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Research Article
Characteristic of Two *Monosporascus* species Associated with Vine Decline of Melon in Kurdistan Region Iraq

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Abstract

Background and Objective: The 2 species of the genus *Monosporascus* (M), *Monosporascus cannonballus* and *Monosporascus eutypoides*, were discovered and characterized in the rhizospheres of melon-producing fields in the Kurdistan area of Northeast Iraq. The primary objective of this study was to characterize and compare the morphology of *Monosporascus cannonballus* and *Monosporascus eutypoides*, both of which were isolated from sick melon roots and to evaluate their pathogenicity on melon seedlings. **Materials and Methods:** A study on vine decline disease was conducted from 2020 to 2022 in 198 cantaloupe fields in 44 locations randomly chosen from 11 districts of the Iraqi Kurdistan Regions. Estimation of disease prevalence based on plant visuals was made. Isolation from the roots was completed by morphological and molecular testing for isolates of *Monosporascus cannonballus* and *Monosporascus eutypoides*. Pathogenicity was tested on greenhouse-inoculated plants. The prevalence percentage was calculated and the results of PCR analysis were evaluated through standard statistical methods. **Results:** Fertile perithecia and globose smooth ascospores varying in color from dark brown to black were common features across melon field isolates and cultures resembling both *Monosporascus cannonballus* and *Monosporascus eutypoides*. Distinct variations in ascospore amount per ascus between the two species were detected. The ITS and LSU sections were amplified using designated primers, allowing for identification. Greenhouse investigations with artificial root inoculation demonstrated sensitivity to *Monosporascus cannonballus* and *Monosporascus eutypoides*, proving they are harmful to melon seedlings. **Conclusion:** The pathogenic effect of *Monosporascus cannonballus* and *Monosporascus eutypoides* on melon seedlings and revealed their presence in melon fields, giving important information for future studies and agricultural management.

Key words: *Monosporascus*, vine decline, muskmelon, *Monosporascus cannonballus*, *Monosporascus eutypoides*, agricultural management

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The muskmelon, scientifically known as *Cucumis melo*, is a famous summertime delicacy because of its succulent, flavorful flesh. However, the loss in muskmelon harvests due to a disease known as muskmelon collapse is a rising concern in the muskmelon business. It may be related to the widespread issue of vine decline in melon harvests[1]. It's reported that common soil-borne infections cause vines to wilt, become yellow and eventually die.

In this regard, the soil-borne ascomycete *Monosporascus cannonballus* (*M. cannonballus*) is responsible for muskmelons collapsing[2]. This infection promotes *Monosporascus* root rot and vine loss, affecting water and mineral intake and leading to wilting and ultimate plant fatality[3]. The principal cucurbit crops affected by this deadly disease are *Cucumis melo* and watermelon *Citrullus lanatus* (Thunb)[4].

In the past 25 years, there has been a rise in vine decline diseases due to changes in melon farming practices, such as the use of seedling transplants and hybrid types[5]. Cantaloupe infections that look like *Stagonospora* infections suggest several causal agents[1].

Globally, it's reported that *M. cannonballus* is connected to cucurbit melon root rot and vine decline (MRRVD) in 22 countries[6-10]. On the other hand, *Monosporascus eutypoides* (*M. eutypoides*) has been traced down to the soil only in the roots of *Cucumis sativus* L. cucumber and watermelon plants in Tunisia[11].

Moreover, new *Monosporascus* species were also discovered on the roots of *Boerhavia diffusa* L. and *Trianthema portulacastrum* L. during recent investigations in Northeastern Brazil[12]. Morphological and multilocus DNA sequence analysis were used to characterize these species. *Monosporascus brasiliensis*, Almada Negreiros, M. León, J. Armengol and Rua Sales Júnior, *Mucor caatinguensis*, Almada Negreiros, Leon M. León, J. Armengol and R. Sales Jnior, *Erythrolamprus mossoroensis,* Almada Negreiros, Leon M. León, J. Armengol and R. Sales Jnior, *M. nordestinus,* A. Negreiros, M. León, Leon, J. Armengol and R. Sales Jnior*, M. semiaridus,* A. Negreiros, M. León, J. Armengol and R. Sales Jnior[12].

