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Isolation, molecular characterization, pathogenicity and host resistance of *Fusarium pseudograminearum*—a soil-borne ascomycete recently discovered in Karbala, Iraq

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Abstract

This study aimed to isolate, identify and characterize *Fusarium pseudograminearum* from wheat fields in Karbala provinces, Iraq with this disease; pathogenicity assessment; genetic diversity estimation among these isolates and finally investigate the resistance of some commonly cultivated wheat cultivars against it. All fungal isolates were collected from symptomatic wheat plants during the growing season of 2023, and identified through morphological features as well as molecular characteristics based on gene sequencing (ITS and TEF-1α.) *F. pseudograminearum* isolates analysis demonstrated 89–99% homology to global strains, which were confirmed by BLAST analysis on all retrieved isolates in the study. Iraqi isolates grouped in a single clade with very low genetic diversity and likely shared ancestral background according to phylogenetic analysis. All isolates caused full inhibition of seed germination and seedling growth, indicating high virulence in pathogenicity assays. All ten wheat cultivars (Sally, Al-Tahaddi, Ashour, Abu Ghraib, Research 22, Iraq, Sabah, Ibaa 99, Latifiyah and Adnaniah) were evaluated for both percent disease incidence and severity of the pathogen categories which showed that one hundred percent (100%) incidence was the extent of susceptibility among all tested genotypes. Reported genetic uniformity of the pathogen population suggests persistence of host–pathogen interactions, which underlines the need for cultivar screening to identify resistant or tolerant ones. It gives us vital information about the epidemiology and management of FCR in Iraq, highlight the importance of breeding programs to improve *F. pseudograminearum* resistance.

Keywords: Fusarium crown rot, *Fusarium pseudograminearum,* Morphological and Molecular identification

Introduction

Wheat (*Triticum aestivum* L.) is a member of the Poaceae family and one of the most important cereal crops globally, being a major staple for humans and livestock [1]. Wheat production in Iraq was estimated at 5,234,171 tons in a mixed governorates of Iraq during the 2024 [2]. However, wheat production is heavily limited by

abiotic and biotic stresses that negatively impact yield and grain quality. One of the major biotic constraints to wheat production is Fusarium crown rot (FCR), a severe disease caused by the plant pathogenic fungus *Fusarium pseudograminearum*. This pathogen is ubiquitous in regions where cereal crops are grown and occurs predominantly under arid or semiarid climatic conditions. The occurrence of FCR has risen in recent years, partly due to moisture-conserving agricultural practices such as minimum tillage and stubble retention [3]. *F. pseudograminearum* has been identified as one of the major Fusarium species causing FCR in surveys of both Basrah (2012) [4] and Karbala Province (2025) [5] in Iraq.

FCR mainly target wheat at the seedling stage, causing loss of seedlings either at emergence or shortly afterwards. which typically includes brown discoloured coleoptile, subcrown internode, lower leaf sheaths and basal stem tissues of the plants. In certain instances, nodes or stems beneath the leaf sheaths develop a pinkish colouration [6]. A consequence of the progress of this disease is that it prematurely induces senescence in wheat heads (also known as "Whiteheads"), which can produce none or shriveled grains [7]. These traits, such as grain yield, tiller height and straw biomass, are all negatively correlated with FCR incidence and severity with potential yield losses as great as 35% [8]. Furthermore, infection by Fusarium spp. and can cause contamination of wheat grains with toxic mycotoxins [9].

Fusarium species were originally identified using morphological characteristics e.g. colony morphology (growth on the different culture media), pigmentation and shape of spore types such as microconidia, macroconidia and chlamydospores [10]. Fungal mycelia and mycelial fragments can also act as sources of infection in addition to spores [11]. Nevertheless, identification based solely on morphological features is typically not effective with such genera. At present, molecular methods (especially DNA sequencing of PCR-amplified regions with gene-specific primers) are used for fast, sensitive and accurate identification of Fusarium species [12]. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA is among the most popular molecular markers for fungal identification and primer design [13,14]. Commonly used primer pairs for the detection of *Fusarium* spp. include those such as ITS1/ITS4 and ITS5/ITS4, in various crops [15,16].

In addition to identification based on ITS, numerous gene regions have been widely employed in molecular characterization and phylogenetic assessment of Fusarium species. Such markers have included the translation elongation factor 1-alpha (TEF1α), mating type (MAT) genes, phosphate permease gene, β-tubulin gene and largest and second-largest subunit of RNA polymerase II (RPB1 and RPB2) [17-18]. Combining multi-locus molecular data with morphological characters is now considered necessary for identifying species correctly and streamline phylogenetic relationships among Fusarium [19–20].

Fusarium spp.-associated diseases are best managed in the long-term with the introduction and use of resistant cultivars. However, there are no fully resistant commercial wheat cultivars worldwide [5]. FCR resistance is in general only partial, pol-

ygenic, and thus very much affected by environmental factors and the interaction of genotype–environment [21,22]. Tolerance, or partial resistance, with reduced disease severity and improved agronomic performance under infection have been described in some cases [23].

