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Molecular Identification of Fusarium solani and β-Aminobutyric Acid (BABA)-Induced Resistance in Tomato (Solanum lycopersicum L.)

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Abstract:

This study aimed to investigate the molecular diversity of the plant pathogen Fusarium solani using molecular techniques, specifically polymerase chain reaction (PCR), due to its high accuracy compared to conventional morphological identification of fungal pathogens in tomato plants. The internal transcribed spacer regions (ITS1 and ITS4) within ribosomal DNA (rDNA) were targeted for identification. Sequence analysis and comparison with reference sequences available in the NCBI GenBank database confirmed that the studied isolate represents a newly recorded isolate in Iraq, registered under the accession number (PV616682). Pathogenicity tests of F. solani showed a significant reduction in seed germination percentage of tomato cultivar Mansoura F1. Germination rates decreased to 73.33% in Petri dishes and 90.00% in plastic pots, compared to the control treatment, which recorded 100% and 96.66%, respectively. Inhibition percentages reached 26.7% in Petri dishes and 20.0% in pots, whereas the control recorded 0% and 3.34%, respectively. The results further demonstrated that treatment with β-Aminobutyric Acid (BABA) effectively induced systemic resistance in tomato plants by activating key defense-related enzymes, namely Peroxidase (POX) and Catalase (CAT). The combined treatment (F. solani + BABA) recorded the highest enzyme activity levels (2.56 and 2.45, respectively), compared to the control (1.49 and 1.38, respectively), at a significance level of 0.05.

Keywords: *Tomato, BABA, PCR, Fusarium solani, Peroxidase, Catalase.*

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1. Introduction

Tomato (Solanum lycopersicum L.) belongs to the family Solanaceae and is cultivated in most regions of the world, with the highest concentration of production in Central and South America. The Solanaceae family comprises approximately 100 genera and about 2,500 species. Among the most important genera is Solanum, which includes several economically significant crops such as tomato, potato, eggplant, and both hot and sweet peppers.(1)

Globally and in the Arab region, tomato is considered one of the most important vegetable crops, ranking second after potato in terms of economic and nutritional importance. Its significance lies in the high nutritional value of its fruits and the diversity of its consumption methods. Tomato is a staple food in many countries due to its richness in vitamins, minerals, amino acids, water, and essential compounds involved in body structure and energy production.(2)

The global production of tomato exceeds 220 million tons annually. China ranks first worldwide, producing more than 77 million tons per year, while Egypt leads Arab countries with an annual production exceeding 6 million tons.(3)

In Iraq, tomato is cultivated in most provinces under two growing seasons: protected cultivation during winter and open-field cultivation in summer. In 2023, the total tomato production in Iraq reached approximately 534,821 tons. In Al-Najaf province, the cultivated area was 18,798 حوالي dunums, producing approximately 98,068 tons.(4)

Like many vegetable crops, tomato is susceptible to a wide range of diseases and pests, including fungal, bacterial, nematode, and viral pathogens, which cause significant economic losses and negatively affect plant health and productivity (5). Crop yield losses due to plant diseases are estimated to range between 10% and 40%.(6)

The fungus Fusarium solani is among the most widespread and destructive soil-borne fungal pathogens worldwide, including in Iraq. It infects plants at various growth stages and can severely damage the root system of tomato plants, impairing water and nutrient uptake and transport, which ultimately reduces both yield quantity and quality.(7)

Due to these substantial yield losses, there is a need to develop effective, environmentally safe, and cost-efficient alternative control strategies. One promising approach involves the use of β-aminobutyric acid (BABA), a non-protein amino acid known for its role in inducing systemic resistance (Induced Systemic Resistance, ISR) in plants. Rather than directly inhibiting the pathogen, BABA enhances the plant’s internal defense mechanisms. The effectiveness of this response depends on factors such as crop type, plant age, and the nature of the pathogen.(9 ,8)

2. Materials and Methods

2.1 Preparation of Culture Media and Fungal Isolation

Potato Dextrose Agar (PDA) was prepared by dissolving 39 g of PDA powder in 1 L of sterile distilled water according to the manufacturer’s instructions. Chloramphenicol was added at a concentration of 250 mg/L to inhibit bacterial growth. The medium was sterilized using an autoclave at 121°C and 15 psi for 20 minutes, then poured into sterile Petri dishes and stored at 4°C until use.

