OPEN ACCESS Plant Protection, 09 (03) 2025. 513-525 DOI:10.33804/pp.009.03.5697

Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

Characterization and Biocontrol Efficacy of Arbuscular Mycorrhizal Fungi from Wheat Roots against Root Rot Caused by *Fusarium culmorum*

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ARTICLE INFO

Article history
Received: 29th April, 2025
Revised: 27th July, 2025
Accepted: 31st July, 2025

Keywords
Arbuscular mycorrhizal fungi
Fusarium culmorum
Biological control
Molecular identification
Induced systemic resistance

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) play a crucial role in enhancing plant growth and resistance to soil-borne pathogens. In this study, five AMF species were isolated based on spore morphology and identified through ITS sequencing as *Acaulospora brasiliensis*, *Entrophospora claroidea*, *Funneliformis mosseae*, *Oehlia diaphana*, and *Rhizophagus intraradices*. The isolates were submitted to GenBank under accession numbers PP329939.1, PP331806.1, PP329940.1, PP329941.1, and PP329943.1, respectively. Mycorrhizal colonization efficiency and plant responses were assessed in five wheat cultivars under *Fusarium culmorum* infection. Colonization rates were highest in Sham 6 inoculated with *R. intraradices* (91.2%) and lowest in Abu Ghraib with *A. brasiliensis* (59.81%) under pathogen stress. Disease severity significantly declined in mycorrhiza-treated plants; Sham 6 inoculated with *R. intraradices* and *F. culmorum* exhibited the lowest severity (29.58%), while the highest (46.72%) was in Abu Ghraib treated with *A. brasiliensis*. Enzyme activities (peroxidase and polyphenol oxidase) were elevated in AMF-inoculated plants, especially in Sham 6 with *R. intraradices* under pathogenic stress (2.309 and 2.402 U/mL, respectively). AMF also improved growth parameters. Under infection, Sham 6 treated with *R. intraradices* showed the highest plant height (69.57 cm), shoot and root dry weights (5.75 g and 2.30 g), and chlorophyll content (34.51 SPAD). These findings suggest that *R. intraradices* is the most effective AMF species for promoting wheat growth and systemic resistance under *F. culmorum* stress. The isolated fungi also represent a new addition to the Iraqi mycoflora and hold potential for sustainable biological disease management.

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INTRODUCTION

Wheat (*Triticum aestivum* L.), a member of the Poaceae family, is one of the world's most important economic crops, cultivated globally due to its high nutritional value (William, 2023). Wheat yield is significantly impacted by root rot diseases, which are caused by a range of fungal pathogens, including *Fusarium culmorum*, *F. graminearum*, *Cephalosporium gramineum*, and *Bipolaris sorokiniana*. In the central and southern regions of Iraq, particularly under irrigated agricultural conditions, additional pathogens such as *Chaetomium elatum*, *C. globosum*, *Sclerotium rolfsii*, *Sclerotium* spp., *Rhizoctonia solani*, *Rhizoctonia* spp., and *Macrophomina phaseolina* have also been implicated in wheat root rot (Hadi et al., 2017).

Various chemical and biological strategies have been explored to manage wheat root rot. Among chemical fungicides, Carbendazim, Hexaconazole, Mancozeb, Tebuconazole, Propiconazole, Prochloraz, Benomyl, and Triadimenol have shown high efficacy in disease control (Shajari and Yahyaei, 2016). However, due to concerns over the environmental impact of chemical fungicides, recent research has focused on developing safer, eco-friendly alternatives. One such promising approach involves the use of biological control agents, which offer a sustainable and environmentally compatible means of disease management, particularly suitable for organic and sustainable agriculture. Suppressive soils can be established by inoculating them with biological agents that inhibit the growth of soilborne pathogens while simultaneously inducing systemic resistance in host plants (Liu et al., 2021).

Arbuscular mycorrhizal fungi (AMF), also referred to as vesicular-arbuscular mycorrhizae (VAM), form mutualistic symbiotic associations with the roots of most terrestrial plant species. AMF have been widely documented to enhance crop growth and productivity, improve tolerance to abiotic stresses and heavy metal toxicity, and provide protection against various fungal phytopathogens. Their plant growth-promoting effects are attributed to their ability to modify root exudation patterns, produce phenolic compounds, antibiotics, and other antagonistic metabolites, and stimulate host defense mechanisms (Hong et al., 2024).

Given the economic importance of wheat and the threats posed by root rot diseases, the present study was undertaken to isolate indigenous arbuscular mycorrhizal fungi associated with wheat roots and evaluate their potential efficacy in managing wheat root rot caused by *Fusarium culmorum*.

Materials and Methods

Laboratory experiment

Wheat seeds

Five Iraqi wheat cultivars, Sham-6, Buhouth, Abu Ghraib, Al-Ezz, and Ibaa-99, were used in this study. These cultivars were obtained from the Seed Technology Center, Directorate of Scientific Research, Iraq.

Pathogenic fungus

A highly virulent isolate of the pathogenic fungus *F. culmorum* was used. This isolate had been previously identified both morphologically and molecularly (GenBank accession number: PP320447.1), as reported by Ismail and Hassan (2024).

Isolation and identification of arbuscular mycorrhizal fungi

Spores of arbuscular mycorrhizal (AM) fungi were isolated using the wet sieving and decanting method, followed by centrifugation in a 50% sucrose solution, as briefly described by Huey et al. (2020). Healthy wheat plants were collected from various locations within Salah Al-Din Governorate, including Ishaqi, Dujail, Dhuluiya, Samarra, Al-Dour, Tikrit, Tuz Khurmatu, Al-Alam, Baiji, and Al-Sharqat.