Further research in the Southwest United States, using genetic and culturing methodologies, has uncovered *Monosporascus* as a widespread root endophyte in numerous plants[13-16]. With an emphasis on its worldwide effect and current results in various plant species and locales, this study intends to give complete insights into the complex dynamics of muskmelon collapse disease and its related infections.

MATERIALS AND METHODS

Study area: Disease studies were conducted throughout 198 melon farms in various parts of the Iraqi Kurdistan Regions (IKR) during May, June and August of 2020-2022.

Study design: The purpose of this study was to document the prevalence and geographic range of vine decline melon disease across a range of melon-growing regions and climatic conditions. A total of 198 cantaloupe fields were analyzed, spread out among 44 collecting locations in 11 districts in the provinces of Sulaymaniyah, Garmian, Halabja and Erbil. These areas were selected on purpose to highlight the climatic and elevational contrasts between them. Disease prevalence was calculated as a percentage using the crossing diameter approach and a random sample of five cantaloupe plants from each field.

Disease assessment and species identification: The proportion of infected plants in a specific melon field was estimated by visually inspecting all of the plants there. The diagnostic samples of melon plants were taken from each field at random. Each plant's roots were exposed by thoroughly washing the dirt away and initial inspections for root colonization were undertaken visually.

In suspected samples, roots with perithecia containing single-spored asci of *Monosporascus cannonballus* and spored asci of *Monosporascus eutypoides* were observed, proving the presence of the pathogen at the root level. For the goal of isolation, various root tissues, including tap roots, crowns, optional and tertiary roots, were used and treated differently depending on the kind of root tissue.

To remove soil particles, a soil molecule was kept under running water for 15 sec[1]. Tap and lateral roots were then surface cleaned with 1% sodium hypochlorite for 2 min after being chopped into little pieces (0.5 to 1 cm). The roots were then filtered on filter paper after being washed in sterile distilled water (SDW) to remove any lingering chemicals.

Fourteen root segments were taken from each plant and placed on a medium containing potato dextrose agar (PDA) and streptomycin sulfate (PDAS) at a concentration of 0.5 mg/mL. Over 7 days, plates were checked daily to see whether the fungi were growing and later, hyphal tips moved from all of the colonies to PDA to continue their development and sporulation. Characteristic morphological traits were used to distinguish between *M. cannonballus* and *M. eutypoides* colonies.

Molecular identification: Following the technique of Favorgen Biotech Company, Taiwan, NA was extracted from the fungal mycelium of *M. cannonballus* and *M. eutypoides*. Using a nano-drop spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA), 18-53 ng/L was determined as the appropriate concentration range for the isolated DNA.

For PCR amplification, a Taq DNA Polymerase kit from Favorgen Biotech Company, Taiwan, was applied. The ITS region was amplified using universal primers for three different kinds of PCR tests. Forward: 5'-TCC GTA GGT GAA CCT GCG-3' and reverse: 5'-TCC GCT TAT TGA TAT GC-3'; these are the ITS1/ITS4 primers. The LSU primers[17] and Bt2 primers (GGTAAC CAA ATC GGT GCT GCT TTC, ACC CTC AGTGTA GTG ACC CTT GGC) were also used to study non-sporulating fungi.

Briefly, small tubes contained the PCR reaction mixture, which included 50 ng/L DNA, 2l forward primer, 2l reverse primer, 18l di-ionic distilled water and 25l master mix. Denaturation at 95°C for 40 sec (40 cycles), annealing at 55°C for 50 sec, extension at 72°C for 1 min and final extension at 72°C for 8 min (1 cycle) were applied to the PCR mixture using universal primers (ITS1/ITS4). Denaturation at 95°C for 40 sec (40 cycles), annealing at 58°C for 50 sec, extension at 72°C for 1 min and final extension at 72°C for 8 min (1 cycle) were applied to the PCR mixture containing the LSU/LR5 and BT2 primers. While electrophoresis (Bio-Rad Laboratories, Hercules, California, USA) of PCR products on a 1.5% agarose gel at 80 V for 45 min was used to analyze the samples, which were then stained with ethidium bromide solution (Sigma-Aldrich, St. Louis, Missouri, USA) and photographed under a UV transilluminator (Biobase, China)[11].

Macrogen Molecular Company, Korea, processed the DNA fragments further and sequenced them using the ABI Prism Terminator Sequencing Kit (ABI Prism Terminator Sequencing Kit, Applied Biosystems, Foster City, California, USA).

