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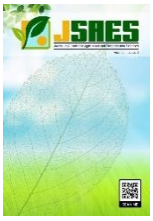

Research Article

Morphological and Molecular Identification of *Fusarium incarnatum* as the Causal Agent of Potato Dry Rot Disease

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Abstract:

Potato (*Solanum tuberosum* L., family Solanaceae), is one of the most widely consumed vegetable crops worldwide. Several phytopathogenic fungi target potato tubers during storage. Dry rot disease caused by several species of Fusarium is considered one of the most serious threats to potato tuber crops under normal or cold conditions. Herein, we isolated, characterized, and identified three isolates of Fusarium associated with dry rot of potato tubers. The morphological characterization and microscopic examination revealed that all isolates belong to the Fusarium genus. Moreover, all isolates were pathogenic and displayed the typical symptoms of dry rot disease on potato tuber 20 days post-inoculation. However, only one isolate (isolate #3), was the most aggressive and caused the maximum lesion diameter (28.00±2.00 mm) and depth (31.67±2.89 mm) in comparison to other isolates (8±2.00, 14.67±1.53, 9.67±0.58 and 13.67±1.53mm for lesion diameter and depth respectively). The most virulent isolate was subjected to further studies such as molecular identification using an internal transcribed spacer (ITS) region and phylogenetic analysis. Molecular identification showed that the tested isolate had high similarity with *Fusarium incarnatum* isolate CM9 (Gen Bank Accession No. MN186812.1; 522pb) subsequently, the sequence of this isolate (isolate #3) was uploaded in the GenBank database under the name *F. incarnatum* isolate AE 2024 (GenBank Accession No. PP086049; 528 pb). Our findings suggest that one of the best ways to control plant diseases such as dry rot disease is to identify (species level) of the pathogen accurately.

1. Introduction

Potato (*Solanum tuberosum* L., family Solanaceae), is one of the most significant crops in the world in addition, it is the main cash crop, consumed by over a billion people in 150 different countries (Erper et al., 2022). In 2022, the global production of potatoes reached 374777763.43 tons and the harvested area was 17788408 hectares (FAOSTAT, 2023). Moreover, Egypt cultivated 213272 hectares of potato, yielding 28.862 tons per hectare with a total production of 6155466.58 tons in the same year (FAOSTAT, 2023). Potato crops are attacked by different species of phytopathogenic bacteria, fungi, and viruses pre- and post-harvest that cause considerable economic losses in potato production. One of the most significant genera of phytopathogenic fungi is Fusarium, which is responsible for storage-related dry rot in potato tubers and field wilt (Azil et al., 2021). Dry rot disease caused by Fusarium species is a major and damaging disease of potato tubers under storage conditions (Batta, 2018; Hay et al., 2019; Tiwari et al., 2022).

Dry rot disease is widespread in all potato-growing regions worldwide and can cause significant losses ranging from 10 to 25% and it can reach 60% in serious infection cases (Wharton and Kirk, 2014). Symptoms of dry rot disease include shrinkage, wilting, and mummification of rotted tubers, with spots and cracks in dead tissues. Additionally, disease symptoms develop during the storage period of the tubers, and cottony growths appear on the affected areas, which are the mycelium of the pathogenic fungus. Eventually, the rotted tubers lose their content of dry and nutritional materials (Heltoft et al., 2016).

More than thirteen species of *Fusarium* are known to cause potato dry rot disease worldwide; however, the distribution of each species depends on the season and geographical location (Cullen et al., 2005). *Fusarium solani*, *F. sambucinum*, *F. oxysporum*, *F. avenaceum*, and *F. culmorum* are the most common species worldwide (Bojanowski et al., 2013; Tiwari et al., 2020). However, *F. sambucinum*, *F. incarnatum*, *F. solani*, *F. oxysporum*, and *F. verticillioide* are the major species causing dry rot of potatoes in Egypt (El-Hassan et al., 2007; Gherbawy et al., 2019).

Properly identifying of the phytopathogenic fungi associated with potato dry rot disease helps design successful management strategies for such a serious disease and reduce the losses caused by it. Consequently, in the present study, the isolation and morphological characterization of the causal agent of potato dry rot disease were done. Furthermore, the most aggressive isolate was chosen for molecular identification.

