Iraqi Journal of Science, 2026, Vol. 67, No. 5, pp: 2565-2574
DOI: 10.24996/ijs.2026.67.5.5

ISSN: 0067-2904

Genetic Variation and Phylogenetic Analysis of *Fusarium* Species Based on Sequencing of Internal Transcribed Spacer (ITS) Region

Sinai W. Mohammed
Tropical Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq

Received: 23/9/2024 Accepted: 5/5/2025 Published: 30/5/2026

Abstract

The Internal Transcribed Spacer (ITS) region is widely used for fungal identification in complex biological specimens. *Fusarium* is an opportunistic fungus responsible for a range of diseases in both plants and humans. This study aimed to identify *Fusarium* species at the species level and investigate their genetic variation. The ITS region of eighteen isolates was amplified by universal ITS primers (ITS1 and ITS4) using PCR. The PCR products of the ITS regions with a molecular weight of 588 bp were sent for sequencing, and then analyzed the sequence results by blast in the National Centre Biotechnology Information (NCBI) online to detect genetic variation in ITS regions. Phylogenetic analysis was compared with NCBI's reference sequences using MEGA X software. According to the NCBI GenBank database, four *Fusarium* species were first isolated in Iraq: *F. pseudoanthophilum, F. fujikuroi, F. luffae*, and *F. boothii*. Eleven isolates were identified as *F. pseudoanthophilum* (NR_163682.1) from the USA, with identities ranging from 98% to 100%. They were assigned the accession numbers MW577709-MW577719. Four isolates were identified as *F. fujikuroi* (NR_111889.1) from the USA, with identities ranging from 99% to 100%. They were assigned the accession numbers MW577720-MW577723. Two isolates were identified as *F. luffae* (NR_164594.1) from China, with an identity of 99%. They were assigned the accession numbers MW577724-MW577725. One isolate was identified as *F. boothii* (NR_121203.1) from the USA, with an identity of 99%. It was assigned the accession number MT658128.1. Thirty-five substitutions were detected by Bioedite software; most of them are deletions, accounting for 51.43%, followed by transitions, 31.43%, and transversions, 11.43%. These substitutions are found in most of the *Fusarium* spp—isolates, except for two isolates, which had no substitutions. The novelty of the present study lies in the first isolation of four *Fusarium* species in Iraq: *F. pseudoanthophilum, F. fujikuroi, F. luffae*, and *F. boothii*.

Keywords: Genetic diversity, *Fusarium spp*., fungal pathogens, phylogenetic tree, point substitutions, Sanger sequencing

التباين الجيني والتحليل التطوري لأنواع الفيوزاريوم بناءً على تسلسل منطقة المباعد الداخلي المنسوخ (ITS)

سيناء وليد محمد

وحدة الابحاث البايولوجية، كلية العلوم، جامعة بغداد، العراق

*Email: sinaiwaleed47@uobaghdad.edu.iq

الخلاصة

منطقة المباعد الداخلي المنسوخ (ITS) تُستخدم على نطاق واسع لتشخيص الفطريات في العينات البيولوجية المعقدة. يُعتبر فطر *Fusarium* من الفطريات الانتهازية المسؤولة عن مجموعة من الأمراض في النباتات والبشر. هدفت هذه الدراسة إلى تشخيص اجناس *Fusarium* الى مستوى النوع والتحري عن التنوع الجيني بينها. حيث تم تضخيم منطقة ITS لثمانية عشرعزلة باستخدام بادئات ITS1 وITS4 بواسطة تفاعل البلمرة المتسلسل (PCR). وارسلت نواتج PCR لمنطقة ITS ذات وزن جزيئي يبلغ 588 زوج قاعدة أساس ، و تم تحليل نتائج التسلسل عن طريق المركز الوطني لمعلومات التكنولوجيا الحيوية (NCBI) عبر الموقع الالكتروني للكشف عن التباين الوراثي في مناطق ITS. تم إجراء تحليل التطور الوراثي ومقارنته بالتسلسلات المرجعية لـ NCBI باستخدام برنامج MEGA X. وكشفت النتائج عن عزل أربعة أنواع من الفيوزاريوم لأول مرة في العراق وفقًا لقاعدة بيانات NCBI GenBank: *F. pseudoanthophilum* ، *F. fujikuroi*، *F. luffae* و *F. boothii*. شخصت إحدى عشرة عزلة تعود للفطر *F. pseudoanthophilum* (NR_163682.1) من الولايات المتحدة الأمريكية، وتتراوح نسبة التطابق بين 98% إلى 100%. وتم تسجيلهم على موقعNCBI GenBank بالارقام:MW577709–MW577719. كذلك شخصت أربع عزلات للفطر *F. fujikuroi* (NR_111889.1) من الولايات المتحدة الأمريكية، وتتراوح نسبة التطابق بين 99% إلى 100%. تم تسجيلها تحـــت الأرقام MW577720–MW577723، وشخصت عزلتين تعود للفطر *F. luffae* (NR_164594.1) من الصين، بهوية 99%. وسجلت بالأرقام: MW577724– MW577725. وشخصت عزلة واحدة تـعود للفــطر *F. boothii* (NR_121203.1) من الولايات المتحدة الأمريكية، بنسبة تطابق 99%. تحت رقم MT658128.1. تم اكتشاف سبعة وثلاثين طفرة باستخدام برنامج Bioedit معظمها عبارة عن طفرة الحذف بنسبة 51.43%، يليها طفرة تبادل بنسبة 31.43%، وطفرة الانقلاب بنسبة 11.43%. توجد هذه الطفرات في معظم عزلات الفطر فيوزاريوم ، باستثناء عزلتين كانت خالية من الطفرات. تعد الدراسة الاولى في عزل اربع انواع من *Fusarium* في العراق: *F. pseudoanthophilum, F. fujikuroi, F. luffae F. boothii*

