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Molecular Characterization and Pathogenicity of Fusarium oxysporum Isolates Causing Wilt in Tomato under Greenhouse and Field Conditions

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Abstract

Background: Fusarium wilt disease, which is caused by Fusarium oxysporum f. sp. lycopersici (Fol) is a destructive soil borne illness that greatly affects tomato production globally. It is important to determine genetic variability and pathogenicity of F. oxysporum isolates to become able to develop effective measures to manage the disease. Objective: The purpose of the study was to describe major tomato producing regions in Iraq in F. oxysporum isolates and determine their pathogenicity in greenhouse and field environments. Methods: In the 2024-2025 tomato growing seasons, forty-five F. oxysporum isolates were obtained in five Iraqi provinces (Baghdad, Babylon, Najaf, Diyala, and Wasit) on symptomatic tomatoes. Species identification and phylogenetic analysis of the species molecular diagnostics was done by use of species-specific primers targeting translation factor 1 alpha (TEF-16) and intergenic spacer (IGS) regions. Pathogenicity was experimented in the greenhouse and field experimental on the weak tomato cultivar of Moneymaker. Morphological characterization and fungus biomass determination through real-time PCR were done. Results: Three physiological races of Fol were determined: race 1, 31.1% (race 2, 48.9% and race 3, 20.0%), race 2 having the majority. Test of pathogenicity showed that there was a broad spectrum of disease severity indices ranging between 22.4 percent and 89.7 percent in the greenhouse and 18.6 percent to 82.3 percent in the field. The most violent isolates (Fol-B12, Fol-N18 and Fol-D23) belonged to race 2 and led to the total wilting of the plants in 21 days. Morphological differences were seen in morphology of the colony, sporulation and morphological growth rates of the colonies. Quantification of fungal biomass in real-time PCR indicated that the highly virulent isolates had a large amount of fungal biomass compared to moderately virulent isolates. Conclusions: The paper brings out the genetic diversity and pathogenicity of F. oxysporum isolates in tomato farms in Iraq. Such results are crucial in the development of disease management strategies, as well as development of resistant tomato species.

Keywords:

Fusarium wilt, fusarium oxysporum, tomato cultivation, pathogenicity, molecular diagnostics, physiological races, fungal biomass.

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Introduction

Tomato (S. lycopersicum L.) is among the economically important vegetables in the world, and it is produced in a large quantity of more than 186 million metric tons annually (Canton, 2021). Tomatoes occupy a total area of about 45,000 hectares in Iraq, which is very important to food security and agriculture in the country (Al-rammahi & Alshimaysawe, 2025). Nevertheless, many biotic factors severely limit tomato production with Fusarium wilt being one of the most destructive that result in a decrease in the yield of between 30 and 80 per cent depending on the environment (Qasim, 2024). In tomatoes, Fusarium oxysporum f. sp. lycopersici (Fol) is a fungal pathogen that causes fol to infect vascular tissues of the plant, thereby resulting into chlorosis, wilting and death of the plant (Xiang et al., 2017). Genetic transmission of this pathogen has resulted in the occurrence of four physiological races (races 1, 4) with each race having the capacity to overcome certain resistance genes in tomato cultivars (Zhang et al., 2022). Race 1 was discovered at the beginning of the previous century, race 2 in 1940s, race 3 in 1960s and race 4 in 1990s (Kanagala et al., 2023). To manage the disease, it is important to identify and describe the F. oxysporum isolates. Conventional morphological identification has not been reliable as the pathogen has been found to be highly plastic and also due to the existence of cryptic species within the F. oxysporum complex (Hudson et al., 2021). Fungal classification and diagnosis of diseases have been transformed using molecular methods. Identification and phylogenetic studies of species are usually done using the translation elongation factor 1-alpha (TEF-1α) gene and intergenic spacer (IGS) regions (Pandey et al., 2024). Studies put emphasis on the need of knowing the population structure and genetic diversity of F. oxysporum in different areas. Due to the increasing frequency of harsh climatic conditions and intensive farming, the new aggressive biotypes and the shift in the geographical distribution of familiar races have become apparent (Hellman et al., 2024). In other parts of the world such as Middle East especially Iraq, little is done in terms of research on the molecular characterization of F. oxysporum population in tomato plants (Martínez-de la Parte et al., 2024). The traditional farming practices in Iraq can offer the possibility to detect new strains of this pathogen that causes the disease.

Key Contribution

1. The paper presents the molecular characterization of the Fusarium oxysporum strains in the five largest tomato-producing provinces in Iraq in terms of species-specific primers of the TEF-1 α gene and IGS regions.

2. Fusarium oxysporum f. sp. lycopersici (Fol) has three physiological races the most prevalent one being race 2 which exhibits considerable variability in virulence and colony properties.

3. The pathogenicity studies conducted in both the greenhouse and field conditions indicate high variability of virulence with the most aggressive being race 2, which underscores the necessity of specific disease management approaches.

4. Findings of the study provide some understanding of the genetic diversity and virulence of the F. oxysporum populations that contribute to the development of the effective disease control strategies and breeding of the disease-resistant tomatoes.

The objectives of this study were to: (1) isolate and identify F. oxysporum from symptomatic tomato plants collected from key production areas in Iraq, (2) characterize the genetic diversity of these isolates using molecular markers, (3) determine the physiological races present in the region, and (4) assess the pathogenicity of representative isolates under greenhouse and field conditions.

Methodology

Sample Collection and Fungal Isolation

The data employed in this research is a biological experimental data that includes the Fusarium oxysporum isolates of diseased tomato plants in Iraq. The number of symptomatic tomato samples collected is 125 composed of 25 commercial fields and greenhouses in five Iraqi provinces, namely, Baghdad, Babylon, Najaf, Diyala, and Wasit. Out of these samples 45 isolates of Fusarium oxysporum were obtained and described. The dataset consists of three significant parts morphological dataset which comprises colony characteristics, growth rates and spore characteristics, molecular which involves sequencing of TEF-1a gene and IGS region to identify the race and build the phylogeny and pathogenicity which involves measurements of disease severity on tomato plants at both greenhouse and field bioassays. These measurements were all based on the 45 isolates of Fusarium oxysporum, which was based on the 125 tomato samples of the five provinces. The given set of data elaborates the information about the genetic, morphological, and pathogenic features of the isolates, which makes it possible to analyse the diversity of Fusarium oxysporum and its effects on tomato plants. The study utilizes the data as the basis of the research and makes the conclusions on the pathogenicity and the genetic variation of Fusarium oxysporum population in Iraq.

