Molecular Identification and First Report of *Fusarium annulatum* Inciting Wheat Root Rot on Bread Wheat in Iraq by Multi-Locus Phylogenetic Analysis

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Abstract

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Fusarium spp. are very important plants, animals, and human pathogens. In this study, field severe wilt infection in bread wheat was observed in January 2021 in the wheat field of the Muradia Agriculture Station, Babylon, Iraq. Disease symptoms on the roots were observed as brown discoloration and root rot. Pathogen isolation, morphological characterization, pathogenicity, and sequencing of ITS, TEF1-α, TUB2, and ACT regions, and the phylogenetic analysis with multi-gene of the isolated pathogen were performed. The results obtained revealed that the multigene phylogenetic trees using Maximum Likelihood phylogenetic analysis in addition to morphological characteristics confirmed the identification of *Fusarium annulatum.* Phylogenetic analysis utilizing Bayesian and Maximum Likelihood trees based on only ITS sequences and ITS data was not sufficient to distinguish this species among *Fusarium* spp. However, the results of phylogenetic analysis using Bayesian and Maximum Likelihood trees based on TEF1-α region or multigene phylogenetic analysis showed that isolates of *F. annulatum* were grouped in a distinct species clade belonging to the *Fusarium* spp. This work is considered as the first molecular identification using 4-gene phylogenetic analysis and first report of *F. annulatum* as a wheat pathogen in Babylon, Iraq.

Keywords: Fungal disease, *Triticum aestivum*, multigene phylogenetic analysis, *Fusarium*, soil borne disease.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops in Iraq and worldwide. It is an essential source of human nutrition. In 2020, wheat was grown in 2,143,421 ha with a production average of 2,910 kg/ha. However, total production is way less than what is needed to feed the population in Iraq. There are two and half million Iraqi individuals in need of humanitarian aid, of whom 960 thousand individuals have urgent needs, based on the 2022 Humanitarian Needs Overview (FAO, 2022). Therefore, reducing yield losses by many plant diseases that attack wheat annually is needed to overcome this food shortage (Al-Maaroof, 2022).

The genus *Fusarium* (Ascomycota, Hypocreales, Nectriacae) is considered one of the very significant plant pathogenic genera worldwide and includes more than three hundred thirty species (Yilmaz *et al.*, 2021). Numerous Fusarium species are economically important as producers of hazardous secondary metabolites such as mycotoxins that lead to significant losses to agricultural crops and threaten worldwide food security (Wu, 2007).

Fusarium has many species complexes, over three hundred phylogenetically different species in the genus, which are dispersed throughout twenty-three clades known as species complexes (Jacobs-Venter *et al.*, 2018). This complexity results from the difficulty of distinguishing species within each species complex using morphological characteristics and molecular identification with a single marker. Several *Fusarium* species have been identified as associated with parts of wheat plants worldwide. Species of this genus caused crown rot and head blight (Matny *et al.*, 2012) and root rot (Minati, 2020).

In Iraq, many *Fusarium* species have been determined associated with wheat and other crops (Al-Maaroof & Rahim, 2024; Hameed 2011; Hameed *et al.* 2012; Khaeim *et al.*, 2019; Mohammed-Ameen, *et al.*, 2021; Minati 2020; Matny *et al.*, 2012; 2017; 2018; Minati & Mohammed-Ammen 2019a; 2019b). Since using multi gene phylogenetic analysis as a tool resolving the problem of complexity and difficulty to distinguish species belonging to each *Fusarium* species complex using morphological characteristics. This study focused on molecular identification of a wheat root rot pathogen using multi-locus phylogenetic analysis and pathogenicity confirmation.

Materials and Methods

Sampling and pathogen isolation

Severe field infection with wilted wheat was observed in January 2021 in a wheat field at Muradia Agriculture Station, Babylon, Iraq. The main symptoms observed on the roots were brown discoloration and root rot. The shoots of the wheat plants were wilted with rapid dropping leaves. Nine samples from five bread wheat with roots infection were randomly sampled from the fields and immediately moved to the Plant Protection Laboratory, Muradia Station, Babylon, Iraq. Small portions of roots from the infected plants were sterilized with ethanol (70%) and sodium hypochlorite (1%) for 30 seconds, followed by rinsing twice with sterile

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distilled water, cultured on nine cm PDA plates, and incubated at 25ºC in the dark. Isolates were purified as hyphal tip isolates by culturing them on water agar, incubated at 25ºC, and transferring hyphal tips of the emerging fungus two days later onto PDA medium. Three *Fusarium* isolates of the prevalent species were maintained for further work.