Knowledge of diversity and pathogenicity in *F. pseudograminearum*, accompanied by evaluations of wheat cultivar responses to the pathogen, will be useful for developing effective management practices for FCR that would be particularly applicable to major sites of wheat production (e.g., Karbala Province). Accordingly, this study was conducted to: (1) isolate and characterize *F. pseudograminearum* morphologically and molecularly and pathogenicity assessment under artificial conditions; (2) analyze the genetic diversity of *F. pseudograminearum* populations from major wheat-growing regions in Iraq; and (3) evaluate resistance/tolerance of ten cultivars of wheat.

Materials and Methods

Isolation, and morphological Identification

During the 2023 growing season, wheat plants that exhibited typical symptoms of crown rot were collected from wheat fields in several locations in Karbala Province. Fields were randomly selected.

Small pieces of tissue (about 5–10 mm) were taken from the interface of healthy and diseased tissue. Surface sterilization of the samples was performed by immersion in 70% (v/v) ethanol for 30 s, followed by a treatment with 1% (v/v) sodium hypochlorite (NaClO) for 2 min, and rinsed three times in sterile distilled water. The cleaned tissues were air-dried placed on sterile filter paper. The obtained paper was cut into small pieces and placed in potato dextrose agar (PDA) medium [24, 25] with 50 µg/mL streptomycin sulfate to prevent bacterial growth, then incubated at 25°C under darkness for 48–72 hours. Identical characteristics were observed in emerging colonies, which were resubcultured to fresh PDA plates and the use of the hyphal tip method produced pure cultures. The isolated Fusarium-like isolates were subsequently stored at −4°C for further analysis. Purified isolates were morphologically characterized from their colony characteristics and pigmentation, as well as the morphology of reproductive structures (i.e., microconidia, and macroconidia) [26–27].

Molecular identification

A representative Fusarium-like isolate was cultured on potato dextrose agar (PDA) plates for 7 days at 25°C in the dark. PCR amplification and sequencing of genomic DNA was performed using genetic markers by Macrogen, Inc. (Seoul, South Korea). The universal primer pair ITS1/ITS4 [28] was used to amplify the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA-ITS). Second, the translation elongation factor 1-alpha (TEF-1α) gene was amplified using primers EF1/EF2 [29]. PCR products were purified and sequenced. The sequences obtained were edited by A Plasmid Editor v3. 1. 6 and subsequently aligned against reference fungal sequences

published in the GenBank database by BLAST. Sequence Demarcation Tool (SDT) v1.3 was used for further sequence analysis.

Genetic diversity and Phylogenetic analyses

Phylogenetic analyses were used to infer relationships among the isolates and branch support was evaluated using 1,000 bootstrap replicates. For the analysis, Spongosporat subterranea sequence was used as an outgroup. Tree Topology and Age Estimation Phylogenetic trees were built using Molecular Evolutionary Genetics Analysis version 11.0.13 (MEGA11). The sequences generated were then submitted to the GenBank database for accession numbers.

Moreover, phylogenetic analysis of the ITS sequences was carried out to analyze the origin and genetic diversity of *Fusarium pseudograminearum* isolates from Iraq. TEF-1α gene sequences were aligned by ClustalX for sequence alignment and characterized using WebLogo3 for *F. pseudograminearum* isolates in collected from Karbala Province [31].

Pathogenicity Assessments

Fungal isolates identification and assessment of pathogenicity were based on plate assay method [32] with ten for *Fusarium* sp. A mycelial disc (0.5 cm apart) was removed from the margin of 7-day-old pure cultures grown on PDA and placed in centre of petri dishes (9 cm × 2 cm) containing WA. The inoculated plates were incubated at 25 ± 2 °C for three days.

Surface-sterilized wheat seeds (Ibaa 99 cv.) were prepared by immersing them in a solution of 1% sodium hypochlorite for 2 minutes, rinsing three times with sterile distilled water and air-drying on sterile filter paper. Ten seeds were subsequently placed at the edge of expanding fungal colonies on each plate. There were three replicates for each isolate. The control treatment with the same number of replicates was sterilized seeds without fungal inoculation. Plates were then incubated at 25 ± 2°C until control treatment germination was complete. Then seed germination percent was measured based on the following equation:

$$\text{Germination \%} = (\text{number of germinated seeds} / \text{total number of seeds}) \times 100$$

Evaluation of resistance/susceptibility of common wheat genotypes to *F.pseudograminearum* in Karbala Province

The soil mixture experienced two consecutive sterilization processes in an autoclave for 1 h. The sterilized soil was transferred into disinfected (70% ethanol) plastic pots (8 cm in depth). A 1×10^6 spores mL^{-1} of each pathogenic isolate of *F. pseudograminearum* was prepared using a hemocytometer. 1 mL of each spore suspension was inoculated into each pot, while control pots were received 1ml sterile distilled water. The experiment was laid out in a randomized complete block design. These pots were then sown with the surface-sterilized wheat seeds (1% sodium hypochlorite) of ten cultivars including Sally, Al-Tahaddi, Ashour, Abu Ghraib, Research 22, Iraq, Sabah, Ibaa 99 Latifiyah and Adnaniah. The control treatment was included and each treatment replicated four times [31]. Four weeks post-sowing, Fusarium

crown rot (FCR) was evaluated and the percentage disease incidence calculated using the following equation:

FCR %= Number of infected seedling/ total number of seedlings

Results and Discussion

Isolation, and morphological Identification

A comprehensive morphological and cultural characterization of the Fusarium isolates from wheat samples with crown and root rot determined characteristics sufficient for genus-level identification [26]. The isolates were fast-growing on potato dextrose agar (PDA) forming profuse, fluffy aerial mycelium with a pink pigment diffusing into the medium (figure 1A).

