INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 13, No. 6, p. 83-88, 2018
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Morphological and molecular identification of *Monosporascus cannonballus* causal agent of melon root rot and plant decline in Iraq

Hanan W. Najem*, T. A. Kareem

Plant Protection Department, Faculty of Agriculture , University of Baghdad, Iraq

Article published December 15, 2018

Key words: Melon, *Monosporascus cannonballus*, Root rot and vine decline.

Abstract

The study was conducted to characterize the causal agent of root rot and melon vine decline based on morphological characteristics and at molecular level. Pieces of melon infected roots were surface sterilized in 1% sodium hypochlorite and placed on Potato Dextrose Agar (PDA) in petri plates and the fungi grown were purified and identified . Based on morphological characteristics six isolate belong to *Monosporascus cannonballus*, Mono1 – Mono6 were identified . The isolates Mono1 – Mono4 were found highly pathogenic to radish and melon seedlings with discoloring root length between 4.45 – 5 mm. The morphological characteristics were confirmed by PCR amplification of isolates DNA using specific and general primers. The analysis of PCR amplification products by agarose gel electrophoresis revealed bands of 112bp long when using specific primers , 600bp with general ITS1/ITS4 primers. The neighbor – joining phytogenetic tree and sequence comparison with M. cannonballus isolates in Gen Bank revealed high identify (Up to 99%) with the isolates from Tunis, Spain, China and clustered in the same group. The Iraqi isolates were recorded in Gen Bank under accession numbers MH179067 – MH179070.

***Corresponding Author:** Hanan W. Najem ✉ hananv153@gmail.com

Introduction

The melon, *Cucumis melo*, is one of the famous vegetables in Cucurbitaceae family, Cultivated at wide areas in the world and in Iraq. It was reported that melon fruits are rich in many nutrients including vitamins, minerals, and folic acid (Geo *et al*, 2007).

Melon plants are subjected to infection with many pathogens, Fungi, Bacteria, Nematodes, and Viruses, Wherever grown in the world causing heavy reduction in yield (Siguenza *et al* , 2005). Of the fungi infecting melon, Monosporascus cannonballus, Soil born pathogen, belong to Ascomycota, is characterized by infecting wide host range in *Graminaceae*, *Leguminaceae* in addition to *Cucurbiaceae* worldwide causing heavy losses in yields (Martyn, 2002, Stanghellini *et al*, 2004, Cintia *et al*, 2013).

The symptoms inducted by *M. cannonballus* on melon plants are manifested on root as rot , brown discoloring , root hairs deterioration and formation of perithecia. The symptoms on root were found associated with foliage yellowing, small fruits formation and plant decline (Martyn, 2002).

Symptoms of yellowing , stunting , reducation in fruit formation associated wih root rot , root browning suspected to be of *M. cannonballus* infection were observed in melon fields. The study was conducted to characterize the causal agent of these symptoms morphologically and at molecular level.

Material and methods

Fungi Isolation and Identification

Samples of melon plants showing root rot symptoms associated with general foliage yellowing and stunting were collected from different location in Iraq in 2017. Stems and roots of the plants were washed with runing water for 30 mins and cut to 0.5 cm pieces. The pieces were surface sterilized with 1% sodium hypochloride for 2 mins, washed with sterile distilled water and dried on filter papers. The pieces were then placed on Potato Dextrose Agar (PDA) amended with 100 mg/l amoxillin in 9 cm dim petriplates (4 sample pieces /=plate) and maintained at 25 ˚c for 3 days. The fungi grown from the sample piece were purified by transferring a small part of colony border into the center of new petriplates containing PDA. The plates were maintained at 25 ˚c for 30 days in the dark and the fungi were identification based on morphological characteristics as described by Cluck *et al*, (2009).

Pathogenicity of M. cannonballus Isolates

Discs of 0.5 cm dim of *M. cannonballus* growth on PDA were inoculated on water agar (WA) in 9 cm dim petriplates and maintain at 25 ˚c for 48 hrs. Surface sterilized seeds of radish and melon were sown in the plates (5 seeds/plate) with four replications. The length discoloring areas on the seedling roots were estimated after one week of sowing and compared with those in non-inoculated plates. The disease severity index was assessed as described by Sneh *et al* , (2004) The virulence of isolates was determined based on the length of discoloring area on melon seedling roots by a scale of 5 degree, where 0 – 0.3mm = Avirulent, 0.5 – 1.9 mm = low virulent, 2 – 2.9 mm = moderate virulent, 3 – 3.9 mm = virulent, 4.0 ≥ 5 = strong virulent.