Morphological characteristics

Mycelial discs (5 cm in diameter) were cut from the margins of actively growing cultures, transferred to 9 cm-diameter plastic Petri dishes containing PDA and incubated at 25°C in the dark. The rates of radial growth for triplicate plates were measured 5 days after incubation. The morphological features were described including the colony color and mycelium texture. The microscopic description was completed by picking a small piece of media with fungal structures and placed on glass slides and inspected under a light microscope (DM5500B, Leica, Wetzlar, Germany) at 400X stained with cotton blue and without stain. Measurements were made for macro- and microconidia (n=50 conidia), phialides, and chlamydospores (n=20 for each measurement). The macromorphological and microscopical characters were captured and identified based on taxonomic keys described by Booth (1971), Gerlach & Nirenberg (1982) and Nelson *et al.* (1983).

Pathogenicity assay

Since the frequency of *Fusarium annulatum* was around 80% among isolated fungi from the wheat fields, this fungus was tested for pathogenicity. A pathogenicity experiment was carried out on three-weeks old wheat plants to fulfil Koch's postulates. The inoculum of *Fusarium* isolate, IRQ2020 was prepared from 5-day-old culture grown on millet seeds and mixed with the sterilized surface soil (5gm/kg soil) in plastic pots with wheat plants (Hameed 2011). The inoculated plants were incubated for one week under controlled conditions at a temperature of 25 ±3°C.

DNA extraction, amplification, and sequencing

The purified representative fungal isolate was grown on potato dextrose agar at room temperature for 7 days, and the mycelium was scrapped from the colony surface. The Genomic DNA of *Fusarium* isolates were extracted from 200 mg scrapped mycelium using the E.Z.N.A. Fungal DNA Mini Kit (Omega Bit-Tek, Georgia, USA) following the manufacture's protocol. After verification of appropriate DNA quantity and quality, DNA samples were stored under -20°C for future use. The polymerase chain reaction (PCR) was conducted using GoTaq PCR master Max (Promega, MD, USA). Four loci were amplified to identify *Fusarium* species, create phylogenetic trees, and define the boundaries of the isolate, as recommended by Udayanga *et al.* (2012). These loci included the ITS of rDNA, beta-tubulin, TUB2 region, the elongation factor 1-alpha, TEF1-α region, and the putative ACT region (Soares *et al.*, 2018) using ITS1 and ITS4, the Bt2a and Bt2b, the EF1-728F and EF1-986R, and the Act783R and Act512F primer pairs, respectively (Glass & Donaldson, 1995).

In each PCR reaction, the mixture of 25 µL contained 20 µl PCR master Max, 0.4 µM each primer (1µl per each primer), 2µl H_2O, and 1µl template DNA. For the ITS and CAL loci, thermocycling included a starting stage of 95°C for 5 min, 40 cycles of 95°C for 30s, 54°C for 50s, and 72°C for 1 min, and a last elongation step of 72°C for 10 min (Udayanga *et al.*, 2012). For the TUB2 and TEF1-α loci, thermocycling included an initiation stage of 95°C for 5min, 40 cycles of 95°C for 30s, 57°C for 50s, and 72°C for 1 min, and a last elongation step of 72°C for 10 min. The PCR amplicons were electrophoresed on 1% agarose gel and stained with RedSafe™ nucleic acid stain (iNtRON Biotechnology, Korea). PCR amplicons were purified and sequenced bidirectionally using Sanger sequencing and carried out by Microgen Company (Microgen, Seol, South Korea).

Molecular identification and phylogenetic analysis

Reverse and forward sequence of markers were combined separately using Geneious software (V 9.1.8) (Biomatters Ltd., Newark, NJ). The consensus sequences were compared with global database using BLASTn queries in NCBI to gain preliminary identifications with high similarity to reference specimens' sequences. Later, the ITS sequence of the identified fungus was deposited in GenBank under the accession number OP739335.1.

ACT, TUB2, TEF1-α, and ITS sequences from *Fusarium* isolate were revised with Geneious version 9.1.8. Using the default settings of the aligning tool, MAFFT version 7.309 (Katoh & Standley, 2013), each locus sequences were matched with GenBank sequences of *Fusarium* spp. (using *F. nirenbergiae* and *F. oxysporum* as an outgroup), and any necessary manual adjustments were made. With Geneious v9.1.8, alignments were concatenated. In order to narrow down the total of GenBank sequences used in Bayesian and maximum likelihood studies, a neighbor-joining tree was created (Table 1).