Microscopically, macroconidia were plentiful and narrow to almost straight, generally 2–4 septate, ranging in size from 21–70 × 1.9–4.6 µm (n = 20). Microconidia were not observed. Morphological characteristics were highly consistent with previously described characteristics for *Fusarium* spp. (Figure 2B).

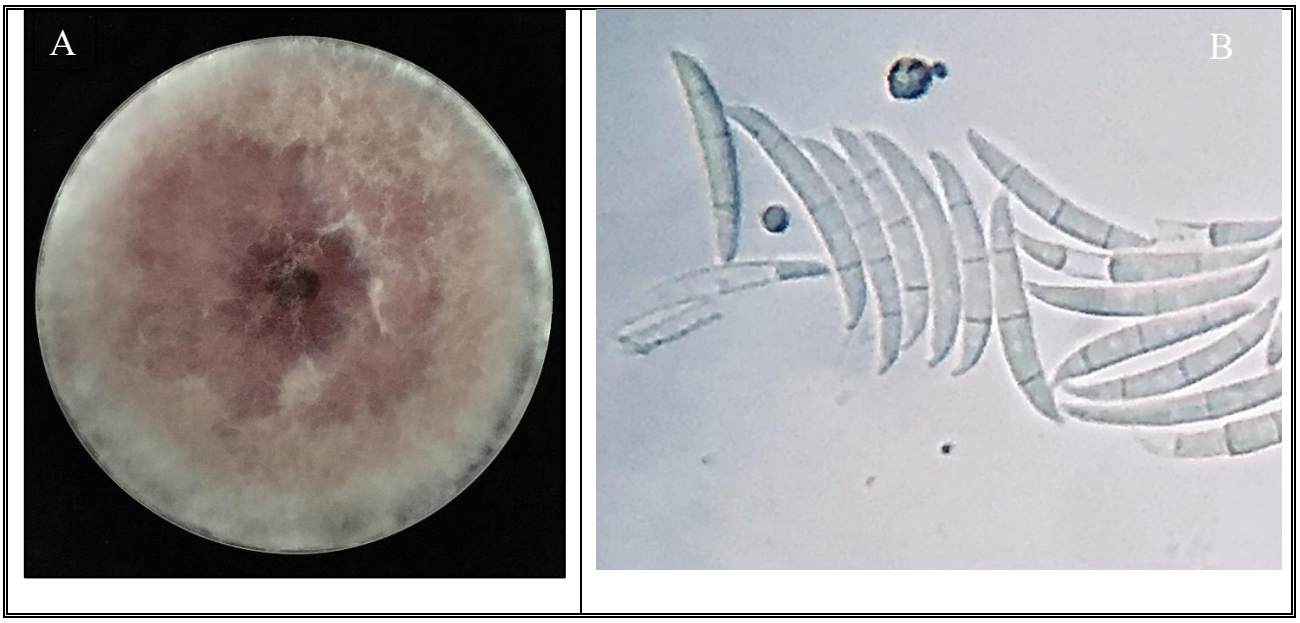

Figure (1): Cultural (A) and microscopic features (B) of the fungus *Fusarium* sp. isolated from infected wheat seedlings.

Molecular identification

All isolates were positive for amplification and sequencing of the rDNA-ITS region, and TEF-1α. BLASTn analysis confirmed the initial morphological identification with all isolates containing sequences originally classified within the genus Fusarium, labelled as *F. pseudograminearum*. Compared to previously characterized global strains of the same species, sequence similarity was between 89% and 99% (Figure 2A–B). In addition, the purified sequences were subsequently deposited to GenBank database and accession numbers assigned for each isolate included in this study (ITS accession number PZ293192.1).