Pathogenicity testing: Greenhouse incubation at 32±2°C for 62 days followed by inoculation of muskmelon seedlings (Taj genotype) with *Monosporascus cannonballus* and *Monosporascus eutypoides* isolates. Specific primers were employed for re-isolation and pathogen identification[18].

Statistical analysis: A phylogenetic tree constructed using the ITS and LSU nucleotide sequences of *Monosporascus cannonballus* and *Monosporascus eutypoides* using the ClustalW algorithm through MEGA×software (11) was statistically evaluated in the following way: Maximum likelihood (ML) trees were constructed with bootstrap analysis (1000 replicates). To find this similarity percentage among the isolates, BLAST searches against the GenBank database were conducted to provide a solid framework to study genetic relationships and similarities among them.

RESULTS

The isolated *M. cannonballus* and *M. eutypoides* were achieved by studying injured melon roots cultivated in the areas of Iraqi Kurdistan. The physical features of the isolates were used for identification and this was confirmed by amplifying 650 and 1200 bp of the ITS and LSU sections, respectively, using specified primers (Fig. 1). Soil-borne fungal pathogens such as *Monosporascus* species, *Acremonium vitellinum*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Fusarium falciforme*, *Fusarium equiseti*, *Pythium hydnosporum*, *Phytophthora colocasiae* and *Macrophomina phaseolina*, were all isolated as contributors to the vine decline complex.

The ascospores of *Monosporascus cannonballus* and *Monosporascus eutypoides* were found in the melon fields that were examined. Characterization of phenotypes in PDA-grown specimens of many species indicated clear differences between them. Globoid perithecia (250-710 m in diameter),

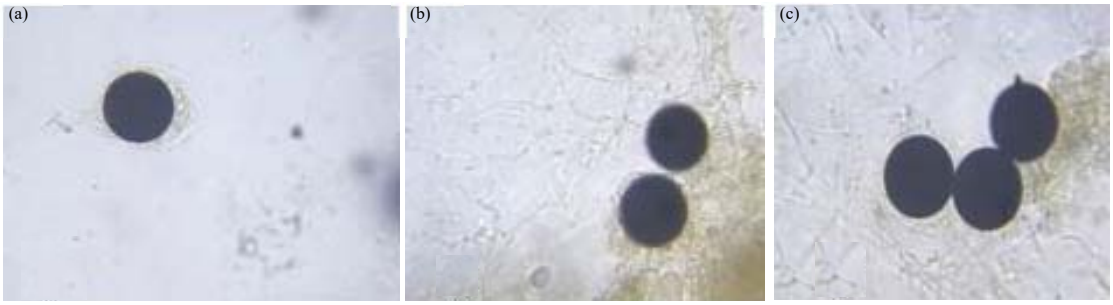

Fig. 1(a-c): *Monosporascus cannonballus* ascus with a (a) Single ascospore, developed on PDA after 35 days of incubation at 27°C. Additional examples of *Monosporascus eutypoides* asci show (b) Two and (c) Three ascospores per ascus, respectively

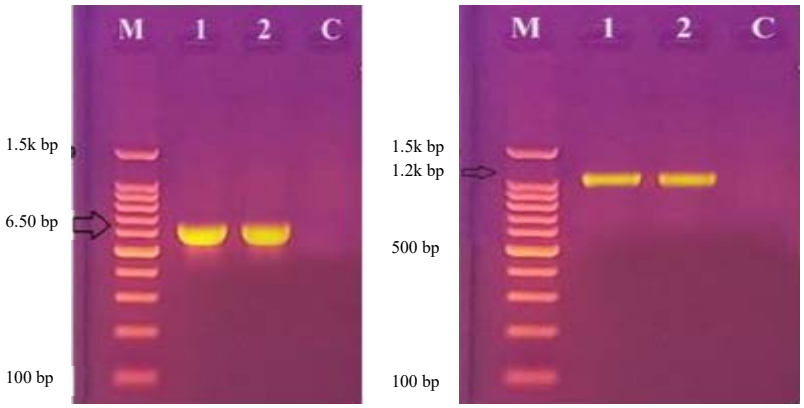

Fig. 2: Amplified 650 and 1200 bp of the ITS and LSU sections using specified primers