The four concatenated loci were used in Bayesian analyses to build phylogenetic trees using MrBayes Version 3.2.6 (Huelsenbeck & Ronquist, 2001) in the workstation Geneious version 9.1.8 (Kearse *et al.*, 2012). The nucleotide substitution model was a general time- reversible (GTR) model chosen by jModel test (Darriba *et al.*, 2012). Each single thousand generations, trees were sampled in a Markov chain Monte Carlo (MCMC) analysis spanning 1.1 million generations, yielding 1100 trees. The majority rule consensus tree's posterior probabilities (PP) were calculated using the remaining 1000 trees after the first 100 were eliminated (Andjic *et al.*, 2016). Maximum likelihood analyses were undertaken in Geneious version 9.1.8 using RAxML Version 7.2.8 (Stamatakis, 2006) and quick bootstrapping for 1000 replicates. Gamma rate heterogeneity (G) modeling and fraction invariable sites modeling were additional options that the RAxML software provided to support the GTR model of nucleotide replacement (I). A phylogenetic tree was visualized in Geneious version 9.1.8.

Table 1. The accession numbers of *Fusarium* spp. derived from GenBank and used for building phylogenetic trees.

Species	Isolate	Type of isolate	Accession No. of ITS	Accession No. of TEF	Accession No. of TUB	Accession No. of Act
F. annulatum	CBS:258.54	T*	NR_138275.1	MT010994	MT011041	MT010861.1
F. annulatum	CBS 115.97			MW401973	MW402173	
F. annulatum	CBS 133.95			MW402040	MW402239	
F. proliferatum	CBS 480.96 ET	ET**		MN534059	MN534129	
F. globosum	CBS:428.97(NRRL26131)	T	GQ495177.1	AF160285.1	MN534124	MT010860.1
F. globosum	CBS 429.97		LT746278.1	MW402130	MW402329	
F. fujikuroi	CBS 221.76	T	MW827608.1	MN534010	MN534130	KU603834.1
F. mangiferae	CBS 120994	T		MN534017	MN534128	
F. concentricum	CBS 450.97	T	MH862659.1	AF160282	MW402334	MT010859.1
F. fractiflexum	NRRL 28852	T	AF158304.1	AF160288	AF160315	
F. sacchari	CBS 223.76 NRRL 1399	ET	NR_174875.1	MW402115	MW402313	KU603825.1
F. dlaminii	CBS 119860 CBS 175.88 NRR13164	T	U34572.1	MN534002	MN534138	
F. nirenbergiae	CBS 744.97	T		AF160312	U34424	
F. oxysporum	CBS 144134 (NRRL22902)	T	MH484862.1	AF160312	U34424	

* = Extype specimen, **= Exepitype specimen.

Results

Morphological characters

Fungal colonies- Fungal colonies grown on PDA often cover themselves with a forest of aerial hyphae after 5 days of incubation. The aerial mycelium was white and cottony, the lower side of colonies had deep shades of purple to eggplant in color at first later turned to a wide variety of medium purple color shades, and then changed to light purple with some dark purple spots on PDA plates. The web of colony filaments filled the airspace above the agar and pushed itself against the plate lid (Figure 1).

Microscopic features- On PDA, mycelium was branched with slender, septate hyphae, 2.5-3 µm in diameter and produced abundant microconidia and macroconidia. These conidia were produced on sympodially proliferating conidiophores. Conidiophores were straight, erect, smooth thin walled, up to 30×2.5µm tall, developed separately or densely aggregated and vertically branched, bearing mono- and polyphialides. Sometimes, conidiophores were reduced to conidiogenous cells, phialides that were smooth- and thin-walled straight and cylindrical 4.6-20.4×2.7-5.3 µm, or 5.0-7.5×2.5–5.0 µm. Microconidia clustered in separate false heads located at the phialides tip (Figure 2). These were hyaline, smooth- and thin- walled, ovoid to elliptical, and aseptate or with one septum, ranging from 5.0-5.4×2.4-5.6 µm in size (n=50) or 2.5 to 10.6×1.4 to 3.3 µm.

Current Name: *Fusarium annulatum* (Bugnicourt, 1952).
Position in classification: Nectriaceae, Hypocreales, Hypocreomycetidae, Sordariomycetes, Pezizomycotina, Ascomycota, Fungi.

Pathogenicity assay

Artificially infected plants revealed same symptom (wilting of leaves and discoloration of roots and crown regions) 10 days after incubation. However, non-inoculated wheat plants (healthy control) did not produce any symptoms. Koch's postulates were successfully met via re-isolation of the pathogenic fungus from infected roots of artificially inoculated plants. Microscopic observations made were found similar to that of the organism from naturally diseased wheat plants and the *Fusarium* species identity was morphologically confirmed. Therefore, the tested pathogen was the causal agent of the observed disease.