Water Agar (WA) was prepared by dissolving 17 g of agar in 1 L of sterile distilled water and sterilized under the same conditions (121°C, 15 psi, 20 min), then stored at 4°C.

For fungal isolation, tomato plant tissues showing wilt symptoms were collected. Tissue segments (0.5 cm; 3–5 pieces) were surface-sterilized in 70% ethanol for 30 seconds, rinsed in sterile distilled water for 1 minute, treated with 2% sodium hypochlorite for 3 minutes, and finally rinsed in sterile distilled water for 5 minutes. Four segments were placed onto PDA plates and incubated at 25 ± 2°C for 3–4 days. Pure cultures were obtained by subculturing hyphal tips onto fresh PDA plates and incubating for 5–7 days at 25 ± 2°C.

2.2 Morphological Identification of Fungal Isolates

Morphological identification was conducted by transferring a 0.5 cm mycelial disc from the actively growing edge of fungal colonies on PDA to the center of fresh PDA plates, followed by incubation at 25 ± 2°C for 2–3 days. Identification to genus and species level was performed based on colony morphology and coloration using standard taxonomic keys (10).

2.3 Molecular Identification Using PCR

Genomic DNA was extracted using a DNA extraction kit (Geneaid) following the manufacturer's protocol. Approximately 100 mg of 4–7-day-old fungal mycelium grown on PDA was used.

The extraction procedure included the addition of FATG buffer (200 µL), incubation at room temperature for 5 minutes, followed by addition of FABG buffer (200 µL) and centrifugation. Samples were then incubated at 70°C for 10 minutes with intermittent mixing. Subsequently, 200 µL of 95% ethanol was added, and the mixture was transferred to a spin column (FAPG). Centrifugation was performed at 14,000 rpm for 5 minutes. The column was washed with 400 µL and then 600 µL of wash buffer, with centrifugation between steps.

DNA was eluted using 100 µL of elution buffer after incubation at room temperature for 3 minutes, followed by centrifugation at 13,000 rpm for 1 minute. DNA concentration and purity were measured using a spectrophotometer, and samples were stored at −20°C until use.

2.4 DNA Quantification and Purity Assessment

DNA concentration and purity were evaluated using a NanoDrop spectrophotometer by measuring absorbance at 260 and 280 nm.

2.5 PCR Amplification

PCR amplification was performed using the Maxime PCR PreMix kit (i-Taq; Intron Biotechnology, Korea). The total reaction volume was 20 µL, consisting of:

- 1 µL of forward primer ITS1 (5′-TCCGTAGGAACCTGCGG-3′)
- 1 µL of reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′)
- 1 µL of DNA template
- Nuclease-free water to complete the volume

PCR conditions are shown in Table (1). Amplified products were sequenced in both directions using ITS1 and ITS4 primers by Macrogen (Korea). Sequence data were analyzed using BLAST and compared with sequences in the NCBI database for identification.

Table 1. PCR Cycling Conditions

Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95	2 min	1
Denaturation	95	1 min	
Annealing	62	1 min	35
Extension	72	1 min	

Final Extension	72	5 min	1
Hold	4	—	—

2.6 Pathogenicity Test in Petri Dishes

WA plates were inoculated with a 0.5 cm fungal disc from a 7-day-old culture and incubated at 25 ± 2°C for 3 days (three replicates). Ten surface-sterilized tomato seeds (cv. Mansoura) were placed in each plate. Control plates contained seeds without fungal inoculation.

All plates were incubated at 25 ± 2°C for 7 days. Germination percentage was calculated, and data were analyzed using GenStat under a Completely Randomized Design (CRD) at a 5% significance level (11, 12).

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

2.7 Pathogenicity Test in Plastic Pots

Plastic pots (5 × 7 cm) were filled with a sterilized soil mixture (sand:peat moss, 1:1). Soil was autoclaved at 121°C for 1 hour (twice). The soil was inoculated with fungus-colonized millet seeds at a rate of 5 g/kg soil, moistened, and covered with plastic to maintain humidity for 7 days.

Ten sterilized seeds were planted per pot (three replicates). Control pots contained non-infested soil. After 14 days, data were recorded and analyzed using CRD at a 5% significance level.

Seedling mortality (%) = (Number of dead seedlings / Total seedlings) × 100

2.8 Field Experiment and BABA Treatment

Field experiments were conducted in Al-Najaf province. Tomato seeds (cv. Mansoura) were planted in mid-July under drip irrigation. At the 2–4 true leaf stage, plants were treated with BABA at a concentration of 20 mM (20 mmol/L).