For each soil sample, 100 g of rhizospheric soil were suspended in 1 L of sterile distilled water with continuous stirring. After allowing the suspension to settle for 1 h, the supernatant was decanted and passed through a series of sieves with mesh sizes of 750 µm, 250 µm, 150 µm, and 50 µm. Spores retained on each sieve were collected separately into Petri dishes and transferred to centrifuge tubes. A 50% sucrose solution was then added, and samples were centrifuged at 2000 rpm for 5 min. Spores from each density layer were collected using a Pasteur pipette, filtered through filter paper, and transferred to Petri dishes.

Preliminary identification to the genus level was conducted based on spore morphology using established taxonomic keys (Kehri et al., 2018). For species-level identification, molecular techniques were employed.

Molecular identification of AMF

Genomic DNA was extracted from ten spores of each AM fungal isolate using the ZR Fungal/Bacterial/Yeast DNA MiniPrep™ Kit (Zymo Research, USA). The internal transcribed spacer (ITS) region of the 5.8S rRNA gene was amplified by polymerase chain reaction (PCR) using the given below primers as described by White et al. (1990). Primers were synthesized by Integrated DNA Technologies (IDT, Canada).

Forward: 5′-TCCGTAGGTGAACCTGCGG-3′
Reverse: 5′-TCCTCCGCTTATTGATATGC-3′

PCR reactions were carried out in a 25 µl total volume containing 1.5 µl of template DNA, 5 µl of Taq PCR PreMix, 1 µl of each primer (10 pmol/µl), and sterile distilled water to complete the final volume. The amplification protocol consisted of 37 cycles with the following conditions:

Initial denaturation at 95°C for 5 min, denaturation at

95°C for 45 sec, annealing at 58°C for 45 sec, extension at 72°C for 45 sec, and final extension at 72°C for 7 min. PCR amplification was performed using a GeneAmp PCR System 9700 (Applied Biosystems, USA).

Amplified products were separated via 1.5% agarose gel electrophoresis and visualized under ultraviolet light (302 nm) after staining with Intron Korea RedSafe™ Nucleic Acid Staining Solution.

Nucleotide sequence analysis

Following successful PCR amplification of the ITS region within the 5.8S rRNA gene, 25 µl of PCR product and 10 µl (10 pmol) of each primer were submitted to the Biotechnology Laboratory Company in Korea for sequencing using the Applied Biosystems 3730XL DNA Sequencer. The resulting sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website. Sequences were compared to registered fungal strains in the NCBI database, and similarity percentages along with the corresponding GenBank accession numbers were recorded (White et al., 1990).

Pot experiment

A pot experiment was conducted on November 30, 2023. The soil was sterilized using 5% formalin and covered with plastic sheets for three days. After sterilization, the soil was thoroughly aerated to remove any residual formalin. Subsequently, 5 kg of sterilized soil was added to each pot. Ten seeds of each of the five wheat cultivars were sown per pot.

The experiment comprised the following treatments:

Pathogenic fungus only

100 ml of spore suspension of the pathogenic fungus was added to each pot at a concentration of 10^8 CFU/ml.

Mycorrhizal fungi only

100 ml of a suspension containing approximately 20 spores of AMF was added to each pot.

Combined treatment (pathogenic + mycorrhizal fungi)

100 ml each of the pathogenic fungal spore suspension and the mycorrhizal fungal suspension were added to each pot.

Control (healthy plants)

100 ml of sterile distilled water was added to each pot.

Each treatment was replicated five times.

Plant height

Plant height was measured using a measuring tape and recorded in centimeters (cm).

Chlorophyll content

Chlorophyll content was measured at the flowering stage using a SPAD chlorophyll meter.

Dry shoot and root biomass

At the flowering stage, plants were uprooted, and the shoot and root systems were separated. The samples were oven-dried to a constant weight and weighed to determine dry biomass.

Estimation of AM colonization

Mycorrhizal colonization was assessed using the method of Phillips and Hayman (1970). One hundred root segments, approximately 1 cm in length, were randomly taken from the terminal parts of the roots. These were cleared in 10% KOH, acidified with 10% HCl, and stained with Trypan Blue. The stained root segments were treated with lactic acid, mounted on glass slides, and examined under an Optica light microscope at 40× magnification.

The percentage of mycorrhizal colonization was calculated using the following formula:

$$\text{Mycorrhizal colonization (\%)} = \frac{\text{Number of infected root segments}}{\text{Total number of root segments}} \times 100$$

Roots were considered colonized if fungal hyphae, vesicles, or arbuscules were observed within root cells, as described by Giovannetti and Mosse (1980).

Estimation of disease severity caused by the pathogenic fungus

Disease severity was assessed based on McKinney's formula (1923) using a disease rating scale described by Gao et al. (1995):

0 = Healthy plant with white roots
1 = Slight root discoloration and yellowing of a few leaves
2 = Complete root discoloration and general leaf yellowing
3 = Discoloration extending from root to stem base
4 = Plant death

The disease severity (%) was calculated as:

$$\text{Disease Severity (\%)} = \frac{\Sigma\ (\text{Number of plants at each rating } \times \text{ rating value})}{\text{Total number of plants assessed } \times \text{ maximum rating value}} \times 100$$

Induction of resistance

Extraction of crude enzyme filtrate

Three plants were randomly selected from each replicate. The roots were thoroughly washed with tap water to remove soil particles, and then cut into small segments. One gram of root tissue was homogenized in 5 ml of 0.05 M acetate buffer (pH 5.6) using a glass mortar and pestle. The homogenate was transferred to 10 ml centrifuge tubes and centrifuged at 5,000 rpm for 5 min. The supernatant was collected and stored at 4°C for further enzymatic analysis.