Molecular Identification

DNA Extration

DNA of *M. cannonballus* was extracted from fungal mycelium according to Corean Bioneer Company instruction. The concentration of DNA was measured by nano-drop spectrophotometer and adjusted to 5 ng/l.

PCR Assays

PCR amplification was performed using taq DNA Polymerase kit (Bioneer Company/Corea) and two type of primers, Specific primers forward CTT ACC TAT GTT GCC TCC GC and reverse, AAG AGT TTA GAT GGT CCA CCG G, (Abolfazl *et al*, 2012) , and general primers ITS1/ITS4 forward TCC GTA GGT GAA CCT GCG G. and reverse AAG AGT TTA GAT GGT CCA CCG G. (Ben *et al* , 2013) PCR reaction mixture consisted of 5ml DNA , 2ml forward primer,

2ml reverse primer, 11ml di ionic distilled water, 5ml master mix in small tubes. The mixture containing the specific primer was pre-incubated at 50 ˚c for 2 mins, pre-denaturated at 95 ˚c for 5 mins, denaturated at 95 ˚c for 15seconds (45 cycles), annealed at 60˚c for one minute and then extended at 72˚c for 10 mins. In a PCR thermal cycler.

The PCR mixture containing the general primers ITS1/ITS4 was denaturated at 95 ˚c for 2 mins, denaturated at 94 ˚c for 30 seconds (35 cycles), annealed at 55˚c for one minute, extended at 72˚c for one minute, and final extention at 72˚c for 10 mins.

The PCR products were analyzed by electrophoresis on 1.5% agarose gel at 100 v. For 30 mins and then visualized by staining the gel in ethidiom bromide solution and photographed under UV trans illuminator (Ben *et al*, 2013).

The DNA fragment was extracted and submitted to sequencing to Bio – Gen Companey.

Result and discussion

Isolation and Identification

Six isolates from infected melon plants showing root rot symptoms and plant decline at different locations in Iraq were obtained. The isolates were firstly identified based on morphological characteristics and then confirmed by PCR analysis using specific primers and universal primers. The results revealed the identity of the isolates and that belong to *Monosporascus cannonballus,* designated as Mono1 – Mono6 (Table 1, Fig 1).

Table 1. Sources and location of *M. cannonballus* isolates in Iraq.

Isolates location	Number of samples	Number of isolates	Name of isolates
Abu-Ghraib	33	3	Mono3, Mono5, Mono6
Al-Jadria	37	4	Mono1, Mono4,
Balad	5	1	Mono2

Table 2. Pathogenicity and disease severity of *M. cannonballus* on radish and melon seedling in petriplates.

M. cannonballus iolates	Mean length of root discoloring/mm		Pathogenicity
	melon	radish	
Mono1	4.45	4.55	Strong virulent
Mono2	4.95	5.00	Strong virulent
Mono3	5.00	4.90	Strong virulent
Mono4	4.70	5.00	Strong virulent
Mono5	3.80	3.90	Virulent
Mono6	3.55	3.80	Virulent

Each value in the table in the mean of 4 replications, five plant in each replication.

Pathogenicity of M. cannonballus Isolates

Results of pathogenicity of *M. cannonballus* isolates revealed that all isolates were pathogenic to radish and melon seedling (Table 2). The isolates, Mono1, Mono2, Mono3, and Mono4, were found highly pathogenic (Strong virulent) with discoloring root lengths, 4.55, 5.00, 4.90, and 5.00mm respectively on radish, 4.45, 4.95, 5.00, and 4.70mm repectively on melon. The isolates, Mono5, Mono6, were considered moderately pathogenic (Virulent), with discoloration root lengths 3.90, 3.80mm respectively on radish and 3.80, 3.55mm respectively on melon (Table 2).A previous study reported that *M. cannonballus* isolates from infected melon plants had the ability to infect melon plants causing considerable economic losses in yield (Alendri *et al*, 2017).