Table 1: Phylogenetic tree of *Monosporascus cannonballus* based on ITS and LSU

Isolate species	GenBank account number	Strain number	Host (source)	Geographic location	Similarity (%)
Monosporascus cannonballus	KT826570	MC-8	*Cucumis melo*	China	100
Monosporascus cannonballus	JQ743053	MC1703	*Cucumis melo*	Spain	99.46
Monosporascus cannonballus	JQ743054	MC2303	*Citrullus lanatus*	Spain	99.45
Monosporascus cannonballus	JQ958966	TX923038-3C	*Cucumis melo*	USA	99.45
Monosporascus cannonballus	JQ958965	MC0399	*Cucumis melo*	Portugal	99.46
Monosporascus cannonballus	JQ743056	MC341-C	*Cucumis melo*	Spain	99.45
Monosporascus cannonballus	MH179068	Mono2	Melon root	Iraq	99.25
Monosporascus cannonballus	JQ762394	HON980076-2	Muskmelon	Honduras	99.45
Monosporascus cannonballus	KY072941	TG-2	Melon	China	99.46
Monosporascus cannonballus	NR111370	ATCC 26931	Melon	USA	99.43
Monosporascus cannonballus	KY072940	TG-1	Melon	China	99.46
Monosporascus cannonballus	OP554759	R-2	Melon root	Iraq	99.39
Monosporascus cannonballus	MN880223	JAHLH10	Plant	Iraq	99.44
Monosporascus cannonballus	MT351999	F_HROOT3_1061	Melon	Occupied Palestine	92.50
Monosporascus cannonballus	OP550087	Re-2	Melon root	Iraq	97.28
Monosporascus cannonballus	MG748823	PEP1	*Cucumis melo* cv. Global F1	Greece	99.29
Monosporascus cannonballus	OP550088	Re-3	Melon root	Iraq	97.28
Monosporascus sp.	KU612440	MH10PH	Moss BSC	USA	

clavate to pyriform asci containing a single spore and globoid, smooth, dark brown to black ascospores (32.5-47.5 m in diameter) were all features of *M. cannonballus* ascomata and sporulation. While the perithecia of *M. eutypoides* were spherical (400-940 m in diameter) and the asci were cylindrical and mostly two-spored, with spherical, smooth, dark brown to black ascospores (34-47.0 m in diameter) within.

Sequencing and phylogenetic analyses: The partial 5.8S rRNA (ITS) gene amplification by Polymerase Chain Reaction (PCR) in fungal samples was shown in Fig. 2. Lanes 1-28 show the PCR result from the fungus; M is the DNA ladder (3K-100 bp) and C is the negative control. Isolated fungus samples (one per lane) are labeled with numbers from 1 to 30, representing 30 different fungal species. The fungal kinds that correspond to the given code numbers were listed in Table 1. Bands of the correct sizes, as shown in Fig. 2, were successfully produced using the primers tested. Table 2 summarised the findings from the species identification and screening processes.

Pathogenicity testing of the isolates: The local Taj muskmelon genotype was used to confirm the pathogenicity of two isolates, i.e., *M. cannonballus* and *M. eutypoides*. These isolates were used to inoculate the native Taj muskmelon genotype gathered from several areas in the Kurdistan Region of Iraq. Symptoms of wilting, stunted development and root rot were seen 4-7 weeks after inoculation, as shown in Fig. 3a-e. The inoculated plants were kept at 30±2°C for 62 days with *M. cannonballus* and *M. eutypoides*, whereas the control plants were not infected. While 7 weeks post-inoculation, the plants infected with *M. cannonballus* and *M. eutypoides* displayed signs such as chlorosis and wilting in the vine and rotten feeder roots. In contrast, control plants that were not injected showed no signs of infection in their roots or leaves.