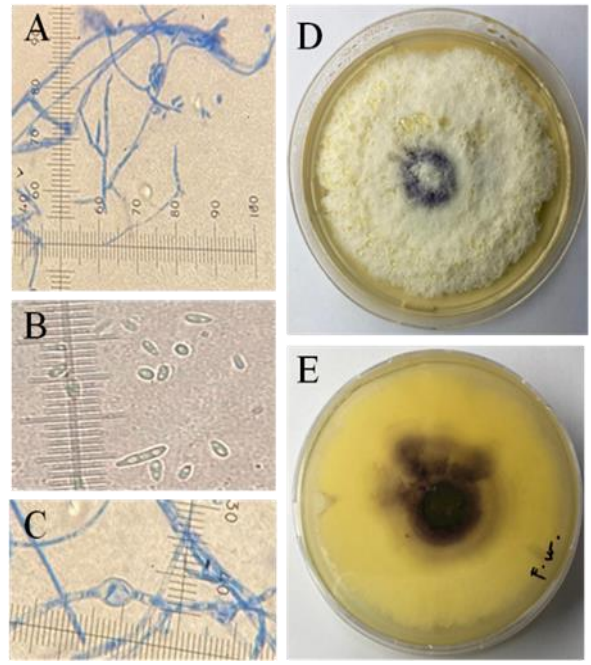

Figure 1. Morphological characters of *F. annulatum* isolate IRQ2020. (A) conidiophore and phialid, (B) macroconidia and microconidia, (C) chlamydospore, (D and E) mycelial growth on PDA.

Molecular identification and phylogenetic analyses

A multigene phylogeny compared with a single gene phylogeny was utilized to show the molecular identification of the isolates studied. The alignment of four genes phylogeny had 14 taxa and was 3870 base pairs long. Results from blast and preliminary phylogenetic trees with single gene (neighbor joining tree constructed based on 99 closely related ITS sequences) showed that the strains of *Fusarium* species in this study had high match to closely related sequences of many *Fusarium* species and clustered in a separate clade that belong to the *Fusarium* spp. Moreover, the phylogenetic analysis results of Bayesian and Maximum Likelihood trees based on only ITS nucleotide sequences and ITS data was not sufficient to distinguish this species among *Fusarium* spp. However, the results of Bayesian and Maximum Likelihood phylogenetic analysis based on TEF1-α region (Figures 2 and 3) or multigene phylogenetic analysis (Figures 4 and 5) revealed that isolates of *F. annulatum* clustered in a distinct clade together with type specimen of this species to be obviously identified as *F. annulatum.*

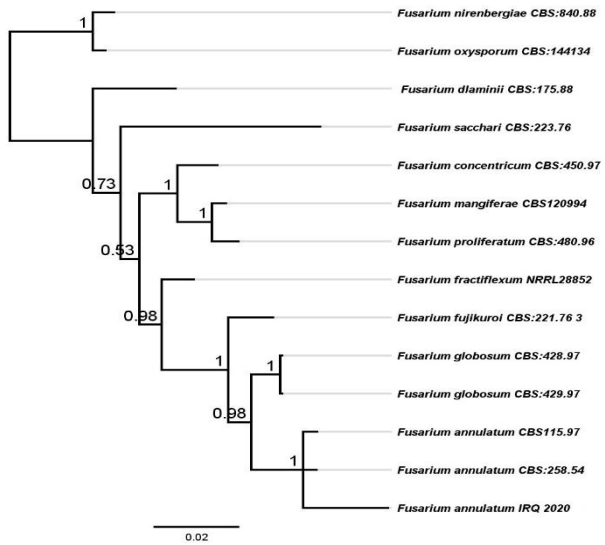

Figure 2. Bayesian phylogenetic tree of *Fusarium annulatum* and related taxa inferred from analysis of the TEF1-α sequences of 14 collections. The nodes display Bayesian posterior probability values. Two outgroup species, *F. nirenbergiae* and *F. oxysporum*, are the roots of the tree. The bar represented the number of nucleotide changes per location.

Discussion

In the present study, multigene phylogeny using ITS, TEF1-α, TUB2, and ACTIN provided high support compared with a single gene phylogeny such as the universal region, ITS. Therefore, phylogenetic analyses of a three-locus dataset powerfully supported the genealogical separation of *Fusarium* species (Taylor *et al.*, 2000).

Due to intraspecific variation and the possibility of striking resemblances among *Fusarium* species over extended evolutionary distances, it is difficult to distinguish between different species by their diagnostic morphological characteristics (Park, 2013).