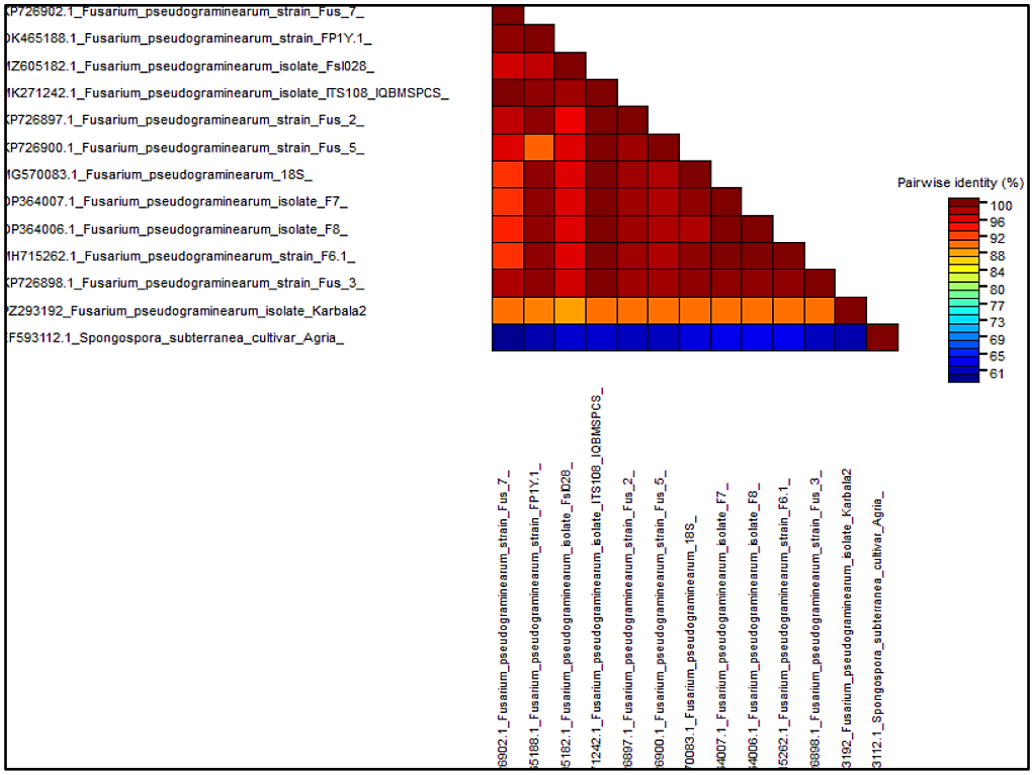

Figure (2) A: Comparative pairwise identity analysis of F. *pseudograminearum* (Karbal-2 isolate) and related taxa based on ITS sequences

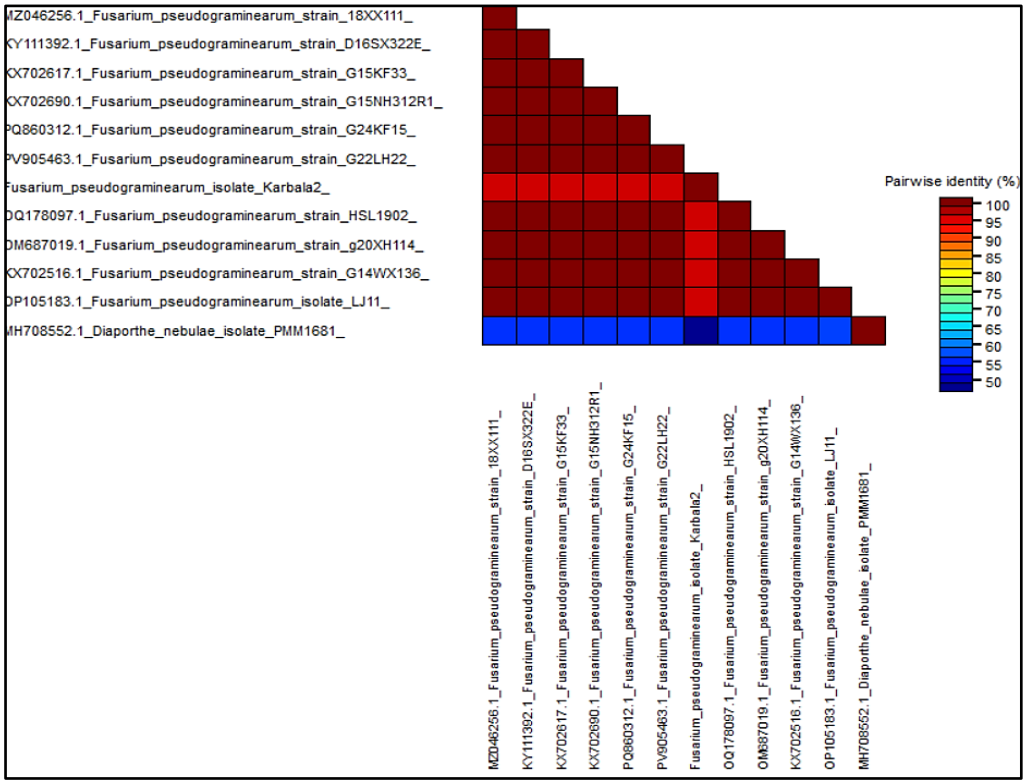

Figure (2) B: Comparative pairwise identity analysis of F. *pseudograminearum* (Karbal-2 isolate) and related taxa based on TEF-1α sequences

Genetic diversity and Phylogenetic analyses

Results Sequence similarity and genetic variation analyses based on the rDNA-ITS region indicated that Iraqi isolates of *Fusarium pseudograminearum* including the two obtained from Karbala Province (Karbala-1 and Karbala-2) are closely related. Despite the presence of minor differences in their sequence, these isolates were highly homologous and therefore genetically stable. Their taxonomic placement in the species *F. pseudograminearum* was confirmed by >90% sequence identity with representative global isolates reported previously.