Fifty milliliters of the solution were applied around each plant near the root zone. After 3 days, fungal inoculum (100 spores/mL) was applied. After 14 days, enzyme activities (POX and CAT) were measured.

2.9 Peroxidase (POX) Activity Assay

POX activity was determined after 14 days following the method described in (14). Approximately 0.5 g of leaf tissue was homogenized in cold 0.1 M phosphate buffer (pH 7.0).

The reaction mixture consisted of:

- 2 mL pyrogallol (0.05 M)
- 0.5 mL H_2O_2 (1%)

Absorbance was measured at 420 nm using a spectrophotometer for 2 minutes.

2.10 Catalase (CAT) Activity Assay

CAT activity was determined following method (15). About 0.5 g of leaf tissue was homogenized in 50 mM phosphate buffer (pH 7.0).

The reaction mixture included:

- 2 mL pyrogallol (0.05 M)
- H_2O_2 (10 mM)

Enzyme activity was measured at 240 nm using a spectrophotometer for 2 minutes.

2.11 **Polyphenol Oxidase (PPO) Activity Assay**

Polyphenol oxidase (PPO) is considered one of the most important chemical defense mechanisms in tomato plants against pathogens. It functions by oxidizing phenolic compounds and converting them into quinones, which are toxic to pathogens. Additionally, PPO contributes to lignin formation, thereby strengthening plant cell walls. Catechol was used as a substrate to estimate the activity of this enzyme. The detection method is similar to that used for peroxidase; however, the difference lies in the substrate used and the absence of hydrogen peroxide (H_2O_2), as PPO depends solely on oxygen. The absorbance is measured at a wavelength of 420 nm using a spectrophotometer after 3 minutes (19). .

3. Results and Discussion

3.1 Morphological and Molecular Identification of *Fusarium solani*

The fungal isolate was identified as *Fusarium solani* based on colony morphology, mycelial characteristics, and spore structure. The fungus produced three types of spores: microconidia (0–2 cells, crescent-shaped), macroconidia (3–5 cells, also crescent-shaped with pointed ends), and chlamydospores, which are spherical and thick-walled. Chlamydospores play a crucial role in survival and preservation of the fungal genetic material under unfavorable conditions, allowing the pathogen to resume activity when environmental conditions become suitable, thereby continuing the infection cycle (11) (Fig. 1).

PCR amplification using ITS1 and ITS4 primers yielded a fragment of 431 base pairs (bp) (16). Sequence analysis confirmed that the isolate represents an Iraqi strain, which was deposited in GenBank under accession number PV616682. Phylogenetic analysis using MEGA7 demonstrated 100% sequence similarity with *Fusarium solani* sequences available in the NCBI database.