A stratified random sampling approach was used and 5-7 fields were sampled per province based on the production area and past disease occurrence. Root and stem tissues (2-3 cm segments) were surface sterilized with 1% sodium hypochlorite for 2 minutes, followed by three rinses in sterile distilled water. Tissue segments were plated on potato dextrose agar (PDA, Difco Laboratories, USA) amended with streptomycin sulfate (100 mg/L) and incubated at 25±2°C for 5-7 days. Emerging fungal colonies resembling Fusarium morphology were purified through single-spore isolation and maintained on PDA slants at 4°C (Gilardi et al., 2024a).

Morphological Characterization

Morphological identification was done using the taxonomic Colony traits such as color, texture, growth rate and pigment production were determined after 7 days of incubation on PDA at 25 o C. Microconidia, macroconidia and chlamydospores were observed with a 100x microscope using lactophenol cotton blue mounting medium. The measurement of growth rate was performed on PDA plates with various temperatures (15 o C, 20 o C, 25 o C, 30 o C, and 35 o C) and measured by collecting the colony diameter after every 24 hours of 5 days.

Molecular Identification and Phylogenetic Analysis

Genomic DNA was extracted from 5-day-old mycelial cultures using the CTAB (cetyltrimethylammonium bromide) method with modifications for filamentous fungi. Using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), the concentration and purity of the DNA were evaluated. The TEF-1α gene was amplified by PCR with the primers EF1-728F (5'-CATCGAGAAGTTCGAGAAGG-3') and EF1-986R (5'-TACTTGAAGGAACCCTTACC-3'), allowing for species identification. The primers CNS1 (5'-GAGACAAGCATATGACTACTG-3') and CNL12 (5'-CTGAACGCCTCTAAGTCAG-3') were also used to amplify the intergenic spacer (IGS) region.

PCR reactions were carried out using a 25-microliter reaction mixture that contained 12.5 micro liters of 2X PCR Master Mix (Promega, USA), 2 micro liters of template DNA (50 ng/uL), 1 0.1 microliter of each primer (10 pmol/uL) and 8.5 2 microliters of nuclease-free water. Amplification conditions were as follows;

initial denaturation at 94 o C for 03 minutes, and subsequently 35 cycles denaturation at 94 o C, 30 seconds; annealing at 58 o C, 45 seconds, and extension at 72 o C, 01 minute, with final extension at 72 o C, 07 minutes.

PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Germany) and subjected to bidirectional sequencing using ABI 3730xl DNA Analyzer (Applied Biosystems, USA). Sequence analysis and phylogenetic reconstruction were conducted using MEGA X software. Multiple sequence alignments were performed using ClustalW algorithm, and phylogenetic trees were constructed using the neighbor-joining method with 1000 bootstrap replications.

Race Determination

Physiological distinction to race was done using different tomato cultivars as per the standard practice. Four differentially susceptible cultivars were used i.e. Moneymaker (susceptible to all races), BonnyBest (resistant to race 1), Cal J VF (resistant to races 1 and 2) and VFNT Cherry (resistant to races 1, 2 and 3). Surface sterilizing of seeds and their germination in sterile vermiculite was done. Seedlings were inoculated by root-dipping (10 min) in spore suspensions (1 x 10 6 conidia/mL) of the pathogens 3 weeks after germination and then transplanted into sterile potting mix. Sterile water was used as control plants. The plants were grown in growth chamber at 25+/- 2 o C with 12-hour stage with a duration of 6 weeks to check the disease symptoms.

Pathogenicity Testing

Pathogenicity testing was performed both greenhouse and, in the field, using a representative of each identifiable race. To conduct greenhouse experiments, tomato seedlings (cv. 'Moneymaker'), 4-week-old, were inoculated by the root-dip inoculation method using conidium suspensions that had been adjusted to 1 x 10 6 conidia/ mL. A set of 15 plants utilized in a completely randomized structure with three replications per isolate was used to test each isolate. Diseases severity was measured on a scale of 0-4 where: 0 = healthy plant, 1 = slight yellowing of the lower leaves, 2 = yellowing and wilting of lower leaves, 3 = severe wilting with vascular browning, and 4 = plant death.

Field experiments were established at the University of Baghdad Agricultural Research Station using a randomized complete block design with four replications. Soil inoculation was performed by incorporating fungal biomass (grown on sterilized sorghum grains for 3 weeks) into planting holes at a rate of 5 g per plant. Disease incidence and severity were monitored weekly for 10 weeks post-transplanting.

Statistical Analysis

Statistical analysis of the data was done using SAS9.4. ANOVA was employed to reveal the significance between treatments. Analysis of means was done using Duncan multiple range test at P 0.05. Correlation analysis was carried out to investigate the dependencies between morphological and virulence characteristics.

The figure 1 gives a descriptive overview of the methodology used in the study, the critical steps that have been undertaken in research process. It starts with sample collection and fungi isolation where tomato samples with typical symptoms of Fusarium wilt were sampled at 5 Iraqi provinces and then they were sterilized and fungal colony purified. The second procedure is molecular identification and phylogenetics, through which the DNA is extracted, amplified through PCR, and the sequence analyzed to determine the isolate of Fusarium oxysporum and place them in their respective categories. Morphological characterization is next that involves investigating the characteristics of the colonies, microscopic observations, and counting the rate of growth of the isolates. Race determination is then done by inoculating the different tomato cultivars

and it will enable the occurrence of the symptoms of the disease and suitability of the particular races of the isolates. Pathogenicity tests involve green house and field tests to determine the severity and isolate virulence of the disease. Last but not least, ANOVA, Duncan test, and correlation analysis are used to conduct statistical analysis and interpret the obtained data and determine whether the results are significant. This paradigm of the methodology gives a clear, organized method of comprehending the experimental procedures in the study (Figure 2).