2. Material and methods

2.1. Plant materials

Diseased potato tubers of the cultivar "Spunta" showing dry rot symptoms were collected from storages at different sites in EL-Gharbia and Beheira governorates, Egypt. The diseased tubers were kept in plastic boxes and stored at 4±1°C and 90% relative humidity until the isolation of the causal pathogens.

2.2. Isolation and purification of the pathogen

The causal agent of potato dry rot disease was isolated from potato tubers that showed typical symptoms of dry rot disease. Briefly, three isolates of the pathogen were obtained from diseased potato tubers as follows; samples were cut into small parts (1x1 cm), surface sterilized for 3 minutes with 2% sodium hypochlorite, then rinsed three times with sterilized distilled water and dried between two layers of sterilized filter papers. Subsequently, small sterilized parts were placed on Petri dishes containing potato dextrose agar (PDA) medium containing 200 mg L^{-1} of streptomycin to prevent bacterial growth and incubated for one week at 25±2°C. Incubated cultures were purified using the hyphal tips method. Purified cultures were placed in fresh PDA plates and incubated for 7 days at 25±2°C.

2.3. Morphological and microscopic characterization of pathogen isolates

Initially, culture morphological features like diameter, texture, surface, and color were used to identify the pathogen isolates. Briefly, a 0.5 cm mycelial plug was taken from areas of active growth close to the borders of 7-day-old cultures that were transferred to PDA. The plug was then cultured for 7 days at 25±2°C in the dark, after which the diameters of growing colonies were measured and recorded, and other colony properties were evaluated visually. Subsequently, the microscopic investigation was done according to the methods underlined by (El-Nagar et al., 2022) as follows, fungal mycelium was put on glass slides, mounted in lactophenol pigment, and examined using a light microscope.

2.4. Pathogenicity test

The pathogenicity test was conducted on freshly harvested, healthy, and uniform-sized potato tubers Cv. "Spunta" using the three pathogen isolates. Potato tubers were washed by running tap water, dipped in sodium hypochlorite (2%) for two minutes, rinsed with sterilized distilled water, and left air-dried for two hours. Subsequently, potato tubers were wounded (0.3x0.3 cm diameter and depth) using a sterilized borer, and 20 µL (1×10^6 conidia per ml) of a conidial suspension of the pathogen isolates were separately injected into each wound. A suspension of Fusarium isolates conidia was prepared from a 7-10 day-old culture grown on PDA plates at 25°C, following the methods described by (Sehim et al., 2023). The culture was submerged in sterilized distilled water and spores were collected using a sterile glass rod. The resulting spore suspension was filtered through sterilized cheesecloth to remove mycelial fragments. Finally, the spore concentration was adjusted to 1×10^6 conidia /mL using sterilized distilled water. Sterilized distilled water was used for control inoculation. Twenty tubers were used for each isolate and the experiment was repeated three times to confirm the results. After 20 days of incubation at 25°±2c, lesion depth and length were measured on rotting tubers as described by (Peters et al., 2008).

2.5. Molecular Identification of *F. incarnatum*

After the isolated pathogens were identified as Fusarium, its highest aggressive isolate was selected for further studies. Briefly, this isolate was cultured on a sterilized potato dextrose broth (PDB) for 10 days at 25 ± 2 ◦C. Subsequently, the mycelium was collected and filtered using cheesecloth, washed three times with sterilized water, and dried using two layers of f sterilized filter paper, according to the protocol described by (Atallah & Yassin, 2020; El-Nagar et al., 2022). Approximately 100 mg of the mycelium was crushed to a fine powder using liquid nitrogen to extract the total DNA using a Quick-DNA™ Fungal/Bacterial Miniprep Kit according to the instructions of the manufacturer then purified, and the targeted sequences of the ITS region (ITS-5.8S rDNA) were amplified using PCR. The purified PCR products were sent for sequencing. DNABASER software was used to process and assemble consensus sequences. An assembled sequence compared with the most recent available data in GenBank and the NCBI using a Nucleotide-Nucleotide Basic Local Alignment Search Tool (BLASTn).

2.6. Phylogenetic tree of *Fusarium incarnatum*

The assembled sequence of the ITS-5.8S rDNA was used to create the evolutionary analysis and phylogenetic trees based on the Maximum Likelihood methods and Tamura-Nei model (Tamura & Nei, 1993) using MEGA 11 software (Tamura et al., 2021). 20 reference isolates (Table 1), additionally, the query sequence was selected for multiple sequence alignment using ClustalW multiple sequence alignment algorithms.