1. Introduction

Fusarium is a genus of filamentous fungi from the class Sordariomycetes, the order Hypocreales, and the family Nectriaceae. *Fusarium* species are widely distributed in soil and associated with plants, causing various diseases that lead to economic losses, such as crown rot, root rot, and vascular wilt [1-3]. They also produce harmful toxins such as zearalenone (ZEA), trichothecenes (TCH), and fumonisins (FBs) that contaminate food and feed and affect human and animal health, causing several diseases, for example, esophageal cancer, liver cancers, neural tube defects (NTD), equine leukoencephalomalacia(ELEA), and porcine pulmonary edema (PPE) [4].

Most conventional methods for identifying fungi require information on colony morphology, microscopic characteristics, and biochemical tests. These methods are time-consuming and necessitate expert staff to identify fungi at the species level, depending on mycological keys and culture techniques. Identifying *Fusarium* species is a complex task due to their morphological diversity. This genus includes numerous closely related species, requiring more accurate morphological methods. Because of this, molecular methods like the intergenic spacer (IGS) region, the internal transcribed spacer (ITS), the beta-tubulin (β-tub), and the translation elongation factor-alpha (TEF) were needed to confirm the identification [5-7].

Recent studies have used the ITS region for fungal identification in complex biological specimens, including plants and the human body [8, 9]. It can also be used to build a

phylogenetic tree and demonstrate evolutionary relationships and genetic diversity among fungi. The ITS region is the widely used DNA sequence for fungal identification and analysis because of a rapid and authoritative method [10,11]. Therefore, this study aimed to identify *Fusarium* species at the species level and investigate their genetic variation.

2. Materials and Methods

2.1 Isolation of Fusarium spp.

Thirty fresh corn samples, each weighing 1 kg, were collected randomly from various local markets in Baghdad city. The surface sterilization of the corn grains with a 2% NaOCl solution for one minute is a commonly used method to eliminate external contaminants that could interfere with the isolation of *Fusarium* spp. The subsequent washing with distilled water and drying at room temperature helps ensure the grains are adequately prepared for culturing[11]. After that, ten grains were randomly selected and cultured on Malachite Green Agar 2.5 ppm plates (MGA 2.5 ppm), which was chosen as a selective medium for *Fusarium* spp. and inhibited the growth of other saprophytic fungi due to the presence of malachite green dye. Plates were incubated at 25°C for seven days. According to colony morphology characteristics, pigment production on Potato Dextrose Agar (PDA), and microscopic characteristics of microconidia and macroconidia by scotch tape preparation and slide culture technique, eighteen distinct isolates of *Fusarium* spp. were identified [12].

2.2 PCR and Sequencing Analysis

The DNA of *Fusarium* spp. was extracted from seven-day-old colonies cultured on Potato Dextrose Agar (PDA) using the extraction kit Z.R. Fungal/Bacterial DNA MiniPrepTM (Zymo/USA), following the manufacturer's instructions. About 50 ng of DNA was obtained from each colony. The amplification of the ITS region is done by PCR assay, which is a well-established technique for identifying fungal species, using primer sets ITS1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3'. The PCR reaction was done as follows: initial denaturation (95°C for 5 min), followed by 30 cycles of denaturation (95°C for 30 sec), annealing (55°C for 30 sec), extension (72°C for 30 sec), and final extension (72°C for 7 min). The amplicons were detected using electrophoresis on 1% agarose gel and examined under UV light [9]. The expected size of the amplicons was about 588 bp. DNA products were purified and quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific/USA). Only DNA products with high purity (A260/A280 ratio >1.8) and concentration (>50 ng/µl) were sent to Sanger sequencing using ABI3730XL automated DNA sequences (by Acrogens Corporation, Korea). Homology searches were conducted using the BLAST program, which is available online at NCBI, against the GenBank nucleotide database.