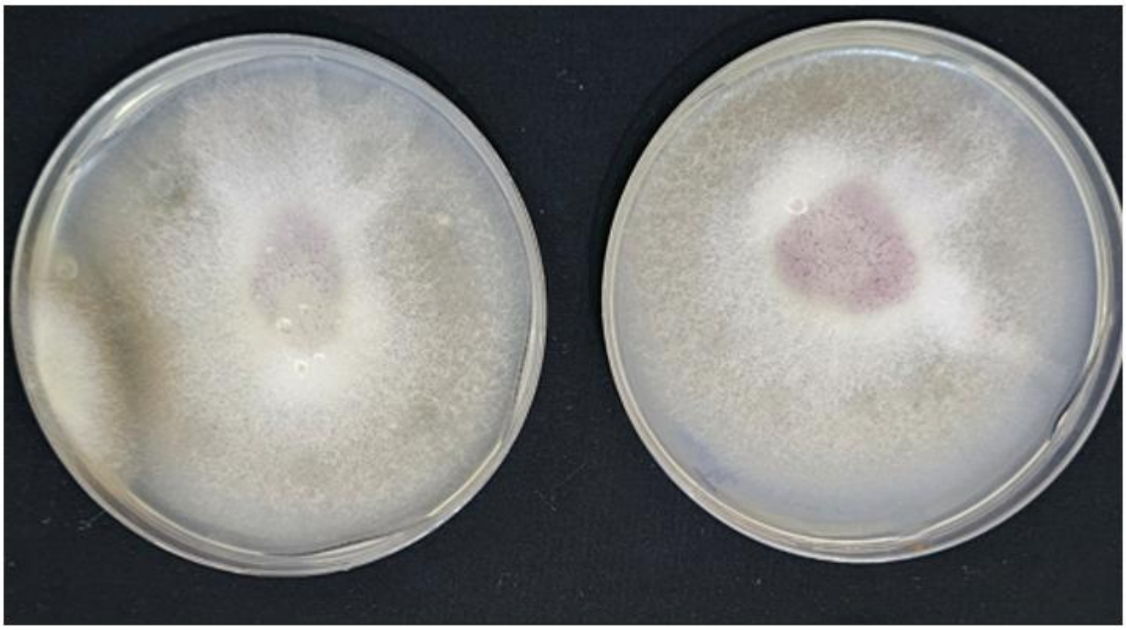
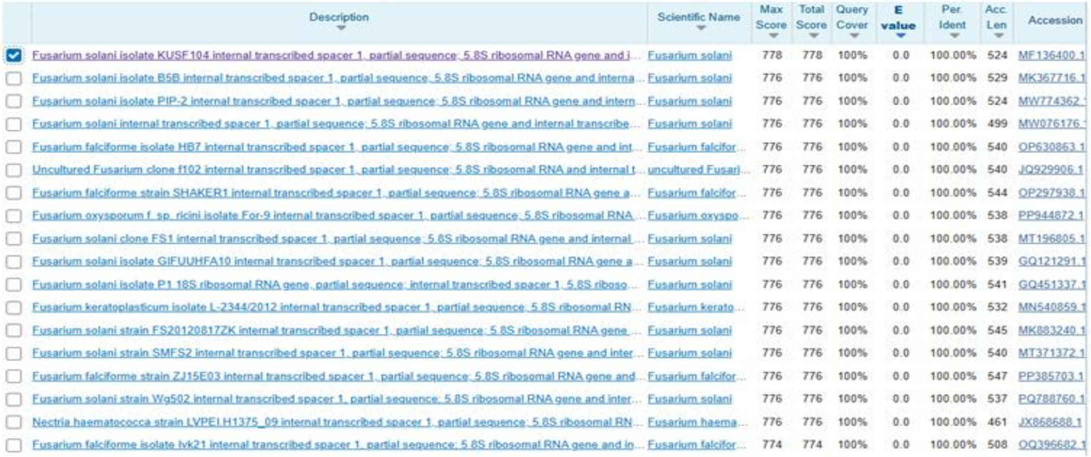


Figure 1. Morphological characteristics of the fungal colony of *Fusarium solani* grown on Potato Dextrose Agar (PDA).

3.2 Genetic Relationships Between the Isolate and Related Strains

The study targeted ribosomal RNA (rRNA) gene regions for molecular identification. Variations in rRNA sequences are widely used for genotyping due to their ability to reflect genetic diversity and adaptability among fungal species.

BLASTn analysis of PCR amplicons showed complete sequence identity (100%) between sample S1 and *F. solani*, confirming its taxonomic classification (17). Alignment results revealed no nucleotide variation compared with the closest reference sequence (GenBank accession no. MF136400.1), indicating complete homology (Fig. 3).



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fusarium solani isolate KUSF104 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...	Fusarium solani	778	778	100%	0.0	100.00%	524	MF136400.1
☐	Fusarium solani isolate B5B internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and interna...	Fusarium solani	776	776	100%	0.0	100.00%	529	MK367716.1
☐	Fusarium solani isolate PIP-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and intern...	Fusarium solani	776	776	100%	0.0	100.00%	524	MW774362.
☐	Fusarium solani internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribe...	Fusarium solani	776	776	100%	0.0	100.00%	499	MW076176.
☐	Fusarium falciforme isolate HB7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...	Fusarium falcifor...	776	776	100%	0.0	100.00%	540	OP630863.1
☐	Uncultured Fusarium clone f102 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA and internal t...	uncultured Fusari...	776	776	100%	0.0	100.00%	540	JQ929906.1
☐	Fusarium falciforme strain SHAKER1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Fusarium falcifor...	776	776	100%	0.0	100.00%	544	OP297938.1
☐	Fusarium oxysporum f. sp. ricini isolate For-9 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...	Fusarium oxyspo...	776	776	100%	0.0	100.00%	538	PP944872.1
☐	Fusarium solani clone FS1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal ...	Fusarium solani	776	776	100%	0.0	100.00%	538	MT196805.1
☐	Fusarium solani isolate GIFUUHFA10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Fusarium solani	776	776	100%	0.0	100.00%	539	GQ121291.1
☐	Fusarium solani isolate P1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S riboso...	Fusarium solani	776	776	100%	0.0	100.00%	541	GQ451337.1
☐	Fusarium keratoplasticum isolate L-2344/2012 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN...	Fusarium kerato...	776	776	100%	0.0	100.00%	532	MN540859.1
☐	Fusarium solani strain FS20120817ZK internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Fusarium solani	776	776	100%	0.0	100.00%	545	MK883240.1
☐	Fusarium solani strain SMFS2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inter...	Fusarium solani	776	776	100%	0.0	100.00%	540	MT371372.1
☐	Fusarium falciforme strain ZJ15E03 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and...	Fusarium falcifor...	776	776	100%	0.0	100.00%	547	PP385703.1
☐	Fusarium solani strain Wg502 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inter...	Fusarium solani	776	776	100%	0.0	100.00%	537	PQ788760.1
☐	Nectria haematococca strain LVPEI.H1375_09 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN...	Fusarium haema...	776	776	100%	0.0	100.00%	461	JX868688.1
☐	Fusarium falciforme isolate lvk21 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and in...	Fusarium falcifor...	774	774	100%	0.0	100.00%	508	OQ396682.1