Estimation of peroxidase and polyphenol oxidase activity

Peroxidase activity was determined following the method of Hammerschmidt et al. (1982). The reaction mixture contained 2.5 ml of substrate solution (guaiacol and hydrogen peroxide) and 0.1 ml of enzyme extract. Absorbance was recorded at 470 nm, and one enzyme unit was defined as an increase in absorbance of 0.01 per minute.

Polyphenol oxidase activity was estimated using the same method, with catechol as the substrate.

Statistical analysis

The experiment was laid out in a Completely Randomized Design (CRD). Data were subjected to analysis of variance (ANOVA) using SPSS software. Mean differences were compared using the Least Significant Difference (LSD) test at a 5% significance level, as described by Al-Rawi and Khalaf Allah (1980).

Results

Isolation and identification of AMF

Five distinct species of AM fungi were isolated based on morphological differences in their spores. Figure 1-A depicts the spores of *Acaulospora* sp., which were dark brown to black, ornamented, and exhibited a visible hyphal attachment scar. The spore diameter ranged from 50-60 µm. Figure 1-B shows spherical spores with smooth edges and a light orange to light brown color, displaying a hyphal attachment in the form of a swelling. Their diameter ranged from 108-122 µm, consistent with certain species of *Funneliformis* sp. Figure 1-C illustrates spores of *Oehlia* sp., which were spherical, yellow, double-walled, and lacked a clearly distinguishable hyphal attachment point. Their diameter ranged from 116-132 µm. Figure 1-D presents spores that were oval to pear-shaped, with smooth margins, double walls, and a coloration ranging from light to dark brown. The hyphal attachment appeared as a swelling, and their dimensions ranged from 45-60 µm in width to 54-116 µm in length, corresponding to the characteristics of *Rhizophagus* sp. Figure 1-E represents spores of *Entrophospora* sp., which were spherical, pale to yellowish, double-walled, and lacked a clearly visible hyphal attachment scar. Their diameter ranged from 88-142 µm.

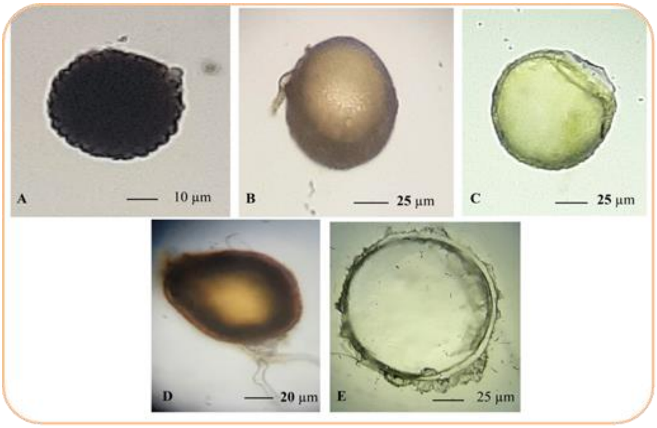

Figure 1. Spores of AMF isolated from wheat roots. (A = *Acaulospora* sp., B = *Funneliformis* sp., C = *Oehlia* sp., D = *Rhizophagus* sp., E = *Entrophospora* sp.).

Molecular identification

Figure 2 shows a single distinct DNA band for each fungal isolate, observed following electrophoretic separation of PCR products, with an approximate molecular size of 600 base pairs. This confirms the successful amplification of fungal DNA using primers specific to true fungi.

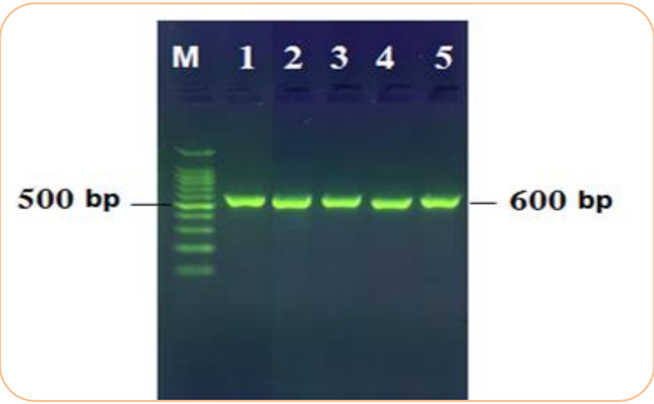

Figure 2. Electrophoretic separation of PCR-amplified

Table 1 summarizes the molecular identification results of AMF, which were identified based on the highest nucleotide sequence similarity within the ITS region of the 5.8S rRNA gene, using reference sequences from the NCBI GenBank database.

The analysis revealed that the fungal isolates corresponded to the following species: *Acaulospora brasiliensis*, *Entrophospora claroidea*, *Funneliformis mosseae*, *Oehlia diaphana*, and *Rhizophagus intraradices*. These isolates exhibited high sequence similarity to their respective reference strains: *A. brasiliensis* isolate W4699/Att1211-0 (United Kingdom), *E. claroidea* clone Clar3 (Czech Republic), *F. mosseae* isolate SP303 (Ethiopia), *O. diaphana* isolate Od_15 (Poland), and *R. intraradices* clone V133 (Iran), with similarity percentages of 99.69%, 97.68%, 99.42%, 96.23%, and 99.43%, respectively.

All isolates were successfully submitted to the NCBI GenBank database under the accession numbers PP329939.1, PP331806.1, PP329940.1, PP329941.1, and PP329943.1. ITS region of the 5.8S rRNA gene from AMF isolated from wheat roots. PCR products were resolved on a 2% agarose gel in 1× TBE buffer at 5 V/cm^2 for 1 h. Lane M: 100 bp DNA ladder.