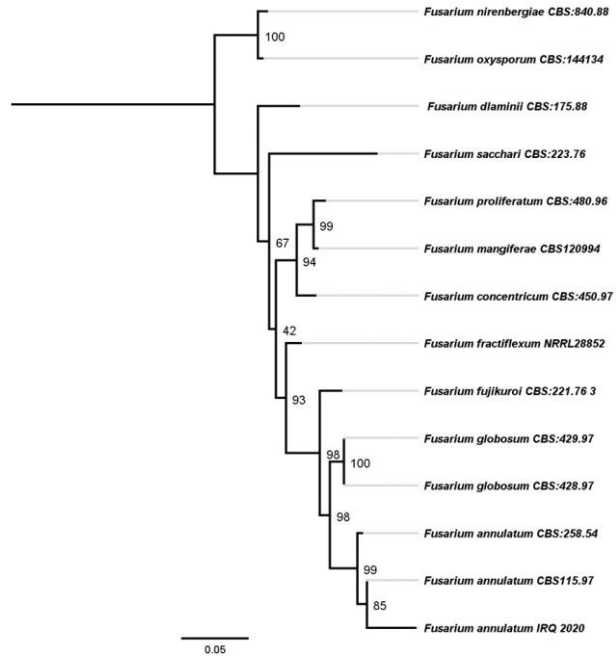

Figure 3. Maximum likelihood (ML) phylogenic tree of *Fusarium annulatum* and related taxa inferred from analysis of the TEF1-α sequences of 14 collections. The nodes display Bayesian posterior probability values. Two outgroup species, *F. nirenbergiae* and *F. oxysporum*, are the roots of the tree. The bar represented the number of nucleotide changes per location.

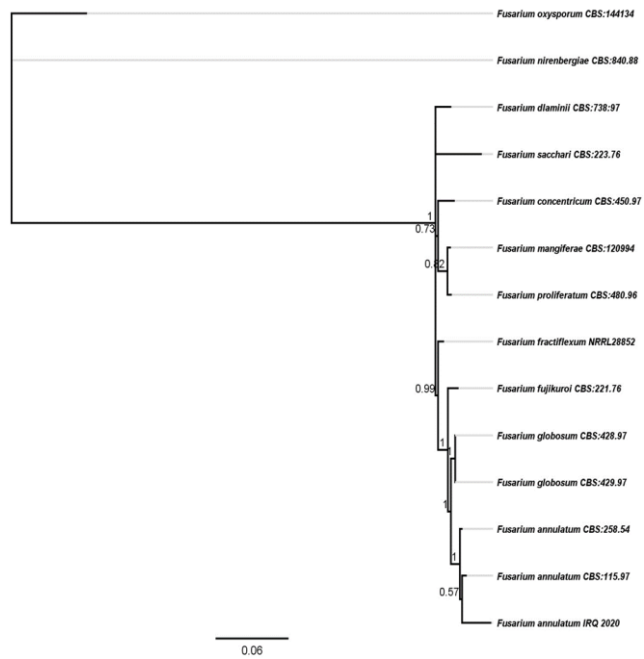

Figure 4. Bayesian phylogenetic tree for *Fusarium annulatum* and related Fusarium taxa inferred from combined analysis of ITS, TEF1-α, TUB2, and ACTIN of 14 collections. The nodes display Bayesian posterior probability values. Two outgroup species, *F. nirenbergiae* and *F. oxysporum*, are the roots of the tree. The bar represented the number of nucleotide changes per location.

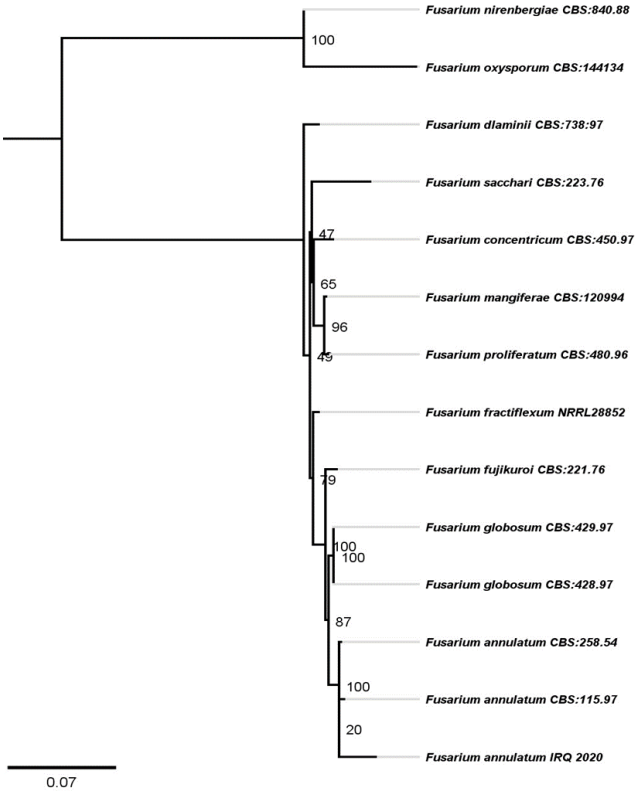

Figure 5. Maximum likelihood (ML) phylogenic tree for *Fusarium annulatum* and related Fusarium taxa inferred from joined analysis of ITS, TEF1-α, TUB2, and ACTIN of 14 collections. The nodes display Bayesian posterior probability values. Two outgroup species, *F. nirenbergiae* and *F. oxysporum*, are the roots of the tree. The bar represented the number of nucleotide changes per location.