2.7. Statistical analysis

The analysis of variance (ANOVA) method was used to statistically evaluate Data. Post hoc pairwise comparisons using the Tukey honestly significant difference test were performed to look at the differences in means of various treatments (HSD; $p \leq 0.05$).

3. Results

3.1. The causal agents of potato dry rot disease

The fungal isolates obtained from rotted tubers showed typical morphological and microscopic characteristics of Fusarium species. On PDA media it formed a fungal colony that looks like cotton with light beige in the front (Figure 1A) and light brown on the back side (Figure 1B). The fungal colony needed seven days to completely cover the surface of a 9-cm Petri dish when cultured on PDA at 25±2°C. Furthermore, microscopic examination revealed the presence of hyaline and separate hyphae, hyaline macroconidia (Figure 1C) that were cylindrical with a slight curvature at the tip, divided into 3-4 cells, and no microconidia were observed in fresh cultures (Figure 1C).

3.2. The pathogenicity test

Generally, all isolates of *Fusarium* sp. were pathogenic and produced typical dry rot symptoms on potato tubers (Figure 2A, B). Necrotic lesions were observed on the skin of inoculated tubers and whitish fungal growth after 20 days post-inoculation at 25°±2 C and 90% relative humidity (Figure 2A, B). No symptoms appeared on control tubers. Isolate #3 was the most aggressive one that caused the largest lesion diameter and lesion depth

on potato tubers (28.00 ± 0.20 and 31.67 ± 0.29 mm, respectively Figure 2c, d). On the other hand, isolate 1 was the least virulence isolate where it recorded the lowest values of lesion diameter and lesion depth (8.00±2.00 and 9.67±0.58 mm, respectively) (Figure 2C, D).

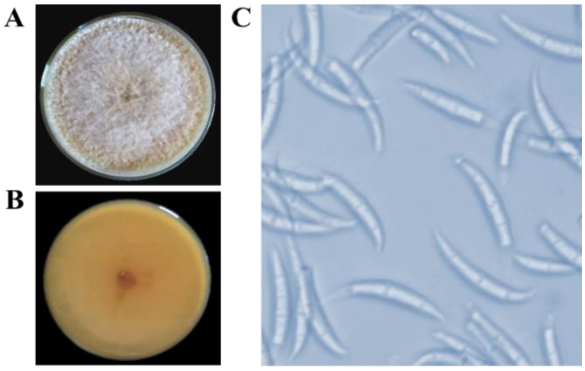

Figure1. The morphological characterization of *Fusarium incarnatum*, the causal agent of potato dry rot disease on PDA plates after incubation 7 days at 25±2°c. (A) the top view, (b) the back view, and (c) the microscopic examination.

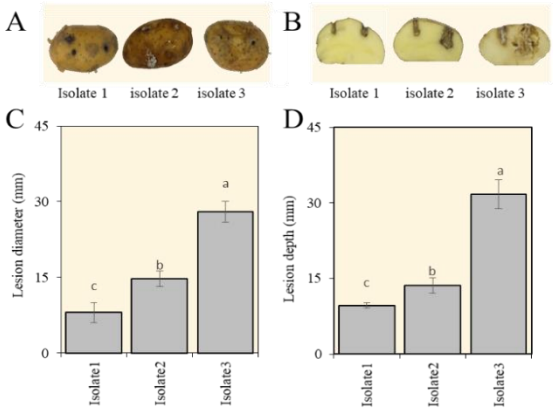

Figure 2. Potato tuber inoculated with different isolates of *Fusarium* Sp. 20 days post-inoculation. (A) the external disease symptoms (B) the internal disease symptoms, (C, D) the Lesion and depth diameter of rotted potato tubers(mm) respectively. values are means + Standard deviations of six biological replicates. Different letters denote significant differences between isolates according to Tukey's HSD test ($p \leq 0.05$).

3.3. Molecular identification and phylogenetic analysis

The most aggressive isolate (isolate #3) was selected for further genetic identification based on the sequence of the internal transcribed spacer (ITS) region (Figure 3). Briefly, the query sequence showed a high degree of similarity with the large subunit ribosomal RNA gene of *Fusarium incarnatum* isolate CM9 (Gen Bank Accession No. MN186812.1; 522 pb) (Figure 3). The new sequence was deposited in the NCBI database and named *Fusarium incarnatum* isolate AE 2024 (GenBank Accession No. PP086049; 528 pb). Furthermore, sequences of 20 reference *Fusarium* species were used to compare the query sequence of the studied isolate, *Fusarium incarnatum*, with them as displayed in Table 1.