Effect of different AMF on mycorrhizal colonization rate in the presence and absence of the pathogenic fungus *F. culmorum*

The results presented in Table 2 indicate significant variation among AMF species in their colonization efficiency across the tested wheat cultivars. Overall, mycorrhizal colonization was reduced in the presence of the pathogenic fungus *F. culmorum*. Under non-infected conditions, *R. intraradices* exhibited the highest colonization rate in the cultivar Sham-6 (91.2%), whereas the lowest rate was observed with *A. brasiliensis* in the cultivar Abu Ghraib (74.06%). Similarly, under *F. culmorum* infection, *R. intraradices* still achieved the highest colonization in Sham-6 (76.95%), while *A. brasiliensis* recorded the lowest colonization in Abu Ghraib (59.81%).

Figure 3 illustrates the colonization of wheat roots by AMF, as evidenced by the presence of arbuscules, vesicles, and fungal hyphae, in contrast to the control (non-inoculated) plants.

Table 1. Molecular identification of AMF isolates based on the percentage similarity of 5.8S rRNA gene sequences with reference strains in the NCBI GenBank database.

Closest Matching Fungal Species	Accession Number (NCBI)	Country of Origin	Similarity (%)	Identified Isolate in this Study	Accession Number in this study
A. brasiliensis isolate W4699/Att1211-0	FN825906.1	United Kingdom	99.69	*A. brasiliensis* isolate Jwan-7	PP329939.1
E. claroidea clone Clar3	MK521698.1	Czech Republic	97.68	*E. claroidea* isolate Jwan-8	PP331806.1
F. mosseae isolate SP303	AY236333.1	Ethiopia	99.42	*F. mosseae* isolate Jwan-8	PP329940.1
O. diaphana isolate Od_15	MG836663.1	Poland	96.23	O. *diaphana* isolate Jwan-9	PP329941.1
R. intraradices clone V133	EF989108.1	Iran	99.43	*R. intraradices* isolate Jwan-11	PP329943.1

Table 2. Effect of different AMF on the mycorrhizal colonization rate (%) in five wheat cultivars under the presence and absence of *F. culmorum*.

Treatments	Abu Ghraib	Ibaa 99	Al-Ezz	Sham 6	Buhouth	Treatment Mean
A. brasiliensis	74.06	80.05	78.08	83.11	77.11	78.48
E. claroidea	74.34	80.33	78.36	83.39	77.39	78.76
F. mosseae	77.52	83.51	81.54	86.57	80.57	81.94
R. intraradices	82.15	88.14	86.17	91.20	85.20	86.57
O. diaphana	79.78	85.77	83.80	88.83	82.83	84.20
F. culmorum (Pathogen only)	0.00	0.00	0.00	0.00	0.00	0.00
F. culmorum + *A. brasiliensis*	59.81	65.80	63.83	68.86	62.86	64.23
F. culmorum + *E. claroidea*	60.09	66.08	64.11	69.14	63.14	64.51
F. culmorum + *F. mosseae*	63.27	69.26	67.29	72.32	66.32	67.69
F. culmorum + *R. intraradices*	67.90	73.89	71.92	76.95	70.95	72.32
F. culmorum + *O. diaphana*	65.53	71.52	69.55	74.58	68.58	69.95
Control (Untreated healthy	0.00	0.00	0.00	0.00	0.00	0.00
Cultivar Mean	58.70	63.70	62.05	66.25	61.25	-
LSD $_{0.05}$	Treatments = 1.11		Cultivars = 1.23		Interaction = 1.36	

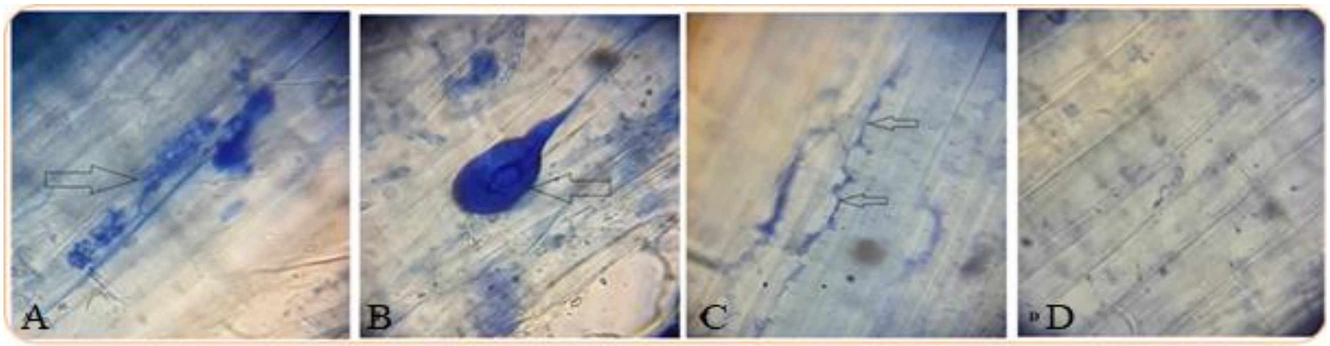

Figure 3. Colonization of wheat roots by AMF. A: Arbuscules; B: Vesicle; C: Fungal hyphae; D: Control plant - non-inoculated.