Molecular Identification

The analysis of PCR amplification products of pathogen isolates DNA using *M. cannonballus* specific primers, by electrophoresis on agarose gel showed a band of 112 bp long of each isolates (Fig. 2.). Similar results were obtained by Abofazl (2012) concerning the amplification of *M. cannonballus*

DNA using specific primer. Bands of 600 bp long were detected on the gel when the amplification of isolates DNA was carried out using the universal primers ITS1/ITS4 (Fig. 3).

Table 3. Comparision of Iraqi fungal isolates to *M. cannonballus* for ITS region and its Gene Bank counterparts.

No.	Fungal isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	MH179067-Mono1-Iraq															
2	MH179068-Mono2-Iraq	99														
3	MH179069-Mono3-Iraq	99	99													
4	MH179070-Mono4-Iraq	99	99	99												
5	JQ771928.1-Tunis	99	99	99	99											
6	JQ771932.1-Tunis	99	99	99	99	99										
7	JQ762382.1-Spain	99	99	99	99	99	99									
8	AF340014.1-Spain	99	99	99	99	99	99	99								
9	JN977054.1-Spain	99	99	99	99	99	99	99	99							
10	JQ958966.1-Tunis	99	99	99	99	99	99	99	99	99						
11	JQ743056.1-Spain	99	99	99	99	99	99	99	99	99	99					
12	JQ743054.1-Spain	99	99	99	99	99	99	99	99	99	99	99				
13	JQ771930.1-Tunis	99	99	99	99	99	99	99	99	99	99	99	99			
14	KY072941.1-China	99	99	99	99	99	99	99	99	99	99	99	99	99		
15	KY072940.1-China	99	99	99	99	99	99	99	99	99	99	99	99	99	99	
16	KF312464.1-*Rhizoctonia solani*	95	95	95	95	95	95	95	95	95	95	95	95	95	95	95

The morphological characteristics with the molecular diagnosis using specific primer confirmed that the isolates obtained from melon plants showing root rot, yellowing and plant decline belong to *M. cannonballus*.

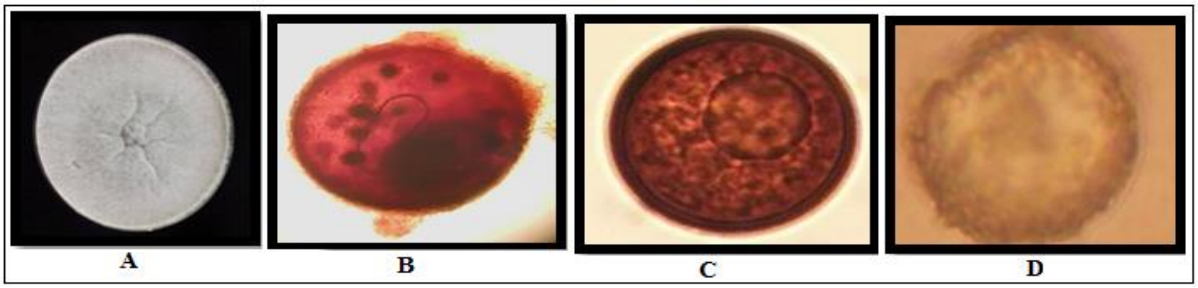

Fig. 1. *M. cannonballus* growth on PDA in petriplates, A= Fungal colony, B= Fruiting body, C= Fruiting ascus D= Ascospore.