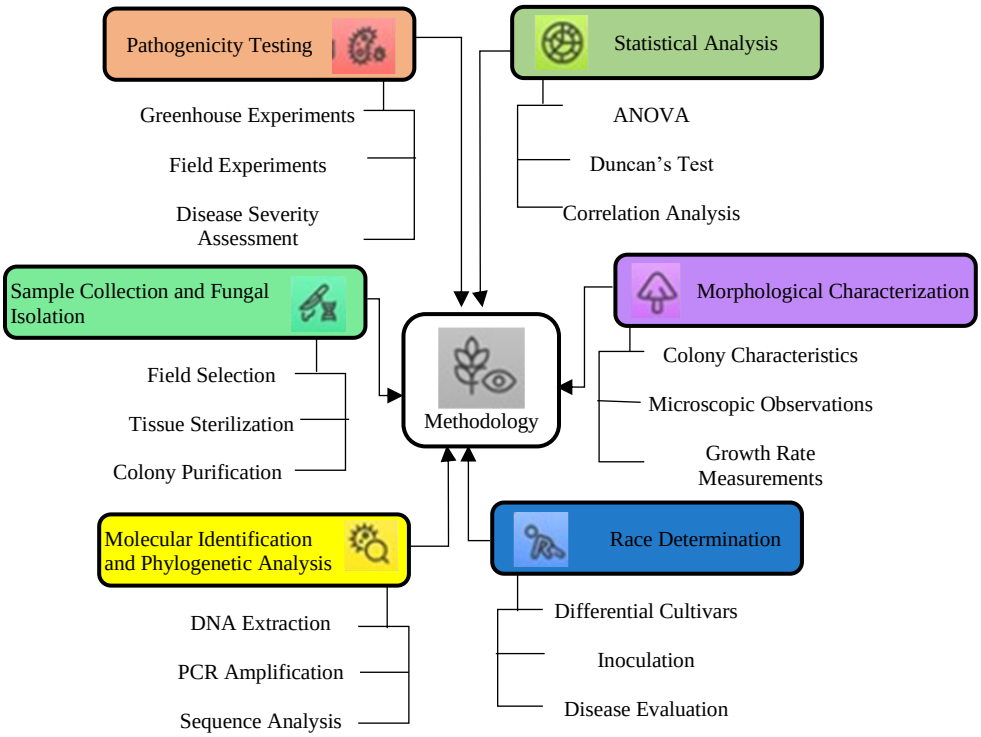

Figure 1. Overview of the research methodology framework

Results

Sample Collection and Fungal Isolation

The fungus was isolated by placing a culture plate on a spore's stationary in the lab, keeping it cold and wet until fungal isolation commenced. Collection of the sample and the isolation of the fungus were done in the following way. A culture plate was placed on a fungal spore's stationary in the laboratory and kept cold and wet until the actual fungal isolation took place.

Table 1. Distribution and isolation frequency of Fusarium oxysporum from tomato samples across Iraqi provinces

Province	Samples Collected	Production Sites	Isolates Obtained	Isolation Rate (%)	Greenhouse (%)	Field (%)
Baghdad	25	5	16	64.0a	71.4	58.3
Babylon	21	4	11	52.4b	60.0	46.7
Najaf	29	6	14	48.3bc	55.6	42.9
Diyala	28	5	10	44.7cd	50.0	40.0
Wasit	22	5	9	40.6d	45.5	36.4
Total	**125**	**25**	**45**	**50.4**	**58.3**	**43.7**

Values followed by the same letter within columns are not significantly different ($P \leq 0.05$)

The table 1 gives the information on the collection and isolation of Fusarium oxysporum on tomato plants with symptoms in five provinces in Iraq. One hundred and fifty-five samples were taken into study, 25 locations were sampled and the greatest number of isolates was taken in Baghdad (16 isolates in 25 samples) and the least in Wasit (9 isolates in 22 samples). The rates of isolation were different among the provinces, Baghdad had the highest rate 64.0, Babylon came second with 52.4 and Wasit with the lowest rate 40.6. The general isolation rate of all the provinces was 50.4%. Isolation rates in green house and field production systems have also been compared in the table 1. All the provinces had higher rates of greenhouse isolation with Baghdad recording the highest greenhouse rate of 71.4. The field isolation rates were also lower in all provinces with the highest rate being 58.3 in Baghdad. The lowest isolation rates in the greenhouse and field settings were in Wasit and were 45.5 and 36.4, respectively. These results underscore the regional differences in isolation success and point to the fact that the conditions in green houses are more conducive to fungal isolation than to those in the field.

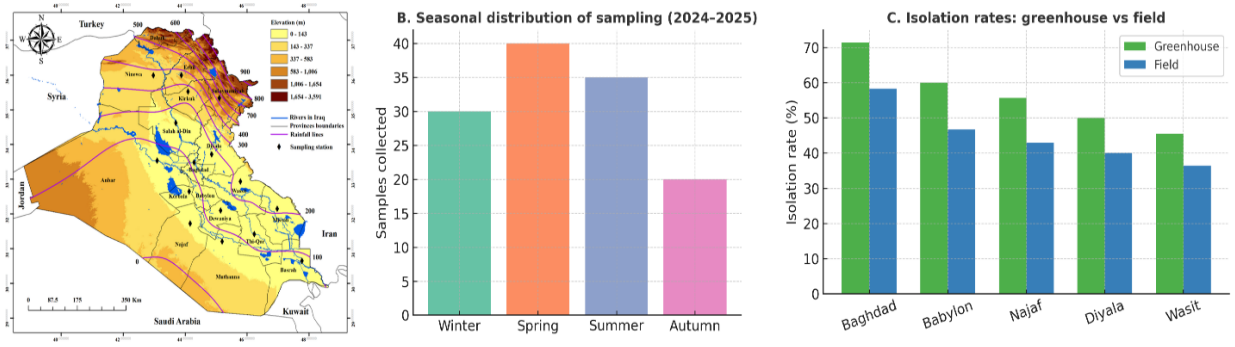

Figure 2. Geographic distribution and sampling strategy

Figure 2 shows a visual interpretation of important data of the research, such as the patterns of geography and season of sampling, and also the rate of isolation of varied environments. The map of Iraq is provided in panel A with the locations of sampling of different provinces outlined. The distribution of samples during the year is shown in the panel B, with the maximum number of samples being gathered during spring and summer. In panel C, the isolation rates are compared under greenhouse and field conditions, the isolation rates are always higher in greenhouse conditions in all the provinces, especially in Baghdad where the isolation rate is more than 70. All this adds up to the differences in geographic and seasonal variation in sample collection, and to the more favorable conditions of isolation in greenhouses.