This study used two software: Bioedite software was used to detect substitutions among isolates sequences against their NCBI references, and MEGA X software was used to perform evolutionary analyses of the *Fusarium* species under investigation. The sequences were aligned using the ClustalW algorithm, and a phylogenetic tree was constructed using the UPGMA method based on the Jukes-Cantor method. Bootstrap analysis with 1000 replicates was performed to assess the reliability of the tree branches[13].

3. Results and Discussion

This study identified eighteen isolates belonging to the *Fusarium* fungi (coded as Fus1-Fus18) based on their colony morphology, micro- and macroconidia, to the genus level. This genus is considered complex due to its many closely related species. Therefore, identification at the species level was based on molecular methods using the ITS region (ITS1 &ITS2) with

a band size of 588 base pairs (bp) (Figure 1). The conventional methods used for fungal identification require much information, such as colony morphology, microscopic characteristics, and biochemical tests. Depending on mycological keys and culture methods, these methods are time-consuming and require expert staff to identify these fungi at the species level [14]. The results revealed four *Fusarium* species that were first isolated in Iraq according to the NCBI GenBank database. Eleven isolates (Fus1, Fus 3, Fus 6, Fus7, Fus 8, Fus 9, Fus11, Fus13, Fus16, Fus17, Fus18) were identified as *F. pseudoanthophilum* (NR_163682.1) from the USA with identity ranging from 98% to 100%. They were assigned the accession numbers MW577709-MW577719. Four isolates (Fus 4, Fus 5, Fus14, Fus15) were identified as *F. fujikuroi* (NR_111889.1) from the USA, with identities ranging from 99% to 100%. They were assigned the accession numbers MW577720-MW577723. Two isolates (Fus 2, Fus12) were identified as *F. luffae* (NR_164594.1) from China, with an identity of 99%. They were assigned the accession numbers MW577724-MW577725. One isolate (Fus10) was identified as *F. boothii* (NR_121203.1) from the USA, with an identity of 99%. It was assigned the accession number MT658128.1 (Table 1).

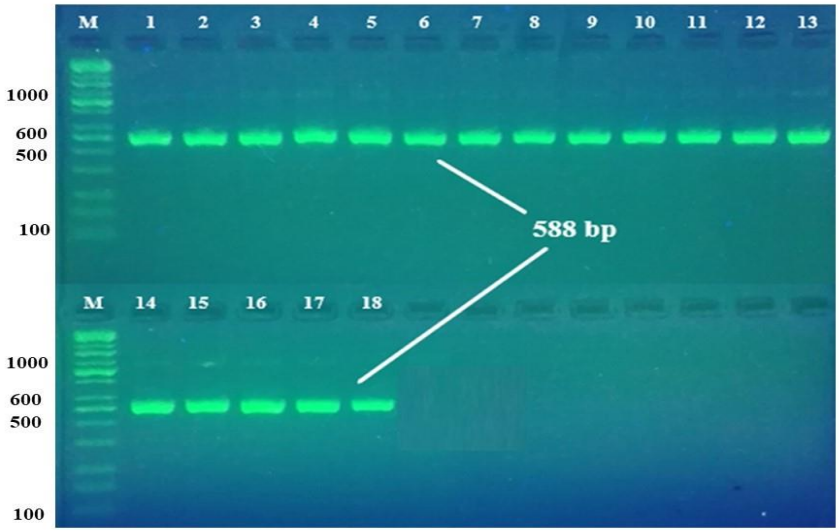

Figure 1: The amplified ITS region (588 bp) of 18 different samples on 1% agarose stained with SYBR green and run at 5 volts/cm² for one hour. M: DNA ladder (100bp), lanes 1–18 show DNA from the fungi isolates.

A comparative study was done between molecular methods depending on ITS sequences and traditional techniques for mycotic keratitis identification. PCR showed greater sensitivity and specificity, detecting fungal pathogens in 64.4% of cases compared to 40.67% identified by culture. Additionally, PCR discovered fungal species that culture methods failed to detect. Therefore, rapid identification of fungi using the PCR technique can lead to early treatment [15]. Internal transcribed spacer (ITS) region was widely used to identify new species of pathogenic fungi as a DNA barcoding marker since it has a conserved region and a high degree of variability between species[16]. ITS1 and ITS4 were valuable tools for identifying *Fusarium* isolates at the species level in this study. This finding aligns with the results of other studies [9,17-19] regarding the presence of fungi in wastewater, tomatoes, rice, maize, and wheat. These studies suggest that ITS sequencing is a powerful identification tool.