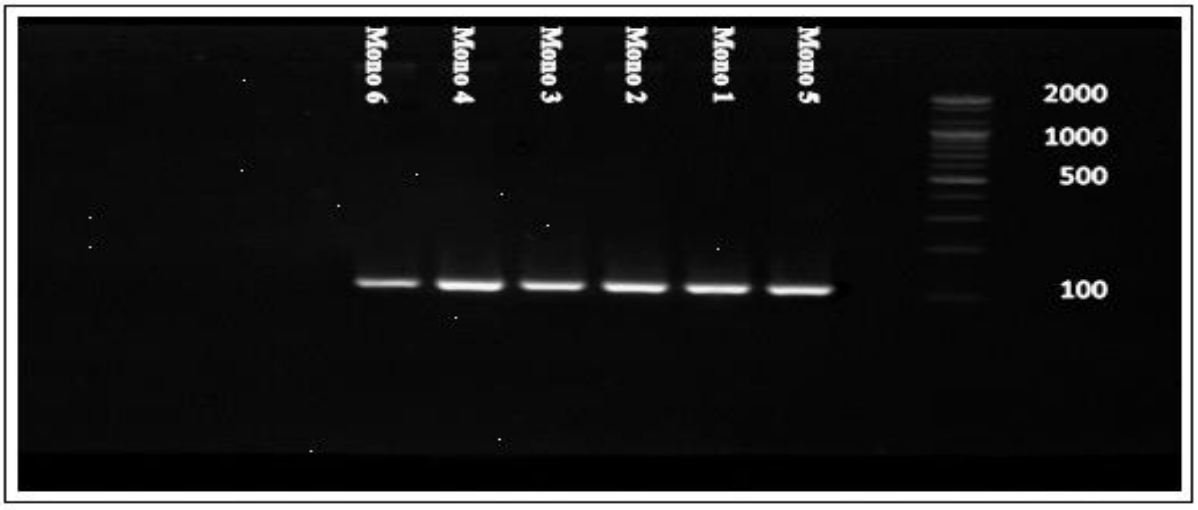

Fig. 2. Agarose gel electrophoresis of *M. cannonballus* DNA resulted from PCR amplification using specific primers.

The Neighbor – Joining phylogenetic tree, done by mega 7 program with comparison with *M. cannonballus* isolates from Tunis, Spain, and China which clustered in same group. The Iraqi isolates were recorded in Gen Bank under accession numbers MH179067 – MH179070. (Table 3, Fig 4).

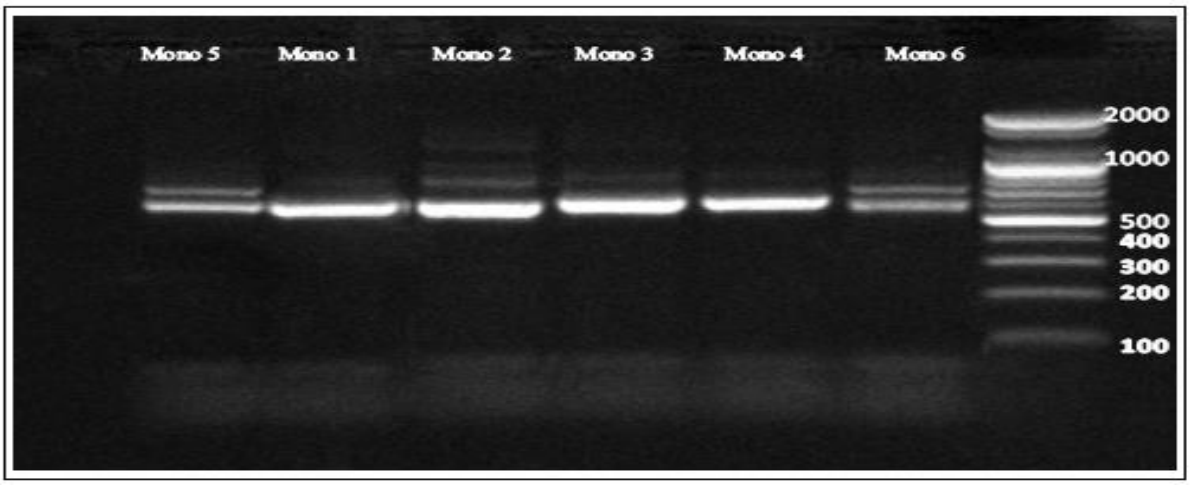

Fig. 3. Agarose gel electrophoresis of *M. cannonballus* DNA resulted from PCR amplification using ITS1/ITS4 primers.