Morphological Characterization

Morphological characteristics of Fusarium oxysporum were typical and observed in all 45 isolates although it was found to be highly phenotypically diverse. Color of colonies differed greatly among isolates with pure white colonies to dark purple with intermediate pink, yellow and violet colors. Growth rate analysis displayed extreme variance between isolates where race 2 isolates grew faster on average as compared to race 1 or race 3.

The table 2 presents morphological features of the Fusarium oxysporum isolates of three different races including Race 1 (n=14), Race 2 (n=22) and Race 3 (n=9) are given in the table 2. There were notable dissimilarities in a number of attributes. Race 2 had the highest growth rate (15.8 ± 1.9 mm/day) and was far higher than Race 1 (12.4 ± 2.1 mm/day) and Race 3 (11.2 ± 2.3 mm/day) with P-value of less than 0.01. The density of sporulation was greatest in Race 2 (high) followed by moderate sporulation in Race 1 and low sporulation in Race 3 with P-value of less than 0.05. Race 2 (26.8 ± 2.8 µm) also had a significant

macroconidia length as compared to Race 1 (24.6 ± 3.2 µm) and Race 3 (23.1 ± 3.5 µm) with the P-value less than 0.05. There were no noticeable differences in the macroconidia septa (3-4 in Race 1 and Race 3, 3-5 in Race 2), and the chlamydospore production was moderate (+) in Race 1 and Race 3, but stronger (+) in Race 2. These results indicate race specific morphological variations in Fusarium oxysporum.

Table 2. Morphological characteristics and growth parameters of F. oxysporum isolates by race

Characteristic	Race 1 (n=14)	Race 2 (n=22)	Race 3 (n=9)	P-value
Colony color	White-Yellow	Pink-Purple	Purple-Violet	-
Growth rate (mm/day)	12.4 ± 2.1b	15.8 ± 1.9a	11.2 ± 2.3b	<0.01
Sporulation density	Moderate	High	Low	<0.05
Macroconidia length (µm)	24.6 ± 3.2ab	26.8 ± 2.8a	23.1 ± 3.5b	<0.05
Macroconidia septa	3-4	3-5	3-4	-
Chlamydospore production	+	++	+	-

Values are means ± standard deviation. Different letters indicate significant differences (P ≤ 0.05)

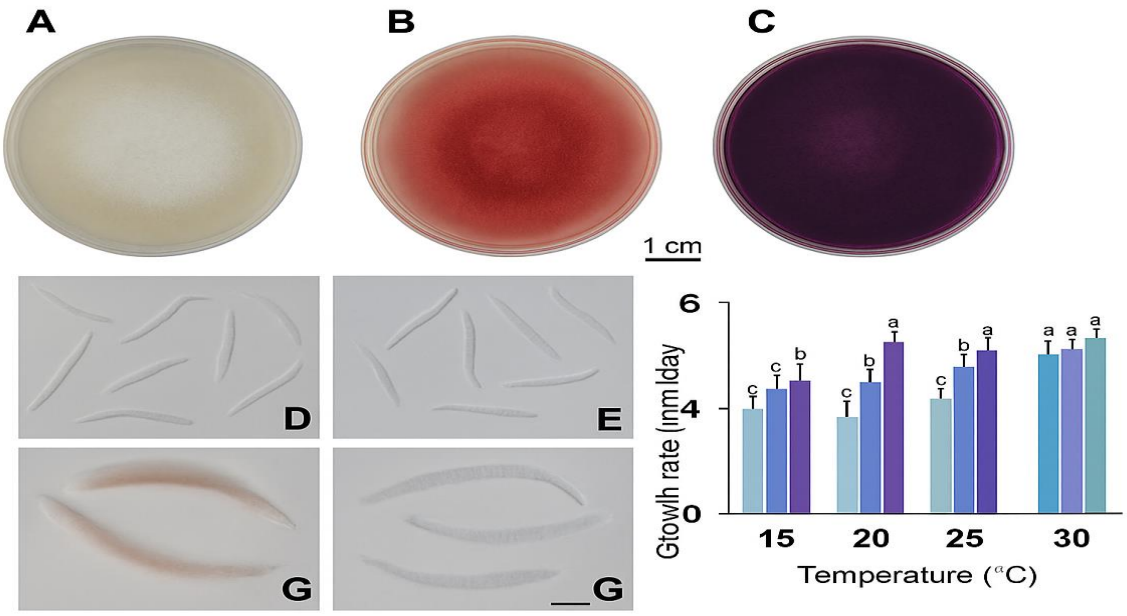

Figure 3. Morphological diversity of fusarium oxysporum isolates

Figure 3 shows morphological features of isolates of Fusarium oxysporum. As depicted in panel (A-C) the representative morphology of the race 1, 2, and 3 of a typical colony on PDA after incubating the medium after 7 days at 25 o C exhibited a clear difference in the colony morphology. The light microscopy images of the morphology of the macroconidia at 400 x magnification were displayed using panels (D-F) to demonstrate the differences in conidia structure between the races. Panels (G-I) are used to draw comparisons between the growth rates of the races under varying temperatures, and therefore, how temperature affects their growth is seen. Panel scale bars (A-C) measure 1 cm, panel scale bars (D-F) measure 20 -1.

Molecular Identification and Phylogenetic Analysis

The TEF-1α gene, which is around 890 base pairs in length, and the IGS region, which is between 1,200 and 1,450 base pairs, were successfully amplified by the utilization of PCR amplification for each of the 45 isolates. The identification of all of the isolates as Fusarium oxysporum was confirmed through the use of sequence analysis, which revealed a similarity of 98-100% to the reference sequences that were located in GenBank.

Phylogenetic research was able to determine that there are three distinct clades that correlate to physiological races 1, 2, and 3 of F. oxysporum f. sp. lycopersici. This was accomplished by employing the neighbor-joining method. Furthermore, the bootstrap support values for major branches were extremely robust, topping 85 percent of the surveyed population.