Effect of different AMF on disease severity (%) caused by *F. culmorum* in five wheat cultivars

Table 3 illustrates significant differences in disease severity caused by *F. culmorum* among wheat plants inoculated with various AMF, compared to plants inoculated with the pathogen alone. The highest disease severity was recorded in plants treated solely with *F. culmorum*, ranging from 88.79% to 91.11% across the tested wheat cultivars. In contrast, the lowest disease severity (29.58%) was observed in the Sham 6 cultivar inoculated with both *F. culmorum* and *R. intraradices*. Among the AMF treatments, the highest disease severity (46.72%) was recorded in the Abu Ghraib cultivar co-inoculated with *F. culmorum* and *A. brasiliensis*.

Effect of different AMF on peroxidase enzyme activity (U/ml) in five wheat cultivars under the presence and absence of *F. culmorum*

The results presented in Table 4 demonstrate that AMF enhanced peroxidase enzyme activity in healthy wheat plants. This activity was further elevated when AMF were applied in conjunction with the pathogenic fungus *F. culmorum*. Under non-pathogenic conditions, the highest peroxidase activity (2.187 U/ml) was recorded in the cultivar Sham 6 inoculated with *R. intraradices*, while the lowest activity (1.378 U/ml) was observed in the cultivar Abu Ghraib treated with *A. brasiliensis*.

In the presence of *F. culmorum*, peroxidase activity increased across all treatments. The highest activity (2.309 U/ml) was again recorded in Sham 6 inoculated with both *R. intraradices* and *F. culmorum*, whereas the lowest activity (1.500 U/ml) was observed in Abu Ghraib treated with *A. brasiliensis* and *F. culmorum*.

Effect of different AMF on polyphenol oxidase activity (U/ml) in five wheat cultivars under *F. culmorum*-inoculated and non-inoculated conditions

Treatments involving healthy wheat plants inoculated with AMF exhibited significantly higher polyphenol oxidase (PPO) activity compared to the untreated control, as shown in Table 5. The same table indicates that co-inoculation with *F. culmorum* and AMF resulted in the highest PPO activity under pathogenic conditions. Among the healthy treatments, the highest PPO activity (2.28 U/ml) was recorded in the Sham 6 cultivar inoculated with *R. intraradices*. In contrast, the lowest activity (0.566 U/ml) was observed in the Abu Ghraib cultivar inoculated with *A. brasiliensis*. Under pathogenic conditions, the Sham 6 cultivar co-inoculated with *F. culmorum* and *R. intraradices* again exhibited the highest PPO activity (2.402 U/ml), while the lowest value (0.688 U/ml) was recorded in the Abu Ghraib cultivar treated with *F. culmorum* and *A. brasiliensis*.

Table 3. Effect of different AMF on disease severity (%) in five wheat cultivars in the presence of *F. culmorum*.

Treatments	Abu	Ibaa 99	Al-Ezz	Sham 6	Buhouth	Treatment Mean
F. culmorum (Pathogen only)	91.11	90.18	90.3	88.79	91.02	90.28
F. culmorum + *A. brasiliensis*	46.72	40.73	42.7	37.67	43.67	42.3
F. culmorum + *E. claroidea*	46.44	40.45	42.42	37.39	43.39	42.02
F. culmorum + *F. mosseae*	43.26	37.27	39.24	34.21	40.21	38.84
F. culmorum + *R. intraradices*	38.63	32.64	34.61	29.58	35.58	34.21
F. culmorum + *O. diaphana*	41.06	35.01	36.98	31.95	37.95	36.59
Control (Untreated healthy plants)	0.0	0.0	0.0	0.0	0.0	0.0
Cultivar Mean	43.89	39.47	40.89	37.08	41.69	-
LSD (0.05)	Treatments = 1.17		Cultivars = 1.12		Interaction = 1.24	

Table 4. Effect of different AMF on peroxidase enzyme activity (U/ml) in five wheat cultivars under *F. culmorum*-inoculated and non-inoculated conditions.

Treatments	Abu Ghraib	Ibaa 99	Al-Ezz	Sham 6	Buhouth	Treatment Mean
A. brasiliensis	0.936	1.535	1.338	1.841	1.241	1.378
E. claroidea	0.964	1.563	1.366	1.869	1.269	1.406
F. mosseae	1.282	1.881	1.684	2.187	1.587	1.724
R. intraradices	1.745	2.344	2.147	2.65	2.05	2.187
O. diaphana	1.508	2.107	1.91	2.413	1.813	1.95
F. culmorum (Pathogen only)	0.658	1.257	1.06	1.563	0.963	1.1
F. culmorum + *A. brasiliensis*	1.058	1.657	1.46	1.963	1.363	1.5
F. culmorum + *E. claroidea*	1.086	1.685	1.488	1.991	1.391	1.528
F. culmorum + *F. mosseae*	1.404	2.003	1.806	2.309	1.709	1.846
F. culmorum + *R. intraradices*	1.867	2.466	2.269	2.772	2.172	2.309
F. culmorum + *O. diaphana*	1.63	2.229	2.032	2.535	1.935	2.072
Control (Untreated healthy plants)	0.072	0.067	0.071	0.068	0.071	0.07
Cultivar Mean	1.184	1.733	1.553	2.013	1.464	-
LSD (0.05)	Treatments = 0.153		Cultivars = 0.158		Interaction = 0.168	

Table 5. Effect of different AMF on polyphenol oxidase activity (U/ml) in five wheat cultivars under the presence and absence of *F. culmorum*.