These results were further supported by the phylogenetic analyses where all *F. pseudograminearum* isolates clustered within a common-supported clade (Figures 3). Such close clustering indicates a common evolutionary ancestor and extreme genetic neutrality among the isolates sampled in this study. On the other hand, *Spongospora subterranea* clearly grouped and was unequivocally placed in a separate and distance clade (as outgroup), thereby testing for the appropriateness of it as an outgroup, while confirming the strength of phylogenetic reconstruction.

The low genetic diversity detected among the Iraqi isolates may indicate a population structure that is quite uniform. This genetic uniformity can be explained by either few introduction events or a common dispersal scheme, like infected seeds or crop residues and agricultural practices designed to favour pathogen survival, such as minimum tillage and residue retention. Low genetic diversity patterns are similarly reported for *F. pseudograminearum* populations in many cereal-growing regions, where both pathogen spread and limited gene flow is often mediated by human activity [3,7].

On a global scale all isolates were clustered in one clade with very high sequence similarity implying low genetic diversity within populations of *F. pseudograminearum* across Iraq and could be derived from the same or low number of introduction events. Genetically uniform populations may confer similar responses to control strategies, including host resistance and cultural practices, making sick farms easier to manage (or conversely more challenging) depending on if the solution requires a synergistic or antagonistic action from the uniform population.

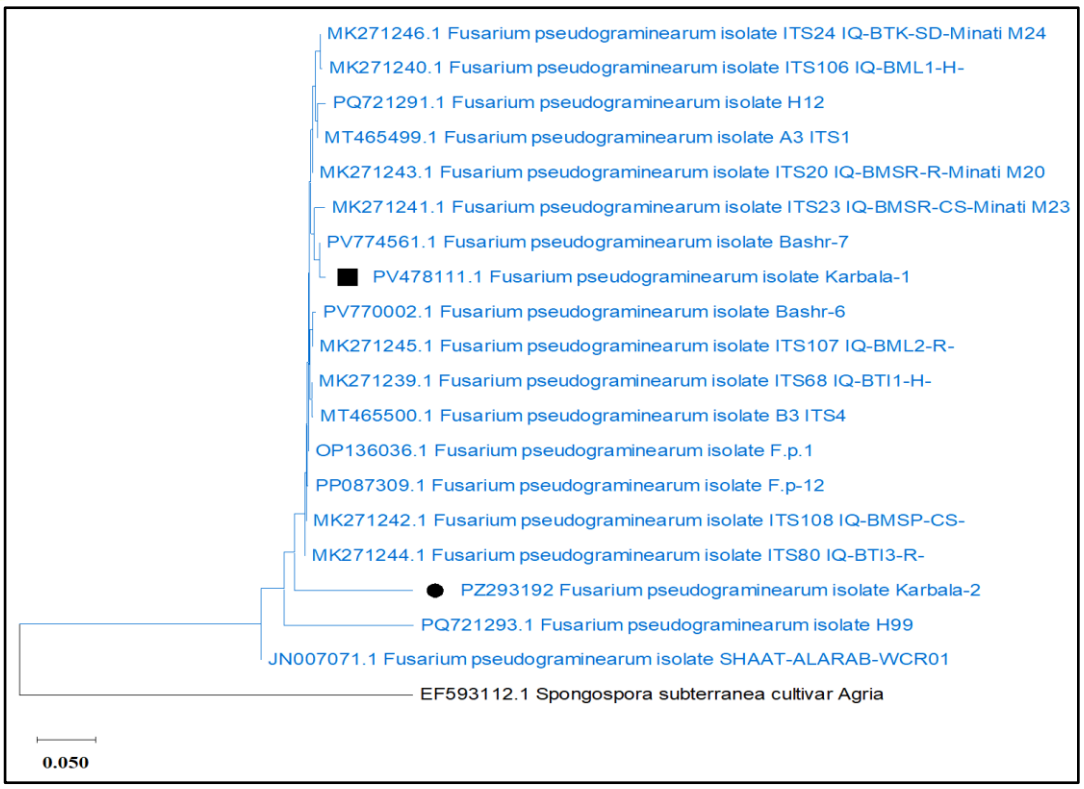

Figure (3): Phylogenetic tree of *F. pseudograminearum* isolates including Karbala-1 (indicated with black square) and Karbal-2 (indicated with black dot) and related Iraqi isolates based on ITS sequences.

Although the ITS region is a fairly conserved genetic marker, it may not provide enough resolution for distinguishing intraspecific variation within Fusarium species. Conversely, multi-locus approaches utilizing greater number of more variable genes; e.g.RPB1, RPB2 and TEF-1α have been shown to provide a higher level of resolution in terms of population structure and genetic diversity analyses [33, 34].

Because the similarity was not 100% among isolates studied, more informative molecular markers are needed to further clarify population structure and evolutionary relationships of *F. pseudograminearum* populations in Iraq. Interestingly, the TEF-1α sequences (only two) had been previously sequenced for *F. pseudograminearum* isolates collected from Karbala Province (Karbala-1 and Karbala-2). Consequently, these fragments were aligned to analyze genetic variability among isolates. The results showed these two isolates almost identical with similarity percentage >99% (Figure 4). Consequently, screening and evaluation of wheat cultivars for resistance or tolerance to this pathogen are likely to be effective, as genetically homogeneous pathogen populations are expected to exhibit relatively consistent responses to host resistance.

