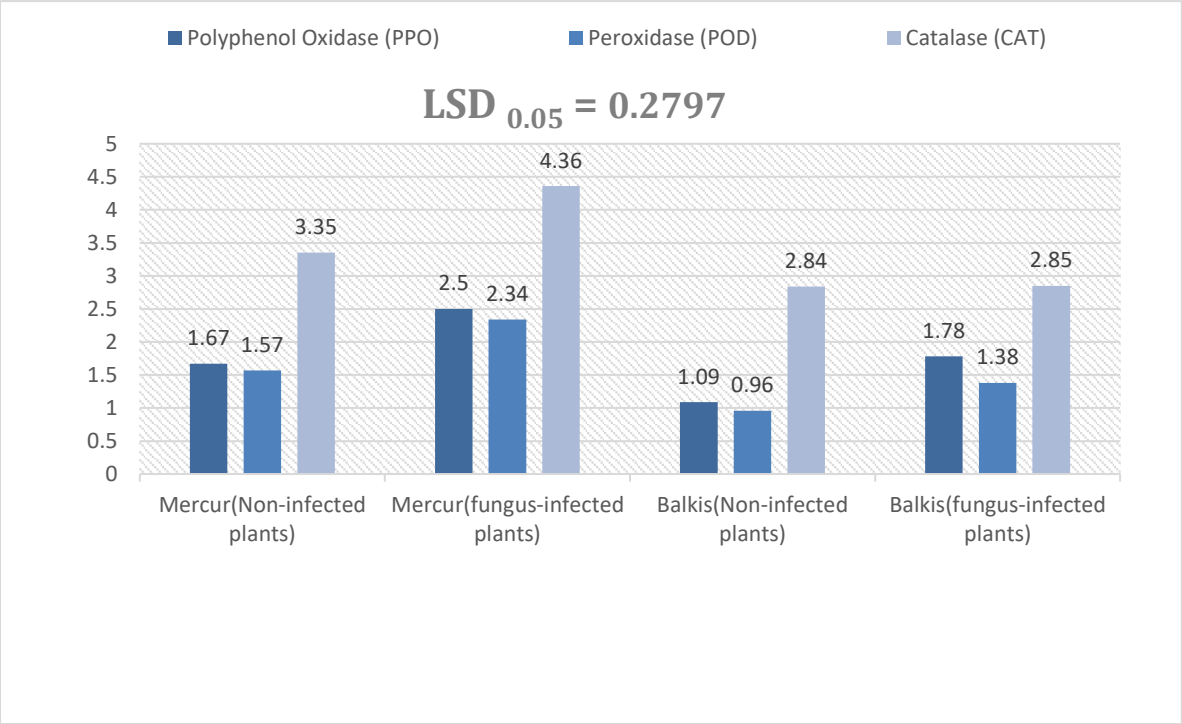


Figure 6. Activity of defense enzymes in the most and least susceptible tomato genotypes following inoculation with *F. solani*.

4. Conclusion

In summary, this study reveals the existence of considerable genetic diversity in the response of tomato genotypes to *Fusarium solani* under greenhouse conditions. The assessment has revealed different degrees of susceptibility, with 'Mercur' and 'Fairuz F1' showing remarkable resistance (0.00% incidence and severity), while 'Balkis F1' was determined as the most sensitive. These results demonstrate a positive association between high genetic resistance and the rapid and strong induction of crucial antioxidant enzymes (PPO, POD and CAT). The findings indicate that these enzymes play an important role as biochemical markers in distinguishing resistant from susceptible germplasm. These results practically suggest the inclusion of the 'Mercur' and 'Fairuz F1' varieties in future tomato breeding programs as a better genetic source to develop resistance against soil-borne fungal pathogens. Moreover, the assessment of defence enzymes is suggested as a physiological approach to screen and select resistant tomato germplasm at an early stage. This strategy provides a promising solution for improving crop tolerance and performance in a pathogen-stressed environment.

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