Fig. 3(a-f): Pathogenicity testing of *Monosporascus cannonballus* and *Monosporascus eutypoides* isolates on muskmelon. The inoculums of *M. cannonballus* and *M. eutypoides* collected from different places in Kurdistan Region of Iraq were prepared and used to inoculate a genotype of muskmelon named locally Taj, (a-d) Inoculated by *M. cannonballus* and *M. eutypoides* and non-inoculated plants and (e-f) Were kept at 30±2°C for up to 62 days
(a-d) Inoculated plants showed chlorosis and wilting in vine and rotted feeder roots at 7 weeks post-inoculation and (e-f) A healthy root and leaves represented of non-inoculated fungi, means control

Table 2: Phylogenetic tree of *Monosporascus eutypoides* based on ITS and LSU

Isolate species	GenBank account number	Strain number	Host (source)	Geographic location	Similarity (%)
Monosporascus eutypoides	OP554778	R-21	Melon root	Iraq	100
Monosporascus eutypoides	MK183805	NQ6GIII15	*Lycium barbarum*	China	100
Monosporascus eutypoides	JQ958962	MT54	*Citrullus lanatus*	Tunisia	94.93
Monosporascus eutypoides	JQ958964	MT47	*Citrullus lanatus*	Tunisia	94.96
Monosporascus eutypoides	JQ958963	MT45	*Citrullus lanatus*	Tunisia	95.44
Monosporascus eutypoides	MH866020	CBS 132472	Watermelon	Tunisia	95.48
Monosporascus eutypoides	OP550106	Re-21	Melon root	Iraq	96
Monosporascus eutypoides	MH877468	CBS 132472	Plant	Tunisia	100
Monosporascus sp.	KU612440	KU612440	MH10PH	Moss BSC	Out group

Evolutionary relationships of taxa: Figure 4 depicted the best-possible tree, with a total branch length of 0.63079189. The bootstrap test (500 repetitions), reveals the proportion of replicate trees in which the linked taxa cluster together along the branches. The tree's branch lengths were shown in units that are analogous to the evolutionary distances used in phylogenetic inference. These distances were calculated using the p-distance approach and expressed as base-per-site differences. A total of eighteen nucleotide sequences were used in the study and the analysis included codon locations from the 1st+2nd+3rd+noncoding areas. After removing all of the places from the dataset that had blanks or missing information, we were left with 194. Evolutionary studies were done using MEGA6. The ITS and LSU gene sequences for our isolate have been submitted to the NCBI Gene Bank nucleotide database with the appropriate accession codes OP554759 and OP550087, respectively. In the ITS gene, the isolate OP554759 matches with an Iraqi counterpart, MN880223, while in the LSU gene, our isolate OP550087 matches with a Greek counterpart, MG748823.

Furthermore, the phylogenetic tree of *M. eutypoides* based on ITS and LSU was also created. The optimal tree with the sum of branch length = 0.62347258 was shown. The bootstrap test was performed 500 times to assess the