Figure (4): Sequence logo representation of TEF-1α gene alignment of *Fusarium pseudograminearum* isolates from Karbala Province

Pathogenicity Assessments

The tested Fusarium isolates caused 100 % inhibition for all wheat seeds and seedlings (Figure 5). No Fusarium isolates were re-isolated from the control seedlings, while Fusarium isolates were consistently re-isolated from wheat seedlings. The identities of the re-isolated fungi were confirmed by molecular characterizations as described above, thus fulfilling Koch's postulates.

The pathogenicity of all tested Fusarium isolates was high (inhibiting seed germination and seedling development totally—100%), independent of wheat cultivar evaluated. Conversely, no visual symptoms of disease were recorded in the control treatment, and non-inoculated seedlings did not yield any Fusarium isolates (not shown).Infected wheat seedlings were then tested at different time points (7 and 15 days post-inoculation) to recover the pathogen, which was consistently re-isolated from diseased samples, with both morphological and molecular characteristics of isolates recovered being identical to those of the original inoculated strains. Molecular confirmation was performed as previously described with verification that the re-isolated fungi identified as *Fusarium pseudograminearum*.

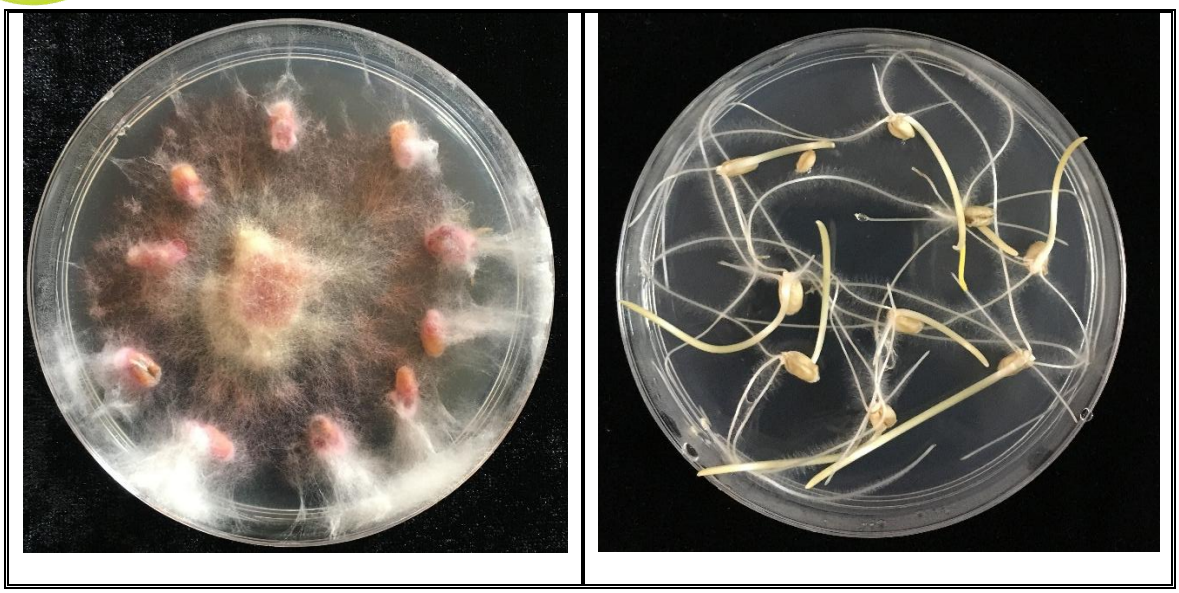

Figure (5): Effect of *Fusarium pseudograminearum* infection on wheat seeds (cultivar Ibaa 99). Diseased seeds are shown on the left, while healthy germinated seeds are shown on the right.

The high virulence of these tested isolates and the ability to induce acute disease in the early stages of development suggest that these isolates could be an important challenge for wheat. Finally, the re-isolation and molecular characterization of the pathogen fulfill Koch's postulates to demonstrate that *F. pseudograminearum* is sufficient under natural transmission conditions to cause symptoms on cereal species.

Evaluation of resistance/susceptibility of common wheat genotypes to *F.pseudograminearum* in Karbala Province

Fusarium crown rot (FCR) incidence was determined at four weeks after sowing on all tested wheat cultivars. *F. pseudograminearum* infection was high in all ten cultivars (Sally, Al-Tahaddi, Ashour, Abu Ghraib, Research 22, Iraq, Sabah, Ibaa 99, Latifiyah and Adnaniah), which yielded a disease incidence of 100% in all treatments. Infected plants showed expected symptoms of crown and root rot, confirming the pathogenicity of this high-virulence pathogen under our experimental conditions.

On the other hand, the control treatment did not show any symptoms with no visible signs of infection (Figure 6), supporting that disease progression was exclusive to fungal inoculation. This is further supported by our findings which show limited resistance among the cultivars we have evaluated, indicating most common wheat grown in this region are potentially at risk to FCR.