Table 3. Molecular characteristics and genetic diversity parameters

Parameter	Race 1	Race 2	Race 3	Overall
Number of isolates	14	22	9	45
Frequency (%)	31.1	48.9	20.0	100.0
TEF-1α length (bp)	887-892	885-895	889-893	885-895
IGS length (bp)	1,245-1,289	1,198-1,367	1,278-1,445	1,198-1,445
GenBank similarity (%)	98.7-99.8	99.1-100.0	98.9-99.6	98.7-100.0
Number of haplotypes	8	15	6	29
Haplotype diversity (Hd)	0.891	0.934	0.867	0.912
Nucleotide diversity (π)	0.0034	0.0067	0.0045	0.0052

The table 3 reveals the molecular properties of Fusarium oxysporum isolates of three F. oxysporum race (Race 1, Race 2, and Race 3). The data comprises 14 isolates of Race 1, 22 isolates of Race 2, and 9 isolates of Race 3 consisting of the total number of 45 isolates. The length of the TEF-1a gene was 887-892base pairs (bp) in Race 1, 885-895bp in Race 2 and 889-893bp in Race 3 with a total length of 885-895bp. The lengths of the IGS regions were Race 1 1,245-1,289 bp, Race 2 1,198-1,367 bp, and Race 3 1, 278-1,445 bp and the range was 1,198-1,445 bp. The similarity of the GenBank was high among the races with percentages of 98.7 and 100. The haplotypes were different with Race 1 containing 8, Race 2 containing 15 and Race 3 containing 6, which made a total of 29 haplotypes. The highest and the lowest reading of haplotype diversity were found in Race 2 (0.934) and Race 3 (0.867) respectively with a total diversity of 0.912. Nucleotide diversity (0) also had more diversity in Race 2 (0.0067) than Race 1 (0.0034) and Race 3 (0.0045). These findings point out to the fact that there was great genetic diversity among the various races of Fusarium oxysporum.

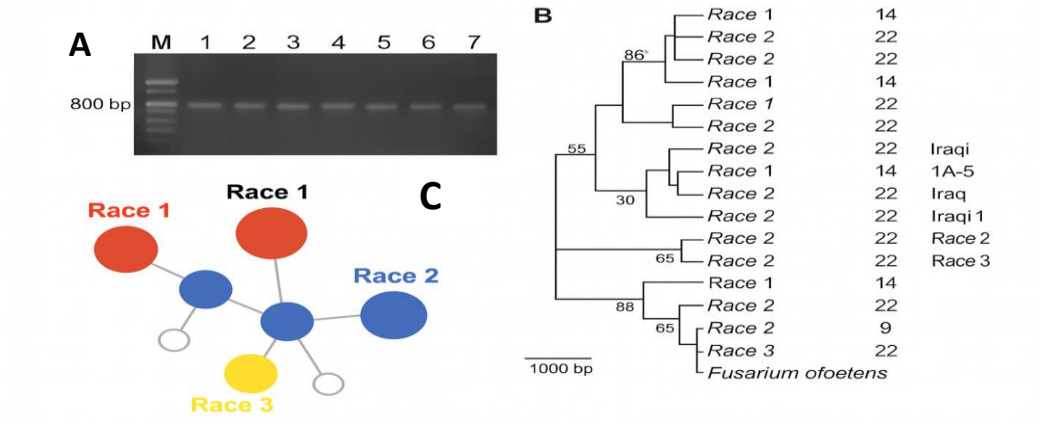

Figure 4. Molecular identification and phylogenetic relationships

In figure 4, Panel (A) is the agarose gel electrophoresis result, which indicates that PCR amplification of the TEF-1 gene was successful in Fusarium oxysporum isolates with a clear differentiation of the band bands depending on the size of the length of the gene. The TEF-1 alpha sequences were clustered using a neighbor-joining phylogeny tree to form a phylogenetic tree that separated the isolates into the various races (Race 1, Race 2 and Race 3) with bootstrap values supporting the reliability of the tree in Panel (B). Figure

(C) shows a haplotype network analysis, based on race, which shows how Iraqi isolates and reference strains are genetically related. The network underlines the genetic variation and reflects the information about the population of Fusarium oxysporum.

Race Determination

In table 4, the identification of mixtures according to the difference between tomato cultivars supported the fact that this Iraqi F. oxysporum population consisted of three physiological races (1, 2, and 3). Race 2 dominated in all the provinces having a percentage of 48.9 whereas race 1 and race 3 had a percentage of 31.1 and 20.0 respectively. This study did not find any race 4 isolates. In the geographic distribution of the races, races 2 took the majority in Baghdad and Diyala, whereas race 1 is predominant in Najaf.

Table 4. Race determination and geographic distribution

Province	Race 1	Race 2	Race 3	Total Isolates	Predominant Race
Baghdad	4 (25.0%)	9 (56.3%)	3 (18.7%)	16	Race 2
Babylon	3 (27.3%)	5 (45.4%)	3 (27.3%)	11	Race 2
Najaf	6 (42.9%)	4 (28.6%)	4 (28.6%)	14	Race 1
Diyala	1 (10.0%)	5 (50.0%)	4 (40.0%)	10	Race 2
Wasit	0 (0.0%)	5 (55.6%)	4 (44.4%)	9	Race 2
Total	**14 (31.1%)**	**22 (48.9%)**	**9 (20.0%)**	**45**	**Race 2**