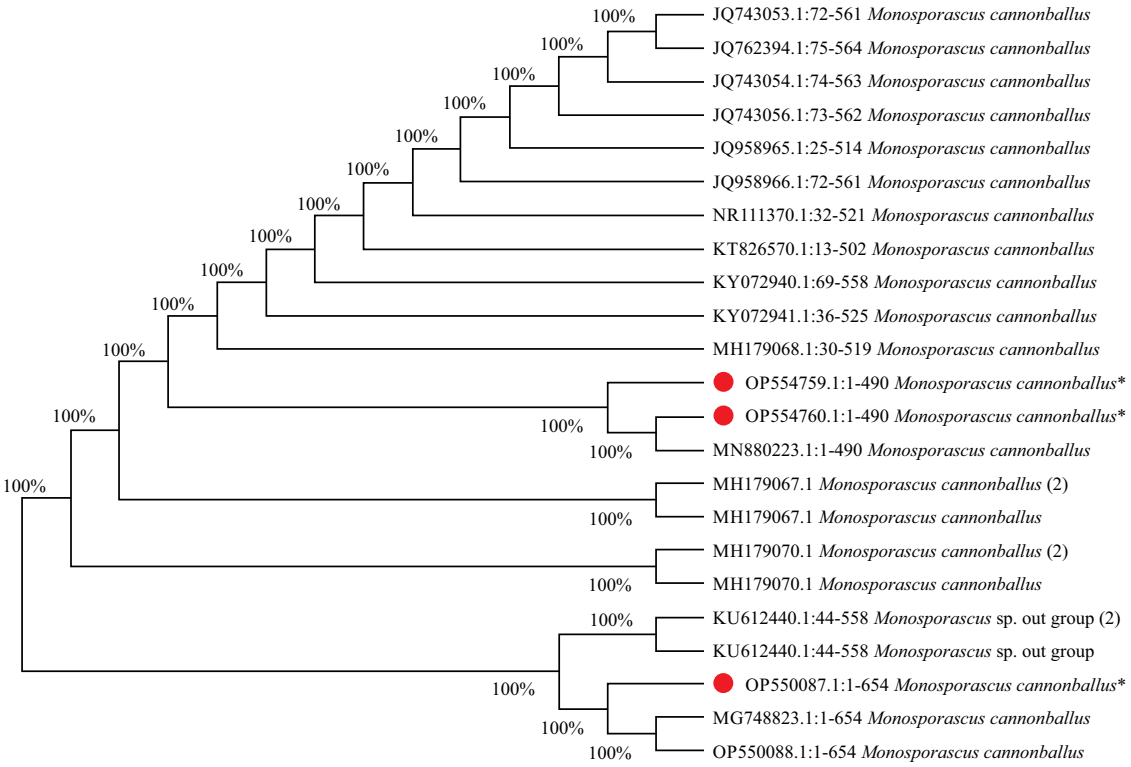

Fig. 4: Evolutionary relationships of taxa

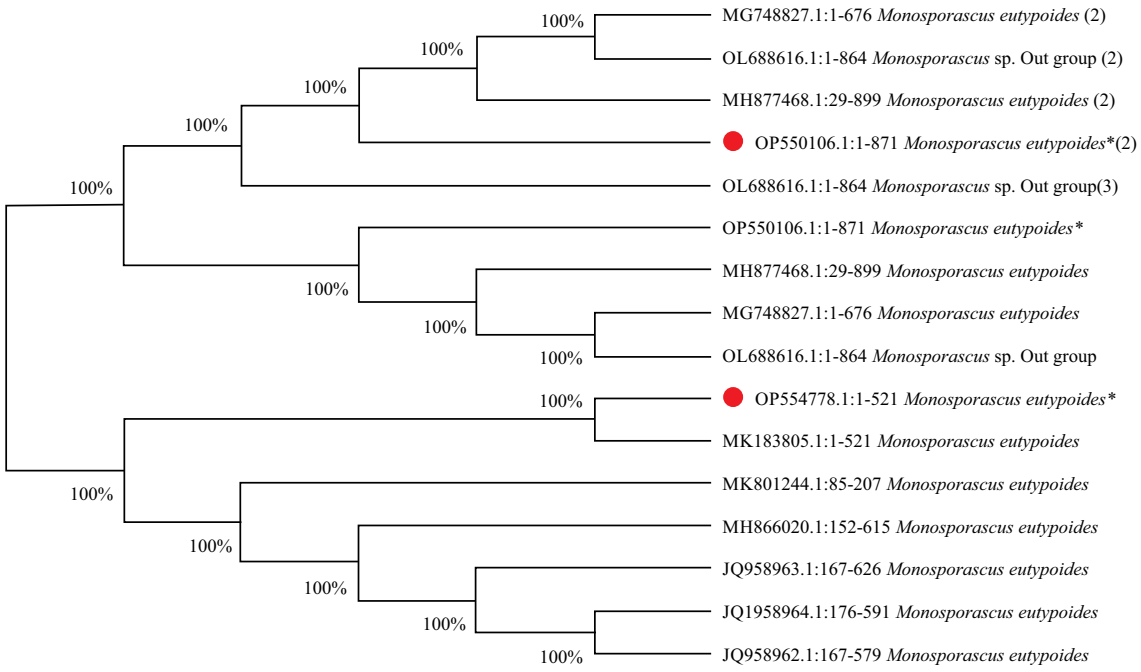

Fig. 5: Evolutionary relationships of taxa

reliability of the tree topology and the results were shown along with the branches. The tree's branch lengths are proportional to the evolutionary distances between nodes, as calculated by the p-distance technique, with distances expressed in terms of base differences per site. The phylogenetic analysis includes nine nucleotide sequences, encompassing codon positions 1st+2nd+3rd+noncoding. After carefully cleaning the data for gaps and missing values, 391 unique locations were left. The MEGA6 was used to perform the evolutionary analyses. The ITS and LSU gene sequences for our isolate have been submitted to the NCBI Gene Bank nucleotide database with the appropriate accession codes OP554778 and OP5500106, respectively.