This result is in agreement with a previous Iraqi study [5] conducted in Karbala province that found all the tested wheat cultivars (Al-Tahaddi, Tamooz, Research 22, Arabyia, Ibaa 99 and wafaia were sensitive to *F. pseudograminearum* infection.

The high susceptibility observed among all tested Iraqi wheat cultivars in the present study is consistent also with previous global reports highlighting the limited availability of strong resistance to *Fusarium pseudograminearum* in wheat

germplasm. A recent study evaluating a wide range of spring wheat lines, including CIMMYT-derived germplasm and local cultivars, demonstrated that while several lines exhibited resistance to *Fusarium culmorum*, only moderate levels of resistance were identified against *F. pseudograminearum*, and no fully resistant genotypes were reported under most environmental conditions. Furthermore, resistance to *F. pseudograminearum* was found to be inconsistent and often restricted to controlled environments, indicating the complexity of host–pathogen interactions and the strong influence of environmental factors on disease expression. These findings support the results of the current study, where all evaluated cultivars showed complete susceptibility (100% disease incidence), emphasizing the lack of effective resistance in commonly cultivated Iraqi wheat varieties. The absence of highly resistant cultivars may be attributed to the quantitative nature of resistance to Fusarium crown rot, which is typically controlled by multiple genes and influenced by environmental conditions. Additionally, the difficulty in identifying stable resistance sources further complicates breeding efforts [35].

Figure (6): Symptoms of crown rot on wheat seedlings caused by *Fusarium pseudograminearum* in this study (left: infected; right: healthy)

Therefore, the integration of resistant or moderately resistant genetic resources, combined with continuous screening under diverse environmental conditions, is essential for developing wheat cultivars with improved tolerance to *F. pseudograminearum*. Ultimately, the deployment of resistant varieties remains a key strategy for reducing disease severity, minimizing yield losses, and limiting mycotoxin contamination in wheat production systems [35, 36].

References

1) Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany, 60*(6), 1537–1553. https://doi.org/10.1093/jxb/erp058
2) Food and Agriculture Organization of the United Nations. (2026). *Database results*. Author.
3) Kazan, K., & Gardiner, D. M. (2018). Fusarium crown rot caused by *Fusarium pseudograminearum* in cereal crops: Recent progress and future prospects. *Molecular Plant Pathology, 19*(7), 1547–1562.
4) Hameed, M. A., Rana, R. M., & Ali, Z. (2012). Identification and characterization of a novel Iraqi isolate of *Fusarium pseudograminearum* causing crown rot in wheat. *Genetics and Molecular Research, 11*(2), 1341–1348.
5) Al-Taie, B. J. (2025). *Phenotypic and molecular diagnosis of the causes of root rot and ear blight in wheat, integration in their control, and estimation of pesticide residues used in their control* (Master's thesis). University of Kerbala, Iraq.
6) Deng, Y. Y., Li, W., Zhang, P., Sun, H. Y., Zhang, X. X., Zhang, A. X., & Chen, H. G. (2020). *Fusarium pseudograminearum* as an emerging pathogen of crown rot of wheat in eastern China. *Plant Pathology, 69*(2), 240–248.
7) Zhou, H. F., He, X. L., Wang, S., Ma, Q. Z., Sun, B. J., Ding, S. L., et al. (2019). Diversity of the *Fusarium* pathogens associated with crown rot in the Huanghuai wheat-growing region of China. *Environmental Microbiology, 21*(8), 2740–2754.
8) Ma, G., Wang, H., Qi, K., Ma, L., Zhang, B., Zhang, Y., Jiang, H., Wu, X., & Qi, J. (2024). Isolation, characterization, and pathogenicity of *Fusarium* species causing crown rot of wheat. *Frontiers in Microbiology, 15*, Article 1405115.
9) Mudge, A. M., Dill-Macky, R., Dong, Y. H., Gardiner, D. M., White, R. G., & Manners, J. M. (2006). A role for the mycotoxin deoxynivalenol in stem colonisation during crown rot disease of wheat caused by *Fusarium graminearum* and *Fusarium pseudograminearum*. *Physiological and Molecular Plant Pathology, 69*(1–3), 73–85.
10) Leslie, J. F., & Summerell, B. A. (2008). *The Fusarium laboratory manual*. John Wiley & Sons.
11) Grausgruber, H., Lemmens, M., Buerstmayr, H., & Ruckenbauer, P. (1995). Evaluation of inoculation methods for testing *Fusarium* head blight resistance of winter wheat on a single plant basis. *Die Bodenkultur, 46*(1), 39–49.
12) van Diepeningen, A. D., Brankovics, B., Iltes, J., van der Lee, T. A. J., & Waalwijk, C. (2015). Diagnosis of *Fusarium* infections: Approaches to identification by the clinical mycology laboratory. *Current Fungal Infection Reports, 9*(3), 135–143.

13) Porter, T. M., & Golding, G. B. (2011). Are similarity- or phylogeny-based methods more appropriate for classifying internal transcribed spacer (ITS) metagenomic amplicons? *New Phytologist, 192*(3), 775–782.

14) White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). Academic Press.