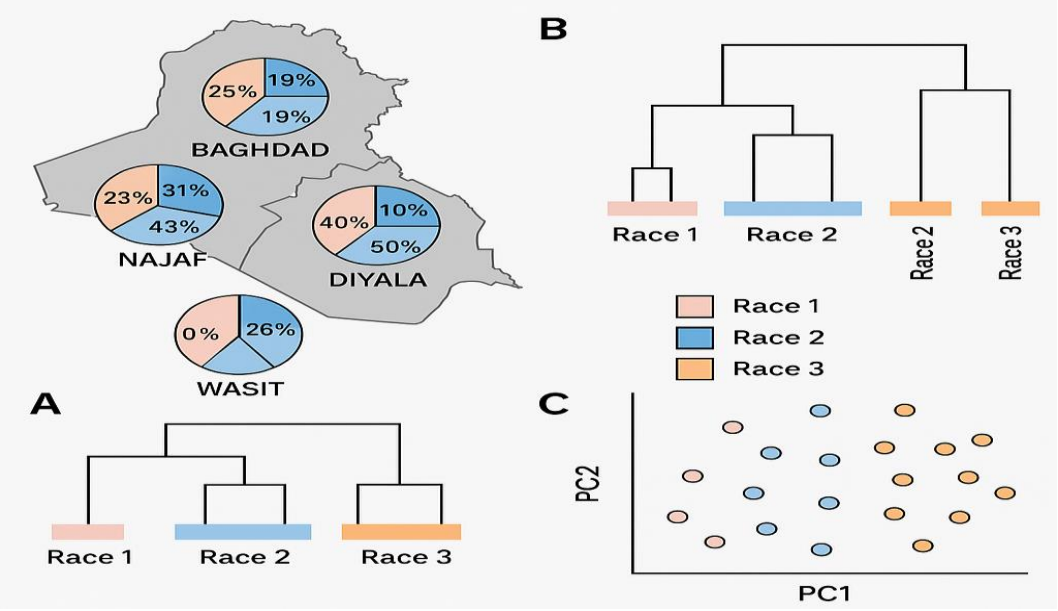

Figure 5. Race distribution and geographic patterns

In Figure 5, Panel (A) gives a map that depicts the distribution of the Fusarium oxysporum races in the various provinces with pie charts depicting the percentage of each race in each province. Hierarchical clustering of the isolates on the basis of the pathogenicity profiles has been shown in panel (B), which illustrates the similarities and differences in the virulence of the isolates. PCA of the morphological and molecular characteristics in panel (C) clearly isolates the races and it is a graphical display of the genetic and phenotypic diversity existing in the Fusarium oxysporum isolates.

Pathogenicity Assessment

In table 5, Pathogenicity tests were used and there was the large variation in the virulence of the isolates under greenhouse and field conditions. In greenhouse experiments disease severity indices were past 22.4, up to 89.7, whereas in the field trials disease severity indices were as little as 18.6 to as high as 82.3. Race 2 isolates were consistently the most virulent followed by race 3 isolates, and race 1. The most virulent isolates (Fol-B12, Fol-N18, and Fol-D23) were race 2 and resulted into complete plant wilting after 21 days of inoculation.

Table 5. Pathogenicity assessment of representative F. oxysporum isolates under greenhouse

Isolate ID	Race	Province	Disease Severity (%)	Days to Symptoms	Root Colonization (CFU/g)	AUDPC
Fol-B12	2	Baghdad	89.7 ± 3.2a	8.3 ± 1.1	2.4×10^7	1,234
Fol-N18	2	Najaf	87.3 ± 2.8a	9.1 ± 1.3	2.1×10^7	1,187
Fol-D23	2	Diyala	84.6 ± 4.1a	10.2 ± 1.8	1.8×10^7	1,145
Fol-Ba15	1	Babylon	72.4 ± 3.6b	12.5 ± 2.1	1.3×10^7	987
Fol-W09	3	Wasit	68.9 ± 2.9b	14.7 ± 1.9	1.1×10^7	923
Fol-N06	1	Najaf	65.2 ± 3.4bc	15.3 ± 2.4	9.8×10^6	876
Fol-B04	3	Baghdad	58.7 ± 4.2cd	17.8 ± 2.7	7.6×10^6	812
Fol-D11	1	Diyala	45.3 ± 3.8de	21.4 ± 3.1	5.2×10^6	634
Fol-W17	1	Wasit	22.4 ± 1.9f	28.6 ± 3.5	2.3×10^6	423
Control	-	-	0.0 ± 0.0g	-	0	0

Values followed by the same letter are not significantly different ($P \leq 0.05$). AUDPC = Area Under Disease Progress Curve