Treatments	Abu Ghraib	Ibaa 99	Al-Ezz	Sham 6	Buhouth	Treatment Mean
A. brasiliensis	0.566	1.165	0.968	1.471	0.871	1.008
E. claroidea	0.594	1.193	0.996	1.499	0.899	1.036
F. mosseae	0.912	1.511	1.314	1.817	1.217	1.354
R. intraradices	1.375	1.974	1.777	2.28	1.68	1.817
O. diaphana	1.138	1.737	1.54	2.043	1.443	1.58
F. culmorum (Pathogen only)	0.288	0.887	0.69	1.193	0.593	0.73
F. culmorum + *A. brasiliensis*	0.688	1.287	1.09	1.593	0.993	1.13
F. culmorum + *E. claroidea*	0.716	1.315	1.118	1.621	1.021	1.158
F. culmorum + *F. mosseae*	1.034	1.633	1.436	1.939	1.339	1.476
F. culmorum + *R. intraradices*	1.497	2.096	1.899	2.402	1.802	1.939
F. culmorum + *O. diaphana*	1.26	1.859	1.662	2.165	1.565	1.702
Control (Untreated healthy plants)	0.042	0.038	0.041	0.04	0.041	0.04
Cultivar Mean	0.846	1.391	1.211	1.672	1.122	-

Effect of different AMF on plant height in five wheat cultivars in the presence and absence of *F. culmorum*

Table 6 presents the effect of various AMF on plant height in five wheat cultivars under both pathogen-free and *F. culmorum*-inoculated conditions. In treatments involving AMF alone, the Sham 6 cultivar inoculated with *R. intraradices* exhibited the highest plant height, reaching 78.35 cm. Conversely, the lowest height (61.21 cm) was recorded in the Abu Ghraib cultivar treated with *A. brasiliensis*.

Under co-inoculation with AMF and *F. culmorum*, the Sham 6 cultivar again showed the greatest plant height (69.57 cm) when treated with *F. culmorum* + *R. intraradices*. The lowest plant height under co-inoculation was observed in the Abu Ghraib cultivar treated with *F. culmorum* + *A. brasiliensis*, measuring 52.43 cm.

Effect of different AMF on dry shoot biomass in five wheat cultivars in the presence and absence of *F. culmorum*

Dry shoot biomass data presented in Table 7 illustrate the effects of AMF on wheat cultivars under both non-pathogenic and pathogenic conditions involving *F.*

culmorum. In the absence of the pathogen, the highest dry shoot biomass (6.33 g) was observed in the Sham 6 cultivar inoculated with *R. intraradices*. Conversely, the lowest biomass (3.31 g) was recorded in the Abu Ghraib cultivar inoculated with *A. brasiliensis*. Under pathogenic conditions, the Sham 6 cultivar treated with *F. culmorum* + *R. intraradices* also exhibited the highest dry shoot biomass (5.75 g), while the lowest value (2.73 g) was again observed in the Abu Ghraib cultivar treated with *F. culmorum* + *A. brasiliensis*.

Table 6. Effect of different AMF on plant height in five wheat cultivars in the presence and absence of *F. culmorum*.

Treatments	Abu Ghraib	Ibaa 99	Al-Ezz	Sham 6	Buhouth	Treatment Mean
A. brasiliensis	61.21	67.2	65.23	70.26	64.26	65.63
E. claroidea	61.49	67.48	65.51	70.54	64.54	65.91
F. mosseae	64.67	70.66	68.69	73.72	67.72	69.09
R. intraradices	69.3	75.29	73.32	78.35	72.35	73.72
O. diaphana	66.93	72.92	70.95	75.98	69.98	71.35
F. culmorum (Pathogen only)	32.27	38.26	36.29	40.32	35.32	36.49
F. culmorum + *A. brasiliensis*	52.43	58.42	56.45	61.48	55.48	56.85
F. culmorum + *E. claroidea*	52.71	58.69	56.73	61.76	55.76	57.13
F. culmorum + *F. mosseae*	55.89	61.88	59.91	64.94	58.94	60.31
F. culmorum + *R. intraradices*	60.52	66.51	64.54	69.57	63.57	64.94
F. culmorum + O. diaphana	58.15	64.14	62.17	67.2	61.2	62.57
Control (Untreated healthy plants)	52.74	58.73	56.78	61.79	55.79	57.17
Cultivar Mean	57.36	63.35	61.38	66.33	60.41	-
LSD (0.05)	Treatments = 1.39	Cultivars = 1.42	Interaction = 1.57			

Table 7. Effect of Different Arbuscular mycorrhizal fungi on Dry Shoot Biomass (g) in Five Wheat Cultivars in the Presence and Absence of Fusarium culmorum

Treatments	Abu Ghraib	Ibaa 99	Al-Ezz	Sham 6	Buhouth	Treatment Mean
A. brasiliensis	3.31	4.89	4.42	5.52	3.72	4.37
E. claroidea	3.33	4.91	4.45	5.55	3.74	4.4
F. mosseae	3.65	5.23	4.76	5.86	4.07	4.74
R. intraradices	4.12	5.69	5.23	6.33	4.53	5.18
O. diaphana	3.88	5.46	4.99	6.09	4.29	4.94
F. culmorum (Pathogen only)	0.77	2.25	1.78	2.88	1.08	1.75
F. culmorum + *A. brasiliensis*	2.73	4.31	3.84	4.94	3.14	3.79
F. culmorum + *E. claroidea*	2.76	4.34	3.87	4.97	3.17	3.82
F. culmorum + *F. mosseae*	3.07	4.65	4.19	5.29	3.48	4.14
F. culmorum + *R. intraradices*	3.54	5.12	4.65	5.75	3.95	4.6
F. culmorum + *O. diaphana*	3.31	4.88	4.41	5.52	3.72	4.37
Control (Untreated healthy plants)	2.87	4.45	3.98	5.08	3.28	3.93
Cultivar Mean	3.11	4.68	4.21	5.32	3.51	-
LSD (0.05)	Treatments = 0.32	Cultivars = 0.36	Interaction = 0.41			