15) Kalman, B., Abraham, D., Graph, S., Perl-Treves, R., Meller Harel, Y., & Degani, O. (2020). Isolation and identification of *Fusarium* spp., the causal agents of onion (*Allium cepa*) basal rot in northeastern Israel. *Biology, 9*(4), Article 69.

16) Chen, Y., Zhou, Q., Strelkov, S. E., & Hwang, S.-F. (2014). Genetic diversity and aggressiveness of *Fusarium* spp. isolated from canola in Alberta, Canada. *Plant Disease, 98*(6), 727–738.

17) Kashyap, P. L., Rai, S., Kumar, S., Srivastava, A. K., Anandaraj, M., & Sharma, A. K. (2015). Mating type genes and genetic markers to decipher intraspecific variability among *Fusarium udum* isolates from pigeonpea. *Journal of Basic Microbiology, 55*(7), 846–856.

18) O'Donnell, K., Rooney, A. P., Proctor, R. H., Brown, D. W., McCormick, S. P., Ward, T. J., Frandsen, R. J. N., Lysøe, E., Rehner, S. A., Aoki, T., et al. (2013). Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important *Fusarium*. *Fungal Genetics and Biology, 52*, 20–31.

19) Dash, A., Gurdaswani, V., D'Souza, J., & Ghag, S. (2020). Functional characterization of an inducible bidirectional promoter from *Fusarium oxysporum* f. sp. *cubense*. *Scientific Reports, 10*, Article 2323.

20) Spanic, V., Lemmens, M., & Drezner, G. (2010). Morphological and molecular identification of *Fusarium* species associated with head blight on wheat in east Croatia. *European Journal of Plant Pathology, 128*(4), 511–516.

21) Kazi, S., Shultz, J., Afzal, J., Johnson, J., Njiti, V. N., & Lightfoot, D. A. (2008). Separate loci underlie resistance to root infection and leaf scorch during soybean sudden death syndrome. *Theoretical and Applied Genetics, 116*(7), 967–977.

22) Weinig, C., & Schmitt, J. (2004). Environmental effects on the expression of quantitative trait loci and implications for phenotypic evolution. *BioScience, 54*(7), 627–635.

23) Zhang, J. X., Xue, A. G., Zhang, H. J., Nagasawa, A. E., & Tambong, J. T. (2010). Response of soybean cultivars to root rot caused by *Fusarium* species. *Canadian Journal of Plant Science, 90*(5), 767–776.

24) Abate, D., Pastore, C., Gerin, D., De Miccolis Angelini, R. M., Rotolo, C., Pollastro, S., et al. (2018). Characterization of *Monilinia* spp. populations on stone fruit in South Italy. *Plant Disease, 102*(9), 1708–1717.

25) Al-Musawi, M. A., Lahuf, A. A., & Jaafar, O. H. (2017). Isolation and diagnosis of the pathogens causing seed decay and damping-off disease on wheat and their control using biological and chemical factors. *Journal of Kerbala for Agricultural Sciences, 4*(1), 112–132.

26) Leslie, J. F., & Summerell, B. A. (2006). *The Fusarium laboratory manual*. Blackwell Publishing Professional.

27) Watanabe, T. (2010). *Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species* (3rd ed.). CRC Press.

28) White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). Academic Press.

29) O'Donnell, K., Whitaker, B. K., Laraba, I., Proctor, R. H., Brown, D. W., Broders, K., et al. (2022). DNA sequence-based identification of *Fusarium*: A work in progress. *Plant Disease, 106*(6), 1597–1609.

30) Carbone, I., & Kohn, L. M. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia, 91*(3), 553–556.

31) Jaber, M. H., & Lahuf, A. A. (2020). Survey, pathogenicity, and molecular identification of novel *Fusarium* species causing seed decay and damping-off disease of wheat in Kerbala Province, Iraq. *Plant Cell Biotechnology and Molecular Biology, 21*(11–12), 1–14.

32) Christensen, M. J., Falloon, R. E., & Skipp, R. A. (1988). A petri plate technique for testing pathogenicity of fungi to seedlings and inducing fungal sporulation. *Australasian Plant Pathology, 17*(2), 45–47.

33) Stoeva, D., Gencheva, D., Radoslavov, G., Hristov, P., Yordanova, R., & Beev, G. (2025). Novel DNA barcoding and multiplex PCR strategy for the molecular identification and mycotoxin gene detection of *Fusarium* spp. in maize from Bulgaria. *Methods and Protocols, 8*(1), Article pending.

34) Bugingo, C., Infantino, A., Okello, P., Pérez-Hernández, O., Petrović, K., Turatsinze, A. N., & Moparthi, S. (2025). From morphology to multi-omics: A new age of *Fusarium* research. *Pathogens, 14*(2), Article pending.

35) Özdemir, F. (2022). Host susceptibility of CIMMYT's international spring wheat lines to crown and root rot caused by *Fusarium culmorum* and *Fusarium pseudograminearum*. *Agronomy, 12*(12), Article 3038.

36) Kazan, K., & Gardiner, D. M. (2018). Fusarium crown rot caused by *Fusarium pseudograminearum* in cereal crops: Recent progress and future prospects. *Molecular Plant Pathology, 19*(7), 1547–1562.