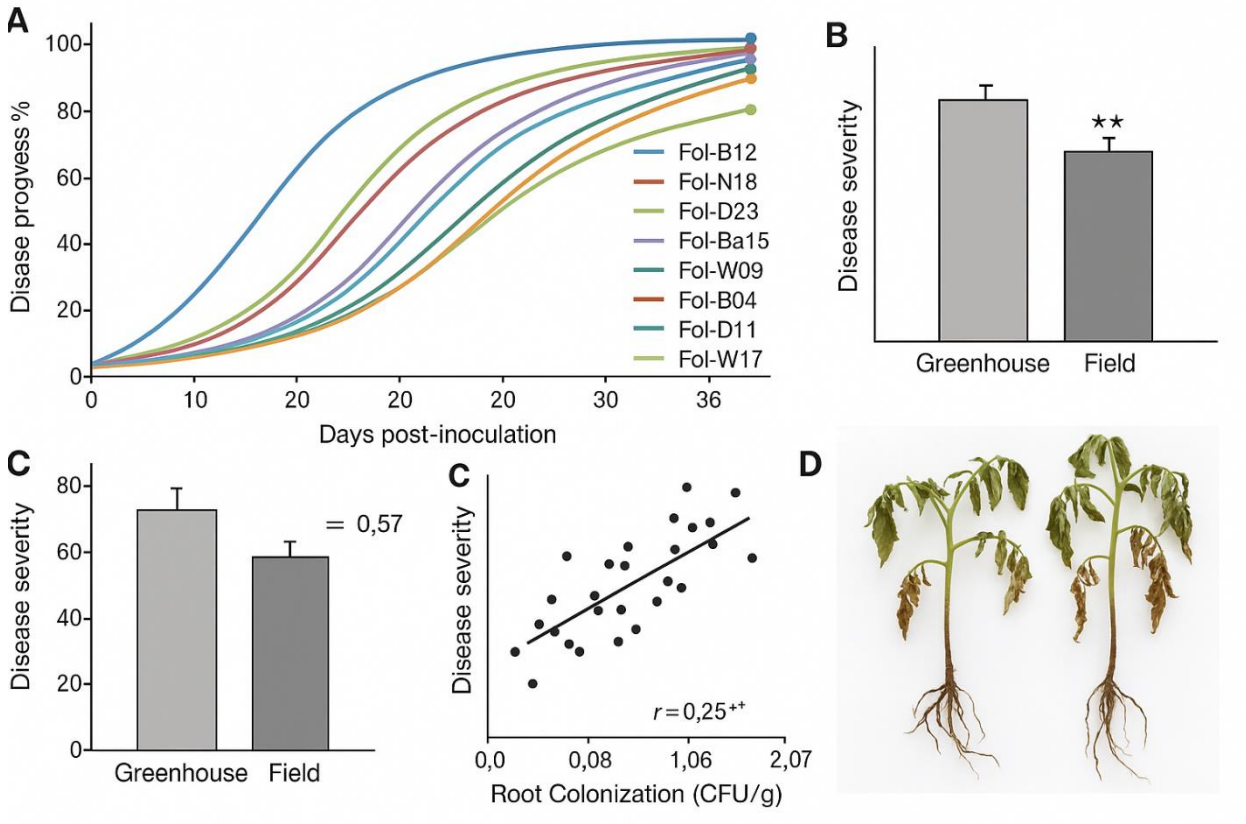

Figure 6. Disease progress and pathogenicity assessment

Figure 6, Panel (A) demonstrates disease progress curves of the representative Fusarium oxysporum isolates in the greenhouse conditions to demonstrate how the disease manifestations change with time. The disease severity in the greenhouse and field experiments is compared in (B) where a discrepancy is observed in the manifestation of the symptoms in both controlled and natural settings. The correlation between root colonization and disease severity is presented in panel (C) with the more importance paid to the correlation between the presence of fungus in the roots and the overall health of the plant. Symptoms of vascular browning of infected tomato plants are the symptom characteristics of Fusarium wilt which are illustrated in panel (D).

Field Pathogenicity and Environmental Factors

In table 6, the greenhouse findings were confirmed under field conditions with slight changes attributed to field conditions. The severity of the disease tended to be weaker in the field conditions and the best disease development was achieved at the soil temperature of 24-28 o C and moisture of 60-70%. The most virulent isolates were able to retain their virulence in the field, though symptoms were delayed 3-5 days as compared to the greenhouse.

Table 6. Field pathogenicity and yield impact assessment

Isolate ID	Race	Disease Incidence (%)	Disease Severity (%)	Plant Height Reduction (%)	Yield Loss (%)
Fol-B12	2	95.8 ± 2.3a	82.3 ± 3.8a	67.4 ± 4.2	78.6 ± 5.1
Fol-N18	2	91.7 ± 3.1ab	79.6 ± 4.2a	63.8 ± 3.9	74.2 ± 4.8
Fol-D23	2	87.5 ± 2.8bc	76.4 ± 3.5ab	59.7 ± 4.6	71.3 ± 5.3
Fol-Ba15	1	79.2 ± 3.6cd	63.2 ± 4.1bc	48.3 ± 5.2	56.7 ± 6.1
Fol-W09	3	75.0 ± 4.2de	58.7 ± 3.7cd	44.6 ± 4.8	52.4 ± 5.7
Fol-D11	1	45.8 ± 5.1gh	34.7 ± 4.6fg	25.4 ± 3.8	29.7 ± 4.6
Fol-W17	1	33.3 ± 4.6hi	18.6 ± 2.8gh	16.7 ± 2.9	18.3 ± 3.2
Control	-	0.0 ± 0.0i	0.0 ± 0.0h	0.0 ± 0.0	0.0 ± 0.0

Values followed by the same letter are not significantly different ($P \leq 0.05$)

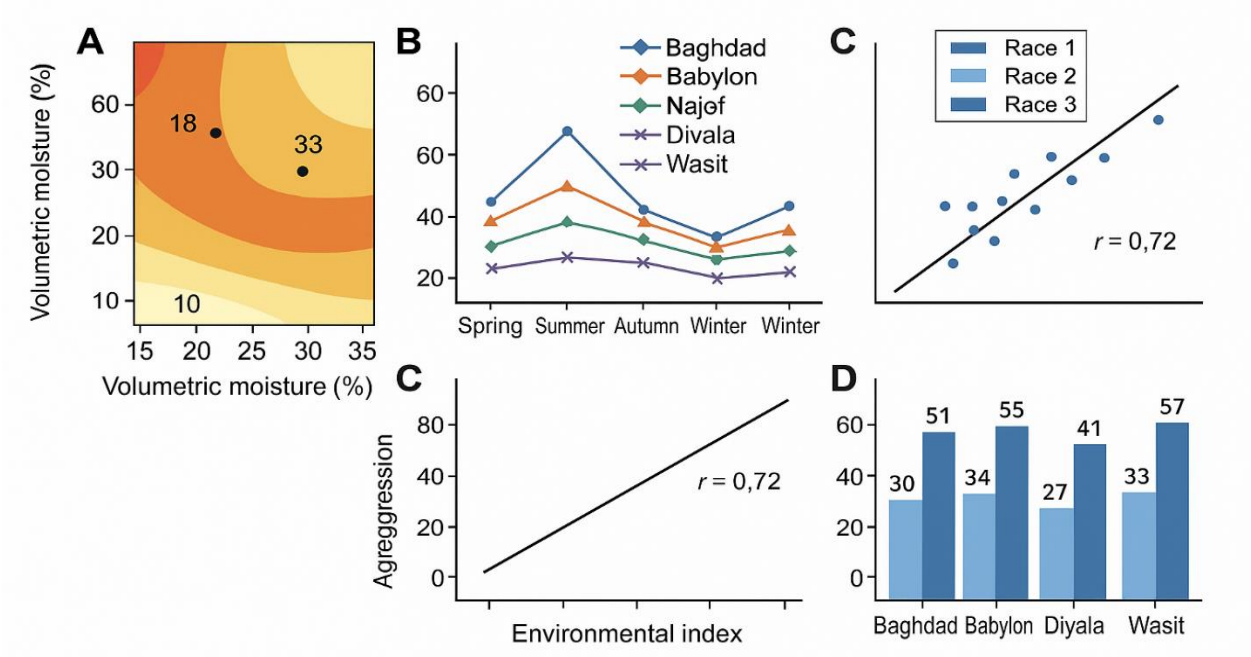

Figure 7. Environmental factors and field performance

Figure 7, Panel (A) shows the effects of soil temperature and soil moisture on the growth of Fusarium oxysporum and this illustrates the effects of these environmental factors on the progress of the disease. In the panel (B), the severity of the diseases in different provinces varies with the seasons, thus demonstrating the alterations in the intensity of the disease over time. The connection between the environment and the aggressiveness of the pathogen is investigated with Panel (C), where such characteristics of the environmental factors as temperature and moisture determine virulence. In panel (D), the economic impact analysis is given, which shows losses in yield by race and province, which shows the financial consequences of Fusarium wilt on the production of tomato crop.