Therefore, in *Fusarium* taxonomy, morphological species concepts are considered as being untrustworthy at the species level (Al-Hatmi *et al.*, 2016).

Based on multigene phylogeny, the *F. annulatum* isolate was clustered with the same species isolates and separated from other *Fusarium* species (Figures 4 and 5). In addition, the current isolates were phylogenetically distinct from *F. proliferatum.* In the past, *F. annulatum* was extensively studied under that previous name. Therefore, this is the first report from Iraq which revealed that *F. annulatum* is associated with wheat in Iraq using three-gene phylogenetic analysis for *Fusarium* species delimitation.

Geographically, *F. annulatum* is commonly isolated from temperate and tropical regions (Domsch *et al.*, 2007), on diverse host plant species with more than 200 reported so far (Yilmaz *et al.*, 2021). This species has been described previously under the name, *F. proliferatum*, separate from *F. moniliforme* isolates (Seifert *et al.*, 2003). The morphological description in the current study is somewhat similar to previously reported research (Yilmaz *et al.*, 2021). However, the sporodochial conidia in the present study were straight macroconidia. which are different from has been reported earlier (Yilmaz *et al.*, 2021), by having strongly curved macroconidia. This observation is in agreement to what has been observed by Nelson *et al.* (1983). Some dissimilarities between reported isolates may be related to distinct environmental conditions between regions or different infected host plants. Furthermore, this fungus has been reported earlier to be associated with young vine decline disease in California (Bustamante, *et al.*, 2022).

Although many *Fusarium* species have already been isolated from wheat in Iraq using molecular identification with a single gene database, this is the first report of *F. annulatum* isolates causing root rot disease of bread wheat in Iraq. In both Al-Hwuir and Al-Qurna regions of Basra province, Iraq, two species, *F. falciforme* and *F. brachygibbosum*, have been recovered earlier from wheat roots and their rhizosphere in wheat fields (Mohammed-Ameen *et al.*, 2021). Another work revealed that nine *Fusarium* species (*F. solani, F. pseudograminearum, F. culmorum, F. cerealism F. nygamai, F. avenaceum, F. graminearum, F. equiseti*, and *F. chlamydosporum*) were associated with wheat in Basra causing varied levels of *Fusarium* root rot disease severity (Minati, 2020).

It can be concluded from this study that genealogical phylogenetic analyses of a three-gene dataset powerfully supported the delimitation and clustering of *F. annulatum* isolates, which were allocated to a distinct phylogenetic clade and identified as a wheat pathogen in Iraq. Therefore, phylogenetic species concept is a robust tool used for *Fusarium* species Taxonomy.

الملخص

الشويلي، فاخر رحيم حميد، حسين علي تمر، زينب مسلم عباس، منتظر قاسم جودي ورجاء عبد الرزاق عباس العنبكي. 2025. التشخيص الجزيئي والتسجيل الأول للفطر *Fusarium annulatum* كمسبب لمرض تعفن جذور القمح في العراق باستخدام تحليل الأصل التطوري متعدد الجين. مجلة وقاية النبات العربية، 43(1):62−68. https://doi.org/10.22268/AJPP-001296

تعدّ أنواع الفطر *Fusarium* spp. من المسببات المرضية المهمة للإنسان والحيوان والنبات، ويتم سنوياً تسجيل أنواع جديدة من الفطر في أماكن مختلفة من العالم. في هذه الدراسة، لوحظ وجود إصابة حقلية شديدة بمرض ذبول القمح في شهر كانون الثاني/يناير لعام 2021 في حقول القمح التابعة لمحطة المرادية في محافظة بابل، العراق، كما لوحظت الأعراض على الجذور كتلون بني وتعفن. تمّ إجراء عزل ووصف مظهري وإثبات القدرة الإمراضية وتحديد تسلسل القواعد النيتروجينية للمناطق الجينية ITS، TEF1-α، TUB2 و ACT وتحليل الأصل التطوري باستخدام الجينات المتعددة. أكدت نتائج أصل شجرة القرابة التطوري للفطر *Fusarium annulatum* باستخدام Bayesian and Maximum Likelihood Phylogenetic Analysis بالإضافة إلى صفاته المظهرية تشخيص الفطر بأنه النوع المسبب لمرض تعفن جذور الحنطة لأول مرة في العراق. إلا أن تحليل الأصل التطوري و Maximum Likelihood اعتماداً على تسلسل قواعد المنطقة الجينية ITS