DISCUSSION

The results confirm the presence of *M. cannonballus* and *M. eutypoides* species in melon fields in Iraqi Kurdistan. The genetic analysis based on ITS and LSU sequences (Fig. 1) supports the identification of perithecia and ascospore morphology. The genetic relationship showed high similarity to some of the strains identified in former studies (Fig. 2).

Strong pathogenicity tests (Fig. 3) revealed the severe impact of these pathogens on melon crops: Infected plants developed very severe symptoms of vine decline and root rot. It has a strong emphasis on the fact that management of soil-borne pathogens is needed to avoid the loss of the crop. On the other hand, comparisons made between the inoculated and the control plants emphasize the pathogenic nature of *M. cannonballus* and *M. eutypoides*, being that the control plants did not develop any infections, confirming the association of these pathogenic fungi in the complex of vine decline in that area.

This is the first in-depth look into *Monosporascus* root rot and vine decline in melons in the Kurdistan area. Still, prior research has shown that both diseases may be caused by either *Monosporascus cannonballus* or *Monosporascus eutypoides*[19-21]. Consequently, morphological characterization allowed us to isolate *M. cannonballus* and *M. eutypoides* from diseased melon roots in sampled areas; PCR testing validated the isolates. One of the key aims of this research was the identification of ascospore features of *M. cannonballus* and *Monosporascus eutypoides* in melon farms around Kurdistan. This piece of the study helps shed light on the pathogen's morphology in its local setting, which in turn improves our comprehension of disease dynamics in the area. Since these infections have been confirmed in local melon crops, it is crucial to keep an eye out for them and implement strategic management plans to lessen the blow of *Monosporascus*-related illnesses on melon farming in this region. Insights into the larger evolutionary context of the investigated gene sequences are provided by these relationships, which emphasize the genetic relatedness of our isolates to counterparts from various geographic locations.

Consequently, a phylogenetic tree of *M. cannoballus* based on ITS and LSU was created. The evolutionary history was inferred using the UPGMA method[22]. The optimal tree with the sum of branch length = 0.63079189 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) shown next to the branches[23]. The phylogenetic tree is inferred using the same units of evolutionary distances and the tree is shown to scale with branch lengths in the same units. Evolutionary distances are measured in base differences per site and were calculated using the p-distance technique[24]. A total of eighteen nucleotide sequences were assessed. Positions 1-3 and noncoding codons were included. Every position that had blanks or incomplete information was removed. The completed dataset had a total of 194 locations. The MEGA6 environment was used for the evolutionary analysis[25]. The ITS and LSU gene sequences for our isolate (*M. cannoballus*) have been submitted to the NCBI Gene Bank nucleotide database with the appropriate accession codes OP554759 and OP550087, respectively, (Fig. 4). In the ITS gene, the isolate OP554759 matches with an Iraqi counterpart, MN880223, while in the LSU gene, our isolate OP550087 matches with a Greek counterpart, MG748823. The ITS sequence OP554759 showed striking similarity to other sequences from all across the world when compared to them. The sequence KT826570 originating from *Cucumis melo* in China showed perfect homology (100%) with our strain, indicating a genetic affinity between these isolates. Sequences JQ743053 and JQ743056 recovered from Spanish *Cucumis melo* showed a high degree of similarity, with a 99.46 and 99.45% degree of identity, respectively. Particularly noteworthy is the 99.25% similarity discovered between sequence MH179068 and Iraqi melon root samples.

The current isolate's LSU sequence OP550087 showed a near-identical (99.29%) match to a sequence MG748823 derived from Greek cucumbers. Furthermore, sequence MN880223, taken from an Iraqi plant, showed a high degree of similarity (99.44%) with other sequences. These results highlighted the importance of both local and global connections since they show that genetic similarities exist among isolates originally discovered in China, Spain, Iraq and Greece. The comparative findings, presented in Table 1, lead to a fuller knowledge of the genetic diversity and relatedness of the investigated sequences (*M. cannoballus)* across various

geographic areas. Considering the context of *Cucumis melo*, current findings provide light on the evolutionary processes and probable dispersion patterns of the analyzed genetic material.