Software Details

A number of software programs and tools were employed in the analysis of the data and molecular characterization in the given study. The phylogenetic analysis, as well as sequence alignment, were conducted with MEGA X (version X), that is a powerful tool in evolutionary biology, such as the neighbor-joining algorithm or bootstrap replication to estimate the confidence. Several sequence alignments were carried out using ClustalW on the TEF-1 alpha and IGS gene sequences. Analysis was done using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and ANOVA was used to analyze the results with multiple range test by Duncan used to test levels of significance (P 0.05). The quality and concentration of the DNA were measured by the NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific) and the PCR products were sequenced with ABI 3730xl DNA Analyzer (Applied Biosystems, USA). To have purified PCR products, QIA quick PCR Purification Kit (Qiagen, Germany) was utilized to get good samples to be used in sequencing.

Discussion

This represents the first extensive molecular characterisation of Fusarium oxysporum f.sp. lycopersici populations in Iraq where significant genetic diversity was observed and where there was some variability of pathogenicity amongst the isolates. The finding that three physiological races (1, 2, and 3) with race 2 dominance (48.9%) is in line with global trends reported in Mediterranean and Middle Eastern regions (Gangwar et al., 2025). The exclusion of the race 4, which is prevalent in California and some select areas across the globe indicates limitations in the geographical spread of pathogen or environmental factors that hinder its proliferation in the agroecosystems of Iraq (Rubayet & Hossain, 2024).

The high genetic diversity of the present study (Hd = 0.912) is greater than that of Turkey (Hd = 0.847) and Iran (Hd = 0.789), suggesting the pathogen population to be more diverse in Iraq (Zakaria, 2024; Ismailaj et al., 2024). This variability can be explained by the fact that the country is located on the border of various biogeographic areas and many types of tomatoes are widely grown during several decades. Nucleotide diversity (2) was in line with other reports on a regional scale of F. oxysporum in the Mediterranean basin and indicated moderate evolution rates in local populations (Srivastava et al., 2024).

The predominance of race 2 isolates is concerning from a disease management perspective, as this race overcomes the I-2 resistance gene commonly deployed in commercial tomato cultivars (Le et al., 2024). Previous studies in Spain and Italy have reported similar race 2 dominance, attributed to widespread use of I-2 resistant cultivars that exerted selection pressure favoring race 2 evolution (Afzalinia et al., 2025; Mohd-Hafifi et al., 2024). The significant pathogenic variation observed among isolates within the same race suggests the presence of pathogenic variants or influence of additional virulence factors not captured by traditional race classification systems (Ali et al., 2024).

The molecular phylogeny based on TEF-1 alpha and IGS markers showed clear distinction of races and the intra-specific relationships among the isolates. These findings are consistent with a recent study by who described the usefulness of multi-locus sequence typing to characterize F. oxysporum (Dong et al., 2025). Length variations in IGS regions represent the dynamism of this non-coding sequence and has been found to have the potential as a population genetic marker (Gilardi et al., 2024b).

Pathogenicity data showed that race 2 isolates were usually more virulent than races 1 and 3, as also reported in (Ruan et al., 2024; Daichi et al., 2025). Virtual virulence of the most virulent isolates was greater than 80, under greenhouse and field conditions respectively and therefore, demonstrating high potential epidemic. Lower disease severity in the field in comparison with greenhouse experiments is the indication of influence of environmental factors and microbial relationships that inhibit the development of the disease (Nel et al., 2024).

The fact that root colonization levels are correlated well with the score of diseases ($r = 0.87$, $P < 0.001$) supports the significance of accumulative funformed biomass in the disease formation. Isolates that caused the highest infection also produced more fungus in infected roots than those with moderate virulence, implying superior root invasion physiology. Such findings were supported by other research that recently identified a relationship between fungal biomass and virulence in vascular wilt disease-causing fungi (Al-Mahmooli et al., 2024; Kumar et al., 2024). It must be noted, however, that yield loss percentage shown in the field experiment conducted in Iraq to be significantly affected by tomato Fusarium wilt disease varied from 18.3% to 78.6$. The results were not very different from other areas under comparable climatic conditions (Larkin, 2024), thus prompting the application of complete disease management strategies (CDMS). Environmental factors such as soil temperature and moisture were shown to favor disease development; optimal values were shown to be 24-28°C and 60-70% moisture (Yatoo et al., 2024). Projections had shown changes to be expected due to climate change to favor increased areas afflicted by tomato Fusarium wilt disease. The results presented here presented critical baseline values needed for management and monitoring changes in disease-causing pathogen development (Mut publi.) (Haruna et al., 2024).

Conclusion

The work presented here contributes significantly to understanding genetic diversity and population structure for F. oxysporum f. sp. lycopersici in Iraq and their pathogenecity. The detection of three physiological races, of which race 2 is dominant, together with their genetic diversity and variability in pathogenicity, emphasizes just how complex fusarium wilt management can be. Techniques of fingerprinting races and analyzing population structure have enabled molecular characterization to provide vital knowledge to aid disease monitoring and resistance development.

The high level of virulence associated with race 2 isolates and their geographical distribution create challenges to the current disease management strategies based solely on I-2-mediated resistance. Therefore, it would be justified to breed varieties combining several sources of resistance and to pursue integrated disease management strategies involving combinations of biocontrol agents, fertilizers, and cultural methods. Setting up a national disease monitoring system involving molecular technologies would ease early detection of new races to guide strategies towards resistance deployment.

The following areas should be explored in future research to determine effector genes present in the Iraqi population of F. oxysporum to understand their relative virulence and to aid in methods to possibly breed for resistance. In addition to these aspects, understanding population dynamics of these fungi under projected

climate change models would be essential to embrace adaptive management approaches. The results generated in this study have direct applications to make decisions related to tomato disease management.

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Author Contributions

All Authors contributed equally.

Conflict of Interest

The author confirms that he has no conflict of interest as far as this research is concerned. The research was carried out objectively and without external factors that may influence the findings and conclusion reached using the data.

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