Table 1: The *Fusarium* isolates identified according to the results of a BLAST search on the GenBank database (NCBI)

Isolates	Accession number in GenBank	Closest species in GenBank database	Identity%	Country	Isolates Accession No.
Fus 1	NR_163682.1	*F. pseudoanthophilum*	99.38	USA	MW577709
Fus 3	NR_163682.1	*F. pseudoanthophilum*	99.79	USA	MW577710
Fus 6	NR_163682.1	*F. pseudoanthophilum*	99.79	USA	MW577711
Fus 7	NR_163682.1	*F. pseudoanthophilum*	99.79	USA	MW577712
Fus 8	NR_163682.1	*F. pseudoanthophilum*	99.79	USA	MW577713
Fus 9	NR_163682.1	*F. pseudoanthophilum*	99.79	USA	MW577714
Fus 11	NR_163682.1	*F. pseudoanthophilum*	99.79	USA	MW577715
Fus 13	NR_163682.1	*F. pseudoanthophilum*	100	USA	MW577716
Fus 16	NR_163682.1	*F. pseudoanthophilum*	99.59	USA	MW577717
Fus 17	NR_163682.1	*F. pseudoanthophilum*	99.19	USA	MW577718
Fus 18	NR_163682.1	*F. pseudoanthophilum*	98.97	USA	MW577719
Fus 4	NR_111889.1	*F. fujikuroi*	100	USA	MW577720
Fus 5	NR_111889.1	*F. fujikuroi*	99.40	USA	MW577721
Fus14	NR_111889.1	*F. fujikuroi*	99.41	USA	MW577722
Fus15	NR_111889.1	*F. fujikuroi*	99.41	USA	MW577723
Fus 2	NR_164594.1	*F. luffae*	99.79	China	MW577724
Fus 12	NR_164594.1	*F. luffae*	99.79	China	MW577725
Fus 10	NR_121203.1	*F. boothii*	99.19	USA	MT658128.1

The type and location of substitutions in ITS sequences among *Fusarium spp.* isolated from corn samples, were analyzed. Figure 2A illustrates that there are thirty-five substitutions in total, most of them are deletions, accounting for 51.43% (18/35), followed by transitions, 31.43% (11/35), and transversions, 11.43% (4/35). Additionally, the highest frequencies of base substitution types were T/C substitutions occurred ten times out of 35 (28.57%), A/T and C/G substitutions occurred twice (5.71 %), and A/G substitution occurred once (2.86%) (Figure 3). However, two isolates (Fus 4, Fus13) did not exhibit any substitutions and were identified as *F. fujikuroi* and *F. pseudoanthophilum* (Figure 2B). Notably, Fus18 had the highest number of substitutions, with five (15.15%) in total. Additionally, Fus17 had four substitutions (12.12%), while Fus 5, Fus10, Fus14, and Fus15 each had three substitutions (9.09%), and Fus1 and Fus16 had two substitutions (6.06%) each. The remaining isolates Fus 2, Fus 3, Fus 6, Fus7, Fus 8, Fus 9, Fus11, and Fus12, each had one substitution (3.03%), as shown in Figure 3.