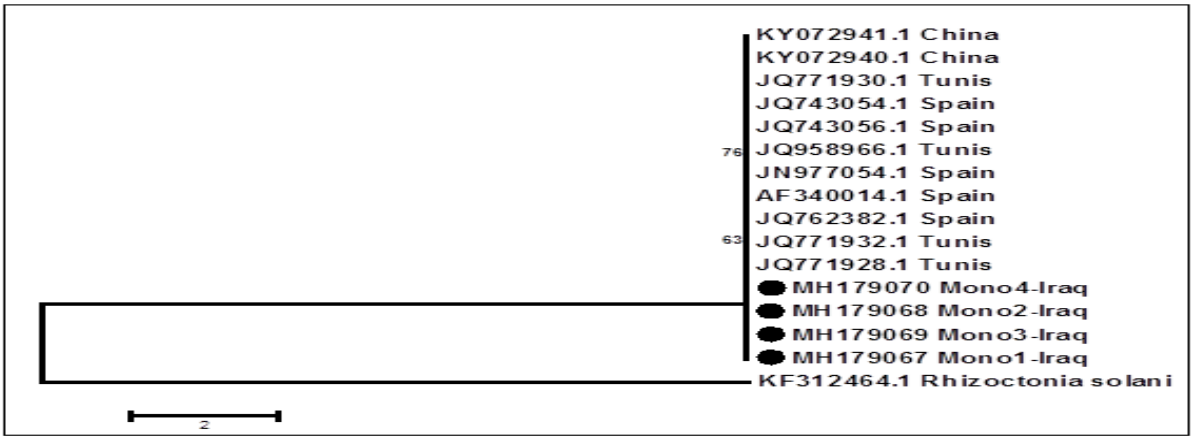

Fig. 4. Neighbor – Joining phylogenetic tree of *M. cannonballus* constructed based on 600 bp sequences obtain using ITS1/ITS4.

The results of this study demonstrated that roots of melon plant are subjected to infection with the fungus *M. cannonballus* causing high damage to root system. The damage of roots may cause a reduction in nutrients uptake that result to plant deterioration and death. These rusults indication the necessity of searching mean to prodect plants against *M. cannonballus* infection.

Refrances

Abolfazl S, Vadieh, Mohammed R. 2012. Detection of Monosporascus cannonballus from melon plants using PCR**.** J. Crop Prot. **1(4),** 349-359.

Alendri MP, Martigononi D, Reda R, Chilosi G. 2017**.** Effect of Preconditioning Through Mycorrhizal Inoculation on the Controlof Melon Root Rot and Vine Decline Caused by Monosporascus cannonballus. Phytopathology Journal **(163),** 898-907.

Ben SI, Correia KC, Boughalleb N, Michereff SJ, Leon M, Abad-Campos P, Garcia-Jimenz J, Armengol J. 2013. M. eutypoides, acause of root rot and vine decline in Tunisia, and evidence that M. cannonballus and M. eutypoides are distinct species. Plant Dis. **97,** 737-743.

Cintia DSB, Kamila CC, Marcos PSC, Rui SJ, Josep A, Sami JM. 2013. Population structure of Monosporascus cannonballus isolated from melons produced in Northeastren Brazil based on mycelium from compatibility groups. Acta Scientiarum **35,** 161-167.

Cluck TW, Biles CL, Duggen M, Jackson T, Carson K, Armengol J, Garcia-Jimenez J, Bruton BD. 2009. Association of dsRNA to Down Regulation of Perthecia Synthesis in Monosporascus cannonballus The Open Mycology Journal **3, 9-19.**

Guo Y, Liu L, Zhao J, Bi Y. 2007. Use of silicon oxide and sodium silicate for controlling Trichothecium roseum postharvest rot in Chinese cantaloupe (Cucumis melo L.). International Journal of Food Science Technology **42(8),** 1012-1018.

Martyn RD. 2002. Monosporascus root rot and vine decline of melons. The Plant Helth nstructor, http://dx.doi.org/10.1094/PH-I-2002-0612-01.

Siguenza C, Schochow M, Turiniand T, Ploeg A. 2005 **.** Use of Cucumis metuliferus as aroot stock for melon to manage Meloidogyne incognita J Nematol **37(3),** 276–280.

Sneh B, Yamoah E, Stewart A. 2004. Hypovirulent Rhizoctonia spp Isolates from New Zealand soils protect Radish seedling against damping – off caused by R. solani. New Zealand Plant Protection Society **(57),** 54–58.

Stanghellini ME, Kim DM, Waugh MM, Ferrin DM, Alcanatara T, Rasmussen SL. 2004