Figure 2. BLASTn analysis of ITS1–5.8S rDNA sequences of *Fusarium solani*, showing sequence similarity with the closest homologous sequences in the NCBI database.

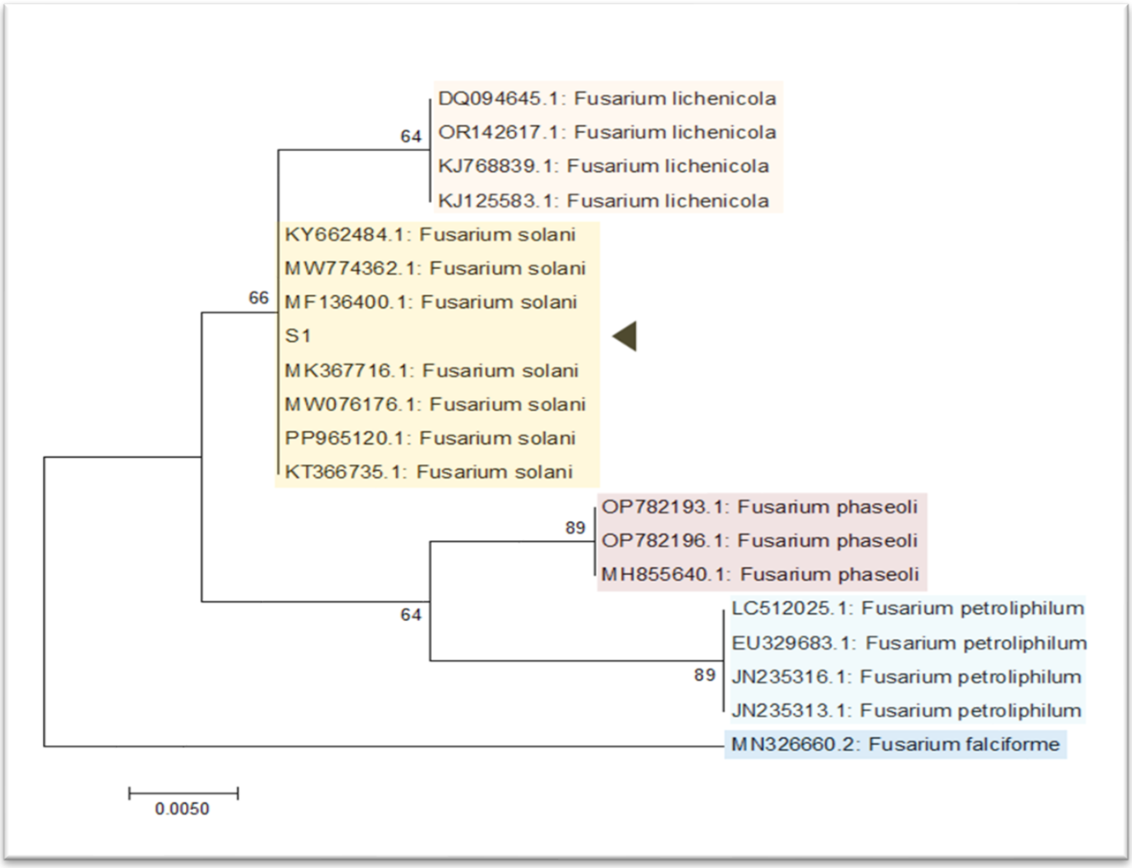


Figure 4. Rectangular cladogram showing the phylogenetic relationships of *Fusarium solani* based on rRNA gene sequences, including the tomato-infecting isolate. Bootstrap values from maximum-likelihood analysis are indicated at the nodes and represent the confidence level of each branch. The scale bar (0.005) represents the number of substitutions per site. To further understand evolutionary relationships, a phylogenetic tree was constructed based on rRNA sequences.