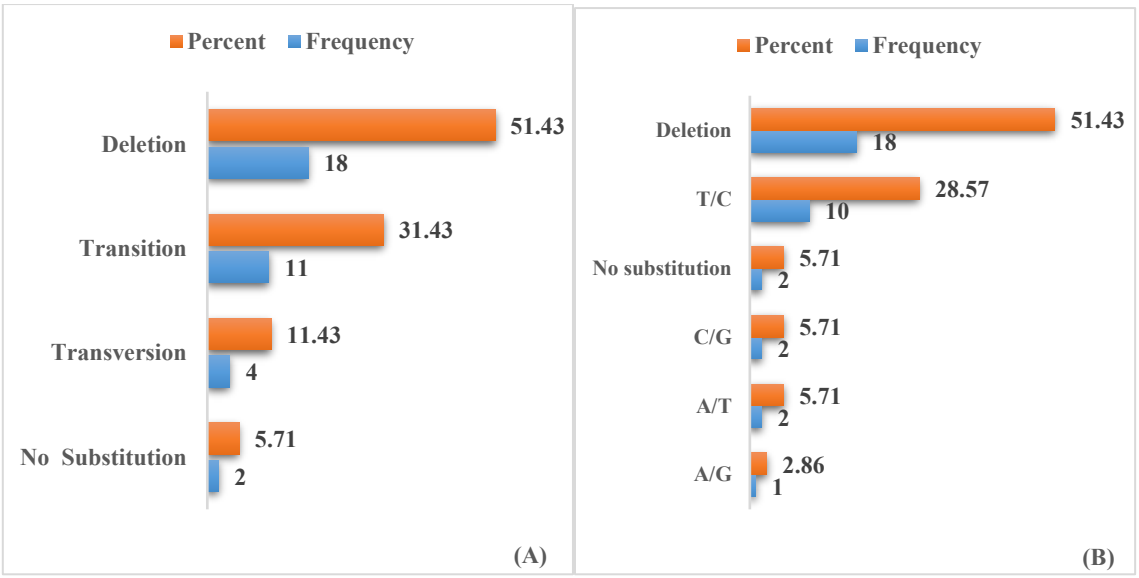

Figure 2: Frequency and percentage of point substitutions in ITS1 sequences of all isolates in this study compared to reference isolates in the GenBank database (NCBI): *F. boothii, F. Fujikuroi, F. luffae,* and *F. pseudoanthophilum.* **(A)** types of substitutions, **(B)** Bases substitutions

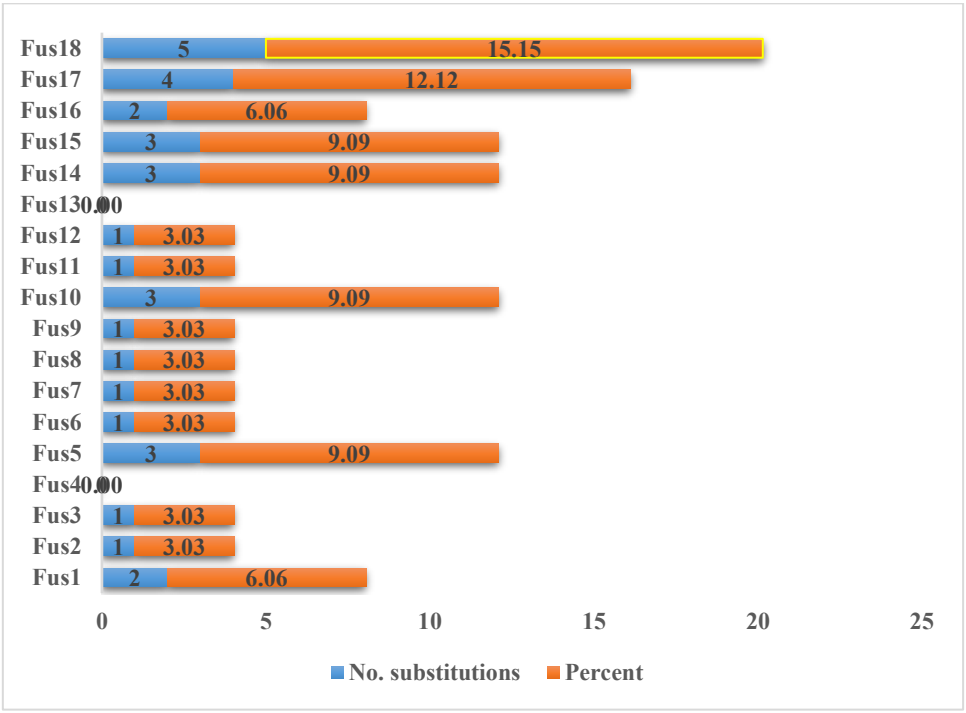

Figure 3: Number and percentage of substitutions in ITS1 sequences in each isolate in this study compared to reference isolates in the GenBank database (NCBI): *F. boothii*, *F. Fujikuroi, F. luffae*, and *F. pseudoanthophilum.*

Figure 4 shows a heatmap that visualizes each isolate's substitution types (transitions, transversions, deletions). The color intensity represents the frequency of each substitution type, providing an easy way to identify hotspots and patterns. The isolates Fus 5, Fus10, Fus14, Fus15, and Fus17 represent hotspots for deletions, while the other isolates appear less

prone to this type of substitution. These five isolates exhibit more deletions, possibly due to environmental stress or genetic predispositions [2].
Few studies have investigated the presence of point substitutions in the ITS region belonging to fungi. Al-Temimay and Aswad studied the substitutions in the ITS regions of six *Cryptococcus neoformans* isolates from clinical samples, they found 38-point substitutions (13 transversions and 25 transitions) and no deletion nucleotides in six *C. neoformans* isolates relative to the reference isolate from GenBank (No.: KJ175191.1)[20]. Also, Jarjees *et al.,* studied polymorphism and substitution in the ITS region of a local *Abortiporus biennis* isolate from Iraq and compared it with a reference isolate from GenBank (No. KP135300.1) [21]. They analyzed the sequence result by blasting it online in the NCBI and found nine-point substitutions in different sites relative to the reference isolate. Most of the substitutions were transitions, where another purine replaced a purine or a pyrimidine with another pyrimidine; only one substitution was a transversion, where a purine was replaced by a pyrimidine or vice versa.