Effect of different AMF on dry root biomass in five wheat cultivars in the presence and absence of *F. culmorum*

Table 8 presents the effects of different AMF on dry root biomass in five wheat cultivars under both healthy and pathogenic conditions. Among the healthy plants, the highest dry root biomass (2.53 g) was observed in the cultivar Sham 6 inoculated with *R. intraradices*, while the lowest (1.32 g) was recorded in the cultivar Abu Ghraib inoculated with *A. brasiliensis*. Under *F. culmorum* infection, the Sham 6 cultivar treated with *F. culmorum* + *R. intraradices* again exhibited the highest dry root biomass (2.30 g). Conversely, the lowest dry root biomass (1.09 g) was observed in the Abu Ghraib cultivar treated with *F. culmorum* + *A. brasiliensis*.

Effect of different AMF on chlorophyll content in five wheat cultivars in the presence and absence of *F. culmorum*

Table 9 presents the effect of AMF, both in the presence and absence of the pathogenic fungus *F. culmorum*, on chlorophyll content (measured as SPAD values) in five wheat cultivars. In treatments without the pathogen, the highest chlorophyll content was observed in the cultivar Sham 6 inoculated with *R. intraradices*, reaching 37.98 SPAD. In contrast, the lowest SPAD value (19.84) was recorded in the Abu Ghraib cultivar treated with *A. brasiliensis*.

Under pathogen-stressed conditions, the highest chlorophyll content (34.51 SPAD) was again recorded in the Sham 6 cultivar co-inoculated with *F. culmorum* and *R. intraradices*. The lowest value (16.37 SPAD) was observed in the Abu Ghraib cultivar treated with *F. culmorum* + *A. brasiliensis*.

Table 8. Effect of different AMF on dry root biomass in five wheat cultivars in the presence and absence of *F. culmorum*.

Treatments	Abu Ghraib	Ibaa 99	Al-Ezz	Sham 6	Buhouth	Treatment Mean
A. brasiliensis	1.32	1.95	1.77	2.2	1.49	1.75
E. claroidea	1.33	1.97	1.78	2.22	1.51	1.76
F. mosseae	1.46	2.09	1.91	2.35	1.63	1.89
R. intraradices	1.65	2.28	2.09	2.53	1.81	2.07
O. diaphana	1.55	2.19	1.99	2.44	1.72	1.98
F. culmorum (Pathogen only)	0.68	0.93	0.91	1.05	0.82	0.88
F. culmorum + *A. brasiliensis*	1.09	1.72	1.54	1.98	1.26	1.52
F. culmorum + *E. claroidea*	1.1	1.73	1.55	1.99	1.27	1.53
F. culmorum + *F. mosseae*	1.23	1.86	1.67	2.12	1.39	1.65
F. culmorum + *R. intraradices*	1.42	2.05	1.86	2.3	1.58	1.84
F. culmorum + *O. diaphana*	1.32	1.95	1.76	2.21	1.49	1.75
Control (Untreated healthy plants)	1.13	1.76	1.57	2.02	1.29	1.55
Cultivar Mean	1.27	1.87	1.7	2.12	1.44	-
LSD (0.05)	Treatments = 0.27		Cultivars = 0.30		Interaction = 0.36	

Table 9. Effect of different AMF on chlorophyll content in five wheat cultivars under the presence and absence of *F. culmorum*.

Treatments	Abu Ghraib	Ibaa 99	Al-Ezz	Sham 6	Buhouth	Treatment Mean
A. brasiliensis	19.84	29.31	26.51	33.12	22.33	26.22
E. claroidea	20.0	29.48	26.68	33.29	22.49	26.39
F. mosseae	21.91	31.39	28.58	35.2	24.4	28.29
R. intraradices	24.69	34.16	31.36	37.98	27.18	31.07
O. diaphana	23.27	32.74	29.94	36.56	25.75	29.65
F. culmorum (Pathogen only)	11.01	19.48	16.68	20.29	13.49	16.19
F. culmorum + *A. brasiliensis*	16.37	25.84	23.04	29.66	18.85	22.75
F. culmorum + *E. claroidea*	16.54	26.01	23.21	29.83	19.03	22.92
F. culmorum + *F. mosseae*	18.44	27.92	25.12	31.73	20.93	24.83
F. culmorum + *R. intraradices*	21.22	30.69	27.89	34.51	23.71	27.6
F. culmorum + *O. diaphana*	19.8	29.27	26.47	33.09	22.29	26.18
Control (Untreated healthy plants)	16.73	26.2	23.4	30.01	19.22	23.11
Cultivar Mean	19.15	28.54	25.74	32.11	21.64	-
LSD (0.05)	Treatments = 0.77		Cultivars = 0.94		Interaction = 1.38	

Discussion

The results of this study demonstrate that the fungal isolates identified are genetically closely related to globally registered fungal strains based on sequence similarity in the 5.8S rRNA gene. The high level of nucleotide sequence identity supports the accuracy of molecular identification. Although the isolates exhibited close genetic similarity to international reference strains, minor genetic variations were observed. These differences may be attributed to natural recombination events or mutations driven by environmental stressors specific to the geographical regions from which the fungi were isolated. Furthermore, prolonged exposure to agrochemicals may increase the frequency of genetic mutations, contributing to population-level variation in fungal communities under diverse environmental conditions (Hassan and Al-Qiassi, 2022; Hassan and Ibrahim, 2022).