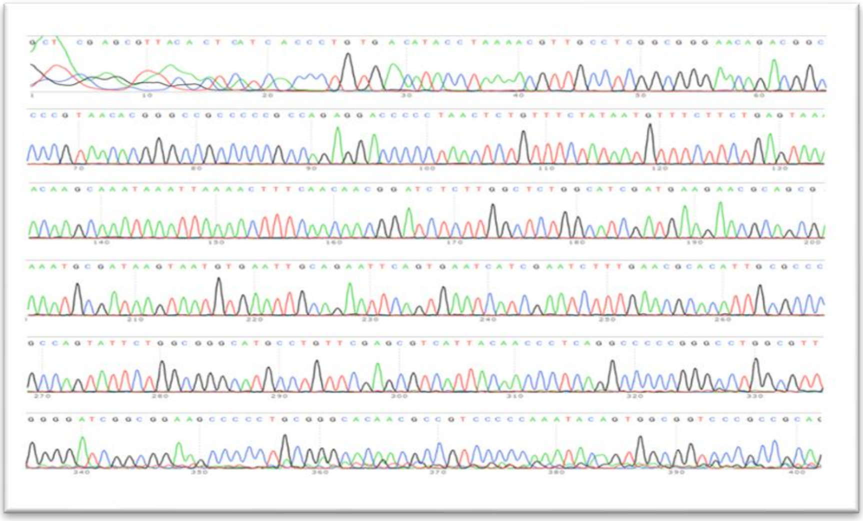


Figure 3. Multiple sequence alignment of the rRNA amplicon from the studied *Fusarium solani* isolate with the reference sequence (GenBank accession no. MF136400.1). The label “S” indicates the investigated sample.

The tree included the studied isolate (S1) along with related *Fusarium* species and an outgroup to assess evolutionary distances. A total of 20 sequences were aligned, and the resulting cladogram (Fig. 4) demonstrated a very close genetic relationship between the studied isolate and reference *F. solani* strains.

3.3 Pathogenicity of *Fusarium solani* on Tomato Seeds (cv. Mansoura)

Results indicated that *F. solani* effectively infected tomato seeds and seedlings under both laboratory (Petri dishes) and semi-field (plastic pots) conditions. The extent of infection depended on environmental conditions, seed characteristics, and pathogen virulence.

In Petri dishes, the control treatment showed 100% germination, whereas seeds inoculated with *F. solani* showed reduced germination (73.33%) and an inhibition rate of 26.7%. In plastic pots, the control recorded 96.66% germination compared to 90.00% in the fungal treatment, while inhibition rates were 3.34% and 20.0%, respectively (Table 2).

Although the Mansoura F1 cultivar is considered relatively tolerant and high-yielding, the results demonstrate that *F. solani* can infect even robust cultivars. The stronger impact observed under pot conditions—closer to field environments—suggests increased pathogen virulence due to enhanced production of enzymes and toxins that promote seed rot, root decay, and seedling mortality (18).

These findings highlight the importance of managing *F. solani* in tomato cultivation to minimize yield losses.