4. Discussion

Postharvest diseases are a major problem facing fruit and vegetable crops, especially during storage conditions, causing huge economic losses, and reducing the quality and quantity of agricultural products. Moreover, the losses resulting from postharvest diseases ranged from 20 to 50% (Cruz et al., 2018). Potato dry rot caused by several species of Fusarium is a major postharvest disease that causes important crop losses under traditional or cold storage conditions (Mejdoub-Trabelsi & Chérif, 2009; Stevenson et al., 2001).

In the present study, three isolates of the ascomycetes Fusarium genus were found to be associated with the postharvest decay of potato tubers. In agreement with these findings, several species of Fusarium such as *F. sambucinum*, *F. oxysporum*, *F. solani*, *F. roseum* var. *sambucinum*, *F. verticillioide*, *F. culmorum* and *F. incarnatum* were previously reported to cause postharvest damage to potato tubers under different conditions (Azil et al., 2021; Gherbawy et al., 2019; Mejdoub-Trabelsi & Chérif, 2009).

Furthermore, *Fusarium* is considered one of the most significant genus of phytopathogenic fungi and is responsible for dry rot disease on potato tubers during storage conditions. Moreover, there are now 17 species and five variations of Fusarium known to cause potato dry rot worldwide, however, the differences between isolated species depend on the geographic location and the season (Tiwari et al., 2020). In addition, the presence of many potato cultivars helps in the emergence of new strains/isolates of Fusarium, which causes dry rot disease in potatoes (Xue et al., 2023). In the present study, morphological characterization indicated that the three obtained isolates belonged to the genus *Fusarium*. Moreover, the pathogenicity test confirmed the virulence of these isolates and their ability to cause dry rot symptoms on potato tubers. However, molecular identification was required to determine the species. Therefore, molecular identification was performed for the most virulent isolate (isolate #3).

Our molecular identification confirmed that the most aggressive of Fusarium isolates had a high similarity with *F. incarnatum* isolate CM9 (Gen Bank Accession No. MN186812.1) and it uploads to NCBI GenBank and named *Fusarium incarnatum* isolate AE 2024 (GenBank Accession No. PP086049; 528 pb). In agreement with the above, 504 isolates were obtained from rotten potato tubers in a previous study conducted by (Gherbawy et al., 2019), all isolates belonged to the genus *Fusarium*. Upon the morphological and molecular identification of these isolates, it was shown that 62.5% of isolates were *F. sambucinum*, 57.5% were *F. oxysporum*, 56.25% were *F. verticillioides*, and 47.5% were *F. incarnatum*. The correct isolation and identification of fungal pathogens using modern methods such as genetic identification is believed to help develop disease control strategies.

5. Conclusions

Three Fusarium isolates were isolated from the rotted potato tubers and identified based on their morphological and microscopic characteristics. Although all isolates were pathogenic, *F. incarnatum* (isolate #3) showed the highest virulence on potato tubers. Therefore, isolate #3 was subjected to further identification based on the sequence of the ITS region. Molecular identification and phylogenetic tree showed that our isolate had high degrees of similarity with other sequences of *F. incarnatum*. Our findings update the information about the phytopathogenic fungi linked to postharvest dry rot of potato tubers and might be useful in the development of an effective strategy to control dry rot disease and reduce its economic losses.

Table 1. Sequences of different *Fusarium* species that significantly align with ribosomal RNA sequences of internal transcribed spacer region from *F. incarnatum*