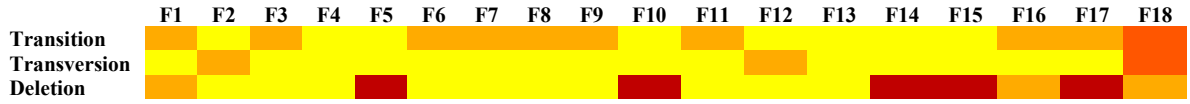

Figure 4: Heat map of point substitution types in each of the eighteen isolates. Yellow denotes no substitution, orange represents one substitution, deep orange indicates two substitutions, and red signifies three substitutions.

A UPGMA phylogenetic tree was constructed and analyzed to show the genetic relationships among different isolates of *Fusarium* spp. based on the sequences against NCBI's reference using MEGA X software. In Figure 5, there were two main clusters visible: the first cluster comprises eleven isolates (Fus1, Fus 3, Fus 6, Fus7, Fus 8, Fus 9, Fus11, Fus13, Fus16, Fus17, Fus18) showed 93% homology to the nucleotide sequence of *F. pseudoanthophilum,* USA (accession no.: NR_163682.1), and four isolates (Fus 4, Fus 5, Fus14, Fus15) showed 94% homology to the nucleotide sequence of *F. fujikuroi,* USA (accession no.: NR_111889.1). Two isolates (Fus 2, Fus12) presented 92% homology to the sequence of *F. luffae*, China (accession no.: NR_164594.1), the high similarity among these *Fusarium* spp. make it difficult to distinguish between them relying on traditional methods [5]. The second cluster includes one isolate (Fus10) that showed 92% homology to the sequence of *F. boothii,* USA (accession no.: NR_121203.1), and *Candida albicans* represented as an outgroup. Studying phylogenetic trees and ITS sequencing can help us better understand the evolutionary relationships among different species. These relationships are based on the similarities and differences in their genetic characteristics, which can help in disease diagnosis and evolution.

The identification of four different species of *Fusarium* in Iraqi corn is important for agriculture because these fungi are known as plant pathogens and produce mycotoxins that are dangerous to food safety [3]. Knowing their genetic diversity is needed to develop management strategies, like effective fungicide treatments or resistant crop varieties. The presence of these species also raises concerns for public health because mycotoxins could get into food and animal feed [22, 23]. The limitation of this study was limited by the small sample size, the possible sequencing or alignment errors, and the need for reference sequences for some *Fusarium* species.

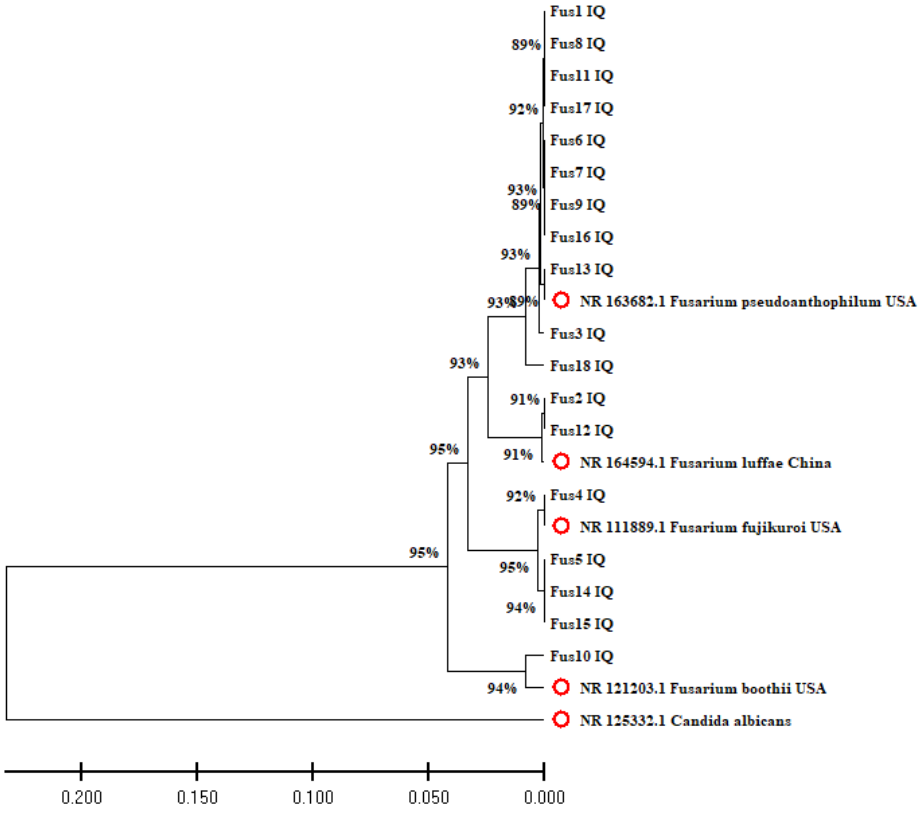

Figure 5: UPGMA phylogenetic tree of *Fusarium* based on ITS1 sequences, constructed using MEGA 11 software. Bootstrap replicates (1000) were used to assess branch support. *Candida albicans* sequence was included as outgroups.