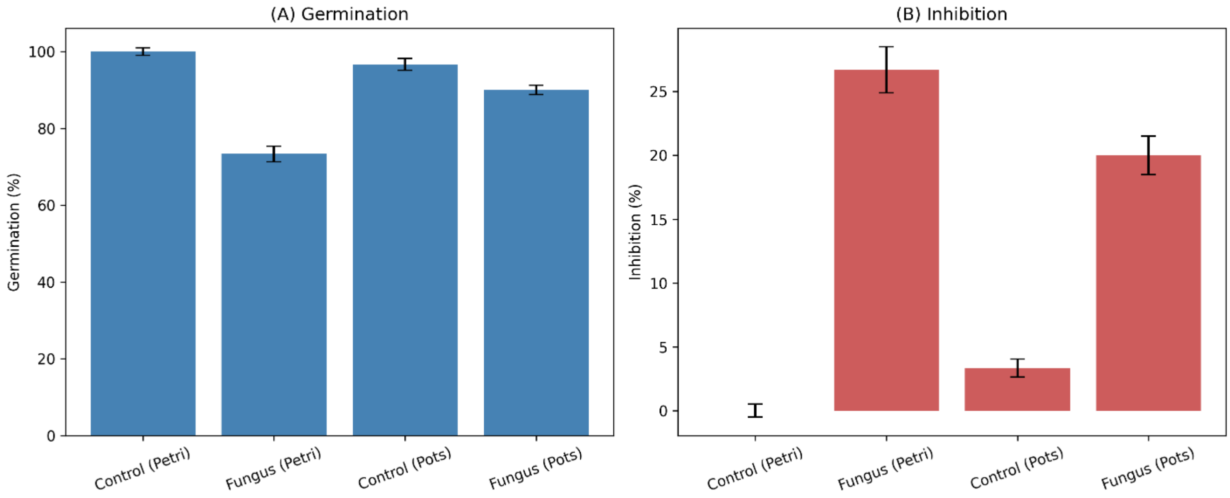


Figure 5. Effect of *Fusarium solani* on tomato seed germination and inhibition under laboratory (Petri dishes) and semi-field (plastic pots) conditions. (A) Germination percentage; (B) Inhibition percentage. Values represent mean ± standard deviation (n = 3).

Table 2. Pathogenicity of *Fusarium solani* on Tomato Seed Germination and Seedling Mortality

Treatment	**Petri Dishes Germination (%)**	**Inhibition (%)**	**Pots Germination (%)**	**Inhibition (%)**

Control	100	0	96.66	3.34
Mansoura F1 + Fungus	73.33	26.7	90.00	20.0
LSD (0.05)	0.925	0.925	—	—

Values represent the mean of three replicates.

3.4 Activity of Peroxidase (POX) and Catalase (CAT) Enzymes and Polyphenol Oxidase (PPO)

The results showed significant differences in enzyme activity among treatments (Table 3). The *F. solani* treatment caused a slight increase in both POX and CATand **PPO** activity (1.52 and 1.45, 1.19 respectively) compared to the control (1.49 and 1.38 and 0.96), indicating activation of plant defense mechanisms in response to oxidative stress caused by pathogen invasion. However, this response alone was insufficient to prevent disease development (14, 15).

Treatment with BABA significantly enhanced enzyme activity (2.33 and 2.21and 1.26), suggesting that BABA primes the plant's defense system by activating genes responsible for antioxidant enzyme production prior to pathogen attack (13).