Description	Max Score	Total Score	Query Cover (%)	E value	Identity (%)	Accession Length (bp)	Accession
F. equiseti isolate ADEL801	976	976	100	0.0	100	583	MN877913.1
F. equiseti isolate QN0826.1	976	976	100	0.0	100	872	MN368509.1
F. equiseti isolate LSU	976	976	100	0.0	100	1035	MN202780.1
F. incarnatum isolate CM9	976	976	100	0.0	100	552	MN186812.1
F. equiseti isolate SU-1	976	976	100	0.0	100	930	MK733313.1
F. incarnatum culture CBS:132194 strain	976	976	100	0.0	100	898	MH877446.1
F. incarnatum culture CBS:130319 strain	976	976	100	0.0	100	898	MH877332.1
F. incarnatum culture CBS:130313 strain	976	976	100	0.0	100	897	MH877326.1
F. incarnatum culture CBS:130312	976	976	100	0.0	100	896	MH877325.1
F. incarnatum culture CBS:131.73 strain	976	976	100	0.0	100	895	MH872348.1
F. incarnatum culture CBS:791.70 strain	976	976	100	0.0	100	921	MH871746.1
F. incarnatum culture CBS:190.60 strain	976	976	100	0.0	100	894	MH869500.1
F. incarnatum culture CBS:189.60 strain	976	976	100	0.0	100	896	MH869499.1
F. incarnatum culture CBS:163.57 strain	976	976	100	0.0	100	895	MH869221.1
F. equiseti culture CBS:186.31 strain	976	976	100	0.0	100	897	MH866628.1
F. equiseti culture CBS:185.31	976	976	100	0.0	100	897	MH866627.1
F. sambucinum culture CBS:135.24 strain	976	976	100	0.0	100	893	MH866280.1
F. lacertarum culture NFCCI:3044	976	976	100	0.0	100	1292	OM837202.1
F. incarnatum strain NU37	976	976	100	0.0	100	546	OL412246.1
F.incarnatum strain 11 28S ribosomal RNA	976	976	100	0.0	100	892	KF181208.1

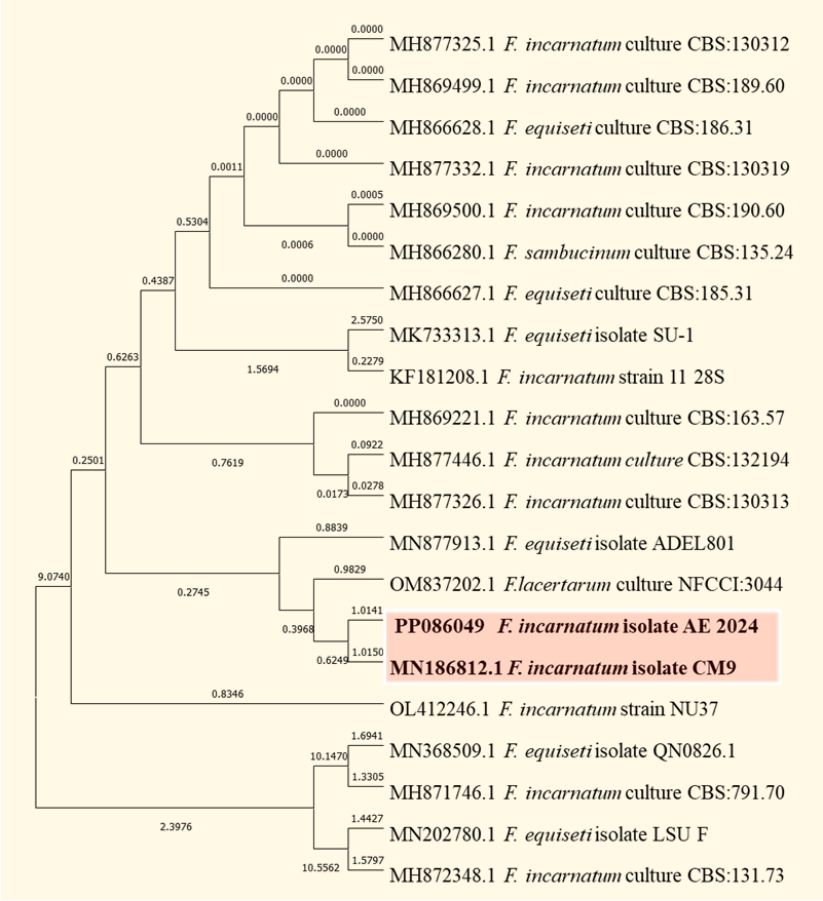

Figure 3. The phylogenetic tree of *Fusarium incarnatum* AE isolate 2024 (GenBank Accession No. PP086049; 528 pb) in comparison twenty references isolate obtained from the NCBI.

Author Contributions: Conceptualization, H.E. and M.H; methodology, A.E.; software, A.E.; validation, A.E-N., A.E and H.E.; formal analysis, A.E-N.; investigation, H.E.; resources, A.E.; data curation, M.H.; writing—original draft preparation, M.H.; writing—review and editing, A.E-N.; visualization, A.E.; supervision, H.E.; project administration, A.E.; funding acquisition, H.E. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: There are no conflicts of interest between the authors.

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