وبيانات الـ ITS فقط غير كافية لتمييز هذا النوع بين أنواع جنس *Fusarium* الأخرى. ولكنّ نتائج تحليل الأصل التطوري لـ Bayesian و Maximum Likelihood اعتماداً على نمط translation elongation factor 1-alpha (TEF1- α) أو تحليل الأصل التطوري متعدد الجينات أثبت أن عزلات الـ *F. annulatum* انضمت إلى عنقود مميز للنوع ينتمي إلى جنس الفطر *Fusarium*. يعدّ هذا البحث أول تشخيص جزيئي باستخدام تحليل الأصل التطوري لأربعة جينات للفطر *F. annulatum*، وأول تسجيل له كمسبب لمرض تعفن جذور القمح في العراق.

كلمات مفتاحية: المرض الفطري، *Triticum aestivum*، تحليل الأصل التطوري متعدد الجين، *Fusarium*، المرض المنقول في التربة.

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References

Al-Hatmi, A.M.S., F. Hagen, S.B.J. Menken, J.F. Meis and G.S. de Hoog. 2016. Global molecular epidemiology and genetic diversity of *Fusarium*, a significant emerging group of human opportunists from 1958 to2015. Emerging Microbes and Infections, 5(12):e124. https://doi.org/10.1038/emi.2016.126

Al-Maaroof, E.M and K.H. Rahim. 2024. Molecular and physiological characterization of *Fusarium oxysporum ciceri* isolates from different chickpea area in IKR, IRAQ. Iraqi Journal of Agricultural Sciences, 55:1-13.

Al-Maaroof, E.M. 2022. Wheat yellow rust in Iraq – current status and future challenges; Pp. 39-48. *In:* M. Li, S. Ali, E. Al-Maaroof, B. Mahto, A. Moghdam and M. Gad (eds.), Wheat Stripe Rust in the Middle East and the Extended Himalayan Regions, The China Agriculture Press. China.

Andjic, V., A. Maxwell, G.E.S. Hardy and T.I. Burgess. 2016. New cryptic species of Teratosphaeria on Eucalyptus in Australia. IMA Fungus, 7(2):253-263. https://doi.org/10.5598/imafungus.2016.07.02.05

Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute Kew, Surrey, England. No. 237.

Bugnicourt, F. 1952. Une espèce fusarienne nouvelle, parasite du riz. Revue Génerale de Botanique, 59:13–18

Bustamante, M.I., K. Elfar, R.J. Smith, L.J. Bettiga, T. Tian, G.A. Torres and A. Eskalen. 2022. First report of *Fusarium annulatum* associated with young vine decline in California. Plant Disease,106(10):2752. https://doi.org/10.1094/PDIS-12-21-2790-PDN

Darriba, D., G. L.Taboada, R. Doallo and D. Posada .2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods, 9(8):772. https://doi.org/10.1038/nmeth.2109

Domsch K.H., W. Gams and T.H. Anderson. 2007. Compendium of Soil Fungi. Second edition. IHW-Verlag, Eching. 672 pp.

FAO. 2022. Crop Prospects and Food Situation – Quarterly Global Report No. 1, March 2022. Rome. https://doi.org/10.4060/cb8893en

Gerlach, W. and H. Nirenberg. 1982. The genus *Fusariurn* - A pictorial atlas. Mitt. Biol. Bundesanst. Land- u, Forstwirtsch. Berlin-Dahlem, 209:1-406.

Glass, N.L. and G.C. Donaldson. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied Environmental Microbiology, 61(4):1323-1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Hameed, F. 2011. Biocontrol of damping off and induced resistance of eggplant cultivars by *Trichoderma harzianum*. Kufa Journal of Agricultural Sciences, 3(1):355-360.

Hameed, M.A., R.M. Rana and Z. Ali. 2012. Identification and characterization of a novel Iraqi isolate of *Fusarium pseudograminearum* causing crown rot in wheat. Genetic and Molecular Research, 11(2):1341-1348. https://doi.org/10.4238/2012.may.15.4

Huelsenbeck, J.P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, 17(8):754-755. https://doi.org/10.1093/bioinformatics/17.8.754

Jacobs-Venter, A., I. Laraba, D.M. Geiser, M. Busman, M.M. Vaughan, R.H. Proctor, S.P. McCormick and K. O'Donnell. 2018. Molecular systematics of two sister clades, the *Fusarium concolor* and *F. babinda* species complexes and the discovery of a novel microcycle macroconidium-producing species from South Africa. Mycologia, 110(6):1189-1204. https://doi.org/10.1080/00275514.2018.1526619