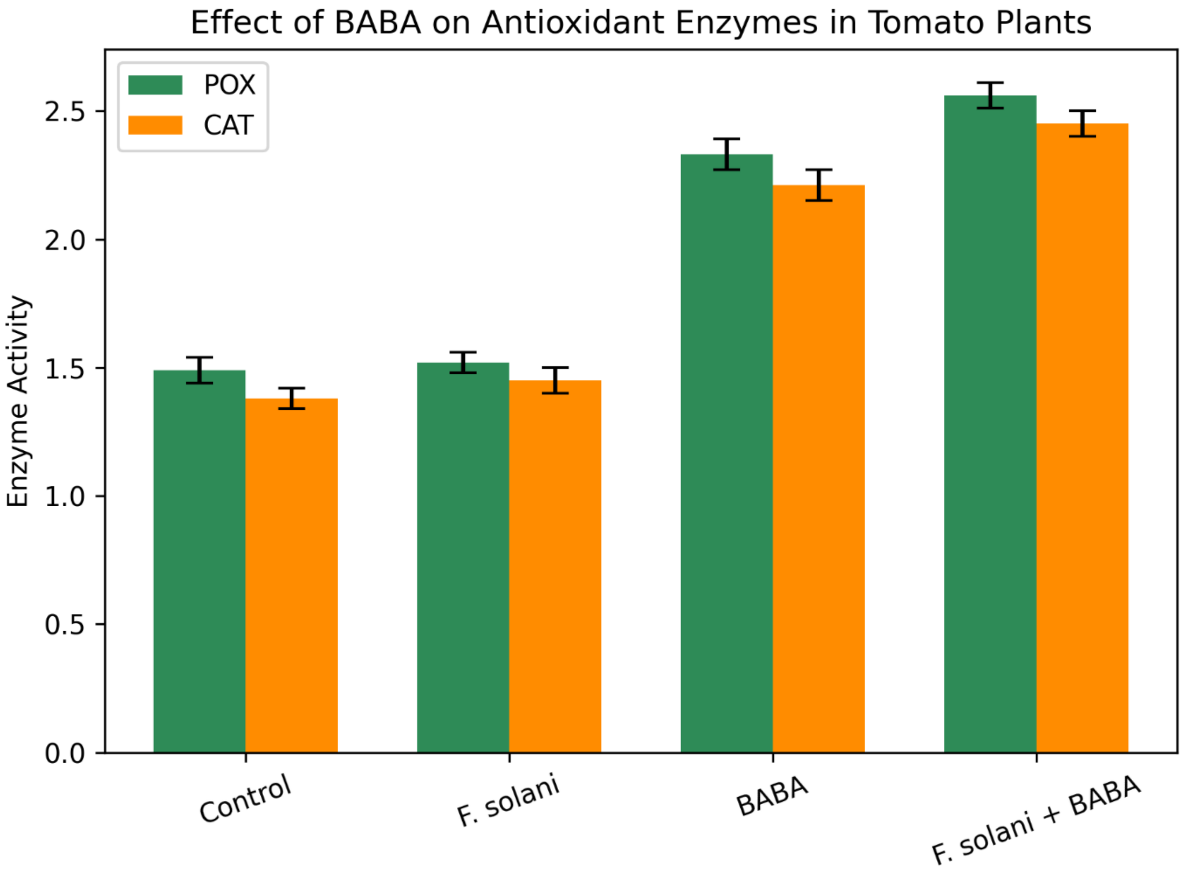


Figure 7. Effect of β-aminobutyric acid (BABA) on antioxidant enzyme activity in tomato plants infected with *Fusarium solani*. Peroxidase (POX) and catalase (CAT) activities were

measured under different treatments. Values represent mean ± standard deviation (n = 3). Different letters indicate significant differences at $P \leq 0.05$ according to the LSD test.

The combined treatment (*F. solani* + BABA) resulted in the highest enzyme activity (2.56 and 2.45 and1.34), indicating a strong induced resistance response. Peroxidase contributes to strengthening cell walls through lignification, forming a physical barrier against pathogen invasion. The PPO enzyme acts by oxidizing phenolic compounds and converting them into quinones, which are toxic to the pathogen. Catalase plays a protective role by detoxifying hydrogen peroxide (H_2O_2), a reactive oxygen species produced during oxidative burst. While H_2O_2 is involved in pathogen defense, its excessive accumulation can damage plant cells; thus, CAT helps maintain cellular balance by converting excess H_2O_2 into water (14).

Table 3. Activity of Peroxidase (POX) and Catalase (CAT) in Tomato Plants (cv. Mansoura)

Treatment	**Peroxidase (POX)**	**Catalase (CAT)**	**Polyphenol Oxidase (PPO)**	**LSD (0.05)**
Control	1.49	1.38	0.96	0.0023
Fusarium solani	1.52	1.45	1.19	
BABA	2.33	2.21	1.26	
F. solani + BABA	2.56	2.45	1.34	
LSD (0.05)	0.0014			0.0040

Values represent the mean of three replicates.

4. Conclusion

This study demonstrated that the fungal isolate *Fusarium solani*, obtained from infected tomato plants, can be accurately identified at the molecular level. The isolate exhibited high pathogenicity under both laboratory and pot (greenhouse) conditions.

The findings confirm that this isolate represents a distinct Iraqi strain closely related to known pathogenic strains. This conclusion was supported by the integration of morphological characterization, molecular identification targeting the ITS region, and phylogenetic analysis. The results suggest the occurrence of continuous microevolution, highlighting the reliability and robustness of the diagnostic approach used in this study.

Furthermore, the observed genetic variability in *F. solani* may influence its virulence and adaptability to host plants, potentially leading to the emergence of more aggressive strains, particularly in major tomato-growing regions such as Al-Najaf.

The application of β-aminobutyric acid (BABA) proved effective in mitigating the impact of the pathogen on plant performance and productivity by enhancing plant defense mechanisms. BABA activates antioxidant enzymes without the need for chemical pesticides, making it an environmentally friendly and sustainable alternative. Unlike conventional fungicides, BABA does not directly eliminate the pathogen but instead induces systemic resistance in plants, strengthening their ability to withstand pathogen attack.

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