The findings also reveal that AMF significantly reduced disease severity caused by the pathogenic fungus *F. culmorum* in all tested wheat cultivars. This biocontrol effect may be attributed to the ability of the fungi to enhance carbohydrate production and improve nutrient uptake, particularly phosphorus, through root colonization, thereby bolstering the resistance of the plant to pathogenic infections (AbdElgawad et al., 2022). Moreover, the results support the role of AMF in inducing systemic resistance, as evidenced by elevated activities of the defense-related enzymes peroxidase and polyphenol oxidase.

The enhanced activity of these enzymes is likely linked to the production of secondary metabolites and signaling molecules by AMF that activate resistance-related genes, forming a key mechanism in systemic acquired resistance. These results align with previous studies demonstrating systemic resistance induction in various plant species by fungal symbionts (Hassan and Yousef, 2023) and diverse mycorrhizal species (Hassan and Al-Samarrai, 2018). Notably, AMF promote pathogen resistance by enhancing the expression of peroxidase, an enzyme regulated by plant defense genes, leading to increased cell wall rigidity that hinders pathogen invasion and colonization.

Peroxidase plays a crucial role in cell wall fortification by catalyzing the oxidation of phenolic compounds into molecules such as hydrogen peroxide and quinones. These compounds generate reactive oxygen species (ROS), including hydroxyl radicals (•OH) and superoxide anions (O_2^-), which in turn activate genes responsible for lignin biosynthesis (Chon et al., 2000; Lavania et al., 2006). Lignin is deposited in the cell walls, especially in xylem vessels, reinforcing structural integrity and enhancing resistance in tissues subjected to mechanical or biotic stress.

Furthermore, peroxidase contributes to plant defense by facilitating the polymerization and deposition of lignin, suberin, and proteins in vascular tissues, thereby limiting the spread of pathogens. It also acts as an antioxidant, mitigating oxidative damage triggered during pathogen attack (Dicko et al., 2006; Fayaz and Zahedi, 2021). Another possible mechanism underlying reduced disease severity in AMF-treated plants is competitive exclusion, where beneficial fungi outcompete pathogenic fungi for colonization sites within the rhizosphere, as supported by Duan et al. (2024).

Moreover, AMF are known to stimulate the production of plant growth-promoting substances, either directly through secretion or indirectly by inducing the host plant to synthesize them (Hong et al., 2024; Pandino et al., 2024). One such key compound is indole-3-acetic acid (IAA), an auxin that enhances root cell division and elongation (Jameson and Horth, 2024). AMF also promote the synthesis of siderophores, organic acids, and exopolysaccharides, which enhance micronutrient availability in the rhizosphere, thereby improving nutrient uptake and plant resilience to abiotic stress (Yadav et al., 2020).

The observed improvement in vegetative growth among wheat cultivars in this study may be attributed to multiple mechanisms, including the formation of an efficient root system with greater water and nutrient absorption capacity. AMF colonization enhances root water retention through arbuscular vesicles, which act as reservoirs, gradually releasing stored water to the host plant (Yadav et al., 2020).

These findings are consistent with those of Arabi et al. (2013), who reported improved nutrient uptake, plant height, shoot and root biomass, and chlorophyll content in AMF-inoculated plants. The increased chlorophyll content enhances photosynthetic capacity and overall metabolic activity, leading to greater resistance and productivity (Pandino et al., 2024). The effectiveness of AMF colonization was found to vary among wheat cultivars, likely due to genetic differences influencing fungal compatibility, colonization efficiency, and host susceptibility. Some cultivars may possess resistance genes that restrict colonization by either symbiotic or pathogenic fungi (Mohammed and Baldwin, 2024).

The variation in AMF colonization observed in this study may be explained by differences in cultivar-specific chemical signals such as sterols and flavonoids, which play key roles in spore germination and hyphal growth toward roots (Smith and Reed, 2008). Moreover, phosphorus availability in the soil can influence colonization; excessive phosphorus levels can inhibit the formation of AMF associations. Repeated exposure of a cultivar to specific AMF species may also increase compatibility through local adaptation (Smith and Smith, 2011).

The role of AMF in suppressing *Fusarium* spp. and other systemic pathogens has been demonstrated in previous studies. For example, Pu et al. (2022) showed that *Glomus versiforme* improved resistance of *Salvia miltiorrhiza* to *F. oxysporum*. Similarly, Meddad-Hamza et al. (2023) reported the biocontrol potential of several AMF species, including *Claroideoglomus claroideum*, *Claroideoglomus etunicatum*, *Funneliformis geosporum*, *F. mosseae*, *Glomus microaggregatum*, and *Rhizophagus intraradices*, against *F. oxysporum* and *Verticillium dahliae* in tomato.

Conclusions

This study confirms the diversity and functional importance of arbuscular mycorrhizal fungi (AMF) associated with wheat roots. The following AMF species were identified and molecularly characterized: *Acaulospora brasiliensis*, *Entrophospora claroidea*, *Funneliformis mosseae*, *Oehlia diaphana*, and *Rhizophagus intraradices*. These species were deposited in the NCBI genetic database, enriching the current knowledge of AMF associated with wheat. Among them, *R. intraradices* demonstrated the highest root colonization potential and was the most effective in reducing disease severity caused by *F. culmorum*. It also significantly induced systemic resistance and improved various vegetative growth parameters. The identified AMF species hold promise for use in the biological control of plant diseases. Furthermore, the wheat cultivar Sham exhibited the best response to AMF inoculation, suggesting its potential suitability for future AMF-based biotechnological applications.

Authors' Contributions

AAH designed and supervised the study; JYI prepared the materials, collected and analyzed the data; Both authors wrote the first draft, proofread and approved the final manuscript.

Research Funding

This research did not receive any grant from funding agencies.

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

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