4. Conclusion

This study reports the first isolation of four *Fusarium* species in Iraq: *F. pseudoanthophilum, F. fujikuroi, F. luffae*, and *F. boothii*. A point substitution analysis in the ITS regions of these species revealed that each isolate possessed a unique ITS sequence with distinct substitutions. The most common substitution type was deletion, followed by transition and transversion. These findings highlight the genetic diversity and evolutionary dynamics of *Fusarium* species, which could aid in disease diagnosis and management strategies for agricultural pathogens. Future studies should expand the sample size and incorporate additional molecular markers to provide a more comprehensive understanding of *Fusarium* phylogeny and diversity.

Acknowledgements

The author thanks all staff at the Tropical Biological Research Unit, College of Science, University of Baghdad, for their assistance in completing this work in their labs.

Conflict of Interest

There are no discrepancies of interest.

References

[1] L. Zakaria, "Fusarium Species Associated with Diseases of Major Tropical Fruit Crops," *Horticulturae*, vol. 9, no. 3, pp. 1–32, 2023, doi: 10.3390/horticulturae9030322.

[2] D. A. Nikitin, E. A. Ivanova, M. V. Semenov, A. D. Zhelezova, N. A. Ksenofontova, A. K.

Tkhakakhova, and V. A. Kholodov, “Diversity, Ecological Characteristics and Identification of Some Problematic Phytopathogenic Fusarium in Soil: A Review,” *Diversity*, vol. 15, no. 1, pp. 1–18, 2023, doi: 10.3390/d15010049.

[3] L. Perincherry, J. Lalak-Kańczugowska, Ł. Stępień, J. Lalak-Kánczugowska, and L. Stepién, “Fusarium-produced mycotoxins in plant-pathogen interactions,” *Toxins*, vol. 11, no. 664, pp. 1–22, 2019, doi: 10.3390/toxins11110664.

[4] M. Bryła, A. Pierzgalski, A. Zapaśnik, P. A. Uwineza, E. Ksieniewicz-Woźniak, M. Modrzewska, and A. Waśkiewicz., "Recent Research on Fusarium Mycotoxins in Maize—A Review," *Food*, vol. 11, no. 21. p. 3465,2022. doi: 10.3390/foods11213465.

[5] S. Yadav, P. Kumari, S. Sharma, V. Kalra, M. Sharma, and B. Batra, “Advanced Molecular Techniques in the Identification of Phytopathogenic Fungi,” in *Molecular and Biotechnological Tools for Plant Disease Management*, J.-T. Chen, M. Khan, A. Parveen, and J. K. Patra, Eds. Singapore: Springer Nature Singapore, 2025, pp. 287–317. doi: 10.1007/978-981-97-7510-1_10.

[6] A. López-Moral, B. I. Antón-Domínguez, M. Lovera, O. Arquero, A. Trapero, and C. Agustí-Brisach, “Identification and pathogenicity of Fusarium species associated with wilting and crown rot in almond (Prunus dulcis),” *Scientific Reports*, vol. 14, no. 1, pp. 1–16, 2024, doi: 10.1038/s41598-024-56350-5.

[7] S. Madhavan, K. Sakthivel, R. V. Dantuluri, S. Tadigiri, U. S. Moturu, L. Muniyappa, P. G. Kawar, R. Pal, P. R. Kudupudi, and P. K. Venkataramana, "Characterization of Fusarium falciforme inciting wilt in peace lily (Spathiphyllum wallisii)," *Scientia Horticulturae*, vol. 339, p. 113834, Jan. 2025., doi: 10.1016/j.scienta.2024.113834.

[8] F. Jabbar, A. A. Al-Attraqchi, and D. Nabeal, “Saccharomyces Cerevisiae as a human pathogen in renal transplant recipients,” *International Conference on Scientific Research & Innovation (Icsri 2022)*, vol. 2820, p. 050017, 2023, doi: 10.1063/5.0150564.

[9] A. Mohammadi and S. Bahramikia, “Molecular identification and genetic variation of Alternaria species isolated from tomatoes using ITS1 sequencing and inter simple sequence repeat methods,” *Current Medical Mycology*, vol. 5, no. 2, pp. 1–8, 2019, doi: 10.18502/cmm.5.2.1154.

[10] Y. MAHMUT, A. M. HAMOODY, and R. S. ABDULQADER, “Morphological and molecular identification of fungi isolated from various habitat in Kirkuk city – Iraq,” *Eurasian Journal of Biological and Chemical Sciences*, vol. 6, no. 1, pp. 26–30, 2023, doi: 10.46239/ejbcs.1276554.