Katoh, K. and D.M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution, 30(4):772-780. https://doi.org/10.1093/molbev/mst010

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, A. Buxton, S. Cooper, Markowitz, C. Duran and T. Thierer. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12):1647-1649. https://doi.org/10.1093/bioinformatics/bts199

Khaeim, H.M., A. Clark, T. Pearson and D. van Sanford. 2019. Methods of assessing *Fusarium* damage to wheat kernels. Al-Qadisiyah Journal for Agricultural Sciences, 9(2):297-308. https://doi.org/10.33794/qjas.Vol9.Iss2.91

Matny, O.N., S. Chakraborty, F. Obanar and R.A. AL-Ani. 2012. Molecular identification of *Fusarium* spp. causing crown rot and head blight on winter wheat in Iraq. Journal of Agricultural Technology, 8(5):1677-1690. https://doi.org/10.13140/RG.2.2.26012.18565

Matny, O.N., S. T. Bates and Z. Song. 2017. Geographic distribution of *Fusarium culmorum* chemotypes associated with wheat crown rot in Iraq. Journal of Plant Protection Research, 57(1):43-49. https://doi.org/10.1515/jppr-2017-0006

Matny, O.N., S.T. Bates, S.A. Shamsallah and Z. Song. 2018. Mating type characterization of *Fusarium culmorum* strains causing wheat crown rot in Iraq. Pakistan Journal of Phytopathology, 30(2):109-114.

Minati, M.H. and M.K. Mohammed-Ameen. 2019a. Interaction between Fusarium head blight and crown rot disease incidence and cultural practices on wheat in the south of Iraq, Basra province. Bulletin of the National Research Centre, 43(1):1-11. https://doi.org/10.1186/s42269-019-0257-9

Minati, M.H. and M.K. Mohammed-Ameen. 2019b. Novel report on six Fusarium species associated with head blight and crown rot of wheat in Basra province, Iraq. Bulletin of the National Research Centre, 43:139. https://doi.org/10.1186/s42269-019-0173-z

Minati, M.H. 2020. First record of nine Fusarium spp. causing root rot on wheat (*Triticum aestivum* L.) in Iraq. AIP Conference Proceedings, 2290(1):020009. https://doi.org/10.1063/5.0027398

Mohammed-Ameen, M.K., M.H. Minati and M. Abbas. 2021. Morphogenetic identification, description and pathogenicity of novel pathogens on Iraqi wheat plant (*Triticum aestivum*) causing head blight and crown rot diseases. Biodiversitas Journal of Biological Diversity, 22(5):2999-3005.

Nelson P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: An illustrated manual for identification. Pennsylvania State University Press, University Park, PA.

Park, B. 2013. Cyber-infrastructure supporting fungal and oomycete phylogenetics and genomics. Ph.D. Dissertation, Pennsylvania State University. 91 pp.

Seifert K.A., T. Aoki, R.P. Baayen, D. Brayford, L.W. Burgess, S. Chulze, W. Gams, D. Geiser, J. De Gruyter, J.F. Leslie and A. Logrieco. 2003. The name *Fusarium moniliforme* should no longer be used-differential use of Termitomyces by termites. Mycological Research, 107(6):643-644. https://doi.org/10.1017/S095375620323820X

Soares, D.A., D.P. de Oliveira, T.T. Dos Santos, P.G. Marson and R.S. Pimenta. 2018. Multiloci identification of *Diaporthe* fungi isolated from the medicinal plant *Costus spiralis* (Jacq.) Roscoe (Costaceae). Journal of applied Microbiology, 125(1):172-180. https://doi.org/10.1111/jam.13769

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22(21):2688-2690. https://doi.org/10.1093/bioinformatics/btl446

Taylor J.W., D.J. Jacobson, S. Kroken, T. Kasuga, D.M. Geiser, D.S. Hibbett and M.C. Fisher. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology, 31(1):21-31. https://doi.org/10.1006/fgbi.2000.1228

Udayanga, D., X.Z. Liu, P.W. Crous, E.H.C. McKenzie, E. Chukeatirote and K.D. Hyde. 2012. A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). Fungal Diversity, 56(1):157-171. https://doi.org/10.1007/s13225-012-0190-9

Wu, F. 2007. Measuring the economic impacts of Fusarium toxins in animal feeds. Animal Feed Science and Technology, 137(3-4):363-374. https://doi.org/10.1016/j.anifeedsci.2007.06.010

Yilmaz N., M. Sandoval-Denis, L. Lombard, C.M. Visagie, B.D. Wingfield and P.W. Crous. 2021. Redefining species limits in the *Fusarium fujikuroi* species complex. Persoonia, 46(1):129-162. https://doi.org/10.3767/persoonia.2021.46.05

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