Furthermore, the phylogenetic tree of *M. eutypoides* based on ITS and LSU was also created. Using the UPGMA method, the evolutionary history was inferred[22]. The optimal tree with the sum of branch length = 0.62347258 is shown. The bootstrap test is conducted with 500 replicates and the percentage of replicate trees in which the associated taxa clustered together is displayed adjacent to the branches[26]. The phylogenetic tree is depicted to scale, with branch lengths in the same units as the evolutionary distances used to infer it[23]. The p-distance method was employed to calculate the evolutionary distances, which are expressed in terms of the number of base differences per site[2,24]. The examination encompassed nine nucleotide sequences. The 1st+2nd+3rd+noncoding positions were included[3]. Gaps and lacking data were eliminated from all positions. The final dataset contained a total of 391 positions. The MEGA6 was employed to execute evolutionary analyses[25]. Complete homology (100%) was found between the *M. eutypoides* ITS sequence OP554778, Fig. 5 and a sequence MK183805 from *Lycium barbarum* in China, suggesting a close genetic kinship. The ITS sequence was also very comparable to that of *Citrullus lanatus* from Tunisia, exhibiting a degree of genetic conservation between species (94.96 and 94.93% similarity, respectively; JQ958962 and JQ958962). In a similar vein, the LSU sequence OP5500106, Fig. 5, was completely identical to and very comparable to MH877468 and MH866020 sequences present in watermelon from Tunisia. These results place our isolates into a larger phylogenetic framework, indicating that they likely share a common genetic ancestor with individuals from other locations.

The genetic similarity found in the sequencing comparison was supported by the phylogenetic tree constructed using the ITS sequence OP554778, which clustered the current isolate with a sequence MK183805 from China. The evolutionary links shown by the sequencing analysis were supported by the fact that the LSU gene sequence OP5500106 clustered with a sequence MH877468 from Tunisia. The comparative findings, presented in Table 2, lead to a fuller knowledge of the genetic diversity and relatedness of the investigated sequences (*M. eutypoides*) across various geographic areas.

This study helps us understand vine decline disease in melon fields by identifying and characterizing *Monosporascus cannonballus* and *eutypoides* from the rhizospheres of melon-producing farms in the Kurdistan Region of Northwest Iraq. All *Monosporascus* species have been shown to have a wide range of variation among their representative isolates, highlighting the importance of these discoveries. These results highlight the growing importance of the soil-borne pathogen *Monosporascus* root rot and vine loss among melon crops in the Kurdistan area of Iraq. Addressing vine decline disease research gaps will improve regional and global melon production and management. However, the study only covers Kurdistan, which may limit its applicability to other regions with different environmental and agricultural conditions. Three-year temporal scope may miss long-term disease prevalence and pathogen behavior changes. The study does not address pathogen management or control, leaving a gap for future research to develop and test disease control methods.

CONCLUSION

This study set out to characterize and compare the morphology of *Monosporascus cannonballus* and *Monosporascus eutypoides*, two pathogens found in sick melon roots and to evaluate their pathogenicity on melon seedlings. All of the melon field isolates that resembled either of these two species shared the presence of fertile perithecia and globose, smooth ascospores of varying colors. Notably, *Monosporascus cannonballus* and *Monosporascus eutypoides* were found to have significantly different ascospore quantities per ascus. The amplification of ITS and LSU regions with specific primers in a molecular analysis setting allowed for precise classification. Greenhouse tests involving artificial root inoculation indisputably confirmed the pathogenic character of both *Monosporascus cannonballus* and *Monosporascus eutypoides*, demonstrating their negative influence on melon seedlings. These results are important for future research and agricultural management practices in melon fields and they also add to our knowledge of the pathogenicity of these newly identified species. In sum, current findings stress the need for ongoing investigation and surveillance of new plant diseases as part of long-term crop management plans.

SIGNIFICANCE STATEMENT

This study presents the discovery and characterization of two new species, *Monosporascus cannonballus* and *Monosporascus eutypoides*, within the *Monosporascus* genus, isolated from the rhizospheres of melon-producing fields in Northwest Iraq. Morphological analysis revealed common features such as fertile perithecia and globose, smooth

ascospores, with distinct variations in ascospore amount per ascus between the two species. Genetic identification through amplification of ITS and LSU sections provided reliable differentiation. Greenhouse investigations demonstrated the pathogenicity of both species on melon seedlings, highlighting their harmful effects on crop production. These findings underscore the importance of further research and effective agricultural management strategies to mitigate the impact of these pathogenic fungi on melon crops in the region.

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