[11] V. D. Navale, A. M. Sawant, and K. R. Vamkudoth, “Genetic diversity of toxigenic Fusarium verticillioides associated with maize grains, India,” *Genetics and Molecular Biology*, vol. 46, no. 1, pp. 1–12, 2023, doi: 10.1590/1678-4685-gmb-2022-0073.

[12] J. Harish, P. P. Jambhulkar, R. Bajpai, M. Arya, P. K. Babele, S. K. Chaturvedi, A. Kumar, and D. K. Lakshman, "Morphological characterization, pathogenicity screening, and molecular identification of Fusarium spp. isolates causing post-flowering stalk rot in maize," *Frontiers in Microbiology*, vol. 14, p. 1121781, Mar. 2023., doi: 10.3389/fmicb.2023.1121781.

[13] K. Tamura, G. Stecher, and S. Kumar, “MEGA11: Molecular Evolutionary Genetics Analysis Version 11,” *Molecular Biology and Evolution*, vol. 38, no. 7, pp. 3022–3027, 2021, doi: 10.1093/molbev/msab120.

[14] K. W. Tarad, H. M. Saleh, and T. S. Rashid, “A survey, Pathogenicity assay, and Molecular Identification of the Pathogenic Fungi Associated with Pistachio in Anbar Province, Iraq,” *Basrah Journal of Agricultural Sciences*, vol. 36, no. 2, pp. 320–333, 2023, doi: 10.37077/25200860.2023.36.2.24.

[15] H. S. Behera and D. Srigyan, “Evaluation of Polymerase Chain Reaction over Routine Microbial Diagnosis for the Diagnosis of Fungal Keratitis,” *Optometry and Vision Science*, vol. 98, no. 3, pp. 280–284, 2021, doi: 10.1097/OPX.0000000000001652.

[16] M. J. Bradshaw, M. C. Aime, A. Rokas, A. Maust, S. Moparthi, K. Jellings, A. M. Pane, D. Hendricks, B. Pandey, Y. Li, and D. H. Pfister, "Extensive intragenomic variation in the internal transcribed spacer region of fungi," *iScience*, vol. 26, no. 8, 2023., doi: 10.1016/j.isci.2023.107317.

[17] L. Al Husnain and M. AlKahtani, “Molecular heterogeneity in the 18s DNA gene of Alternaria sp. and Fusarium sp. producing mycotoxins in rice and maize grains,” *Saudi Journal of Biological Sciences*, vol. 26, no. 2, pp. 368–372, 2019, doi: 10.1016/j.sjbs.2017.12.006.

[18] I. M. Singha, Y. Kakoty, B. G. Unni, J. Das, and M. C. Kalita, “Identification and

characterization of Fusarium sp. using ITS and RAPD causing fusarium wilt of tomato isolated from Assam, North East India," Journal of Genetic Engineering and Biotechnology, vol. 14, no. 1, pp. 99–105, 2016, doi: 10.1016/j.jgeb.2016.07.001.

[19] D. Gencheva and G. Beev, "Molecular identification of fusarium spp. Isolated from wheat grain based on sequencing of internal transcribed spacer (its) region," *Acta Microbiologica Bulgarica*, vol. 37, no. 1, pp. 27–33, 2021, doi: 10.20944/preprints202010.0426.v1.

[20] I. A. Al-Temimay and E. M. Aswad, "Identification of cryptococcus neoformans isolates by PCR-ITS regions," *Iraqi Journal of Science*, vol. 59, no. 4, pp. 1792–1805, 2018, doi: 10.24996/IJS.2018.59.4A.4.

[21] R. M. Jarjees, T. O. Al-khesraji, and S. Y. Abdul-hadi, "First recording and molecular identification of two basidiomycetous macrofungi from Mosul / Iraq," *Journal of Advanced Education and Sciences*, vol. 3, no. 1, pp. 1–7, 2023.

[22] A. H. Ibrahima, D. D. Taherb, O. S. Abbas, and D. A. Abdul-Shaheedb "Determination of some mycotoxins from poultry feed in Baghdad city," *Asia-Pacific Journal of Molecular Biology and Biotechnology*, vol. 32, no. 4, pp. 10–15, 2024. https://doi.org/10.35118/apjmbb.2024.032.4.02

[23] H. S. Alnaemi, T. N. Dawood, and Q. T. Algwari, "Investigation of Aflatoxin B1, Ochratoxin A, and Fumonisin B1 in Poultry Feeds in Nineveh Province," *Iraqi Journal of Veterinary Medicine*, vol. 47, no. 2, pp. 37–43, 2023, doi: 10.30539/ijvm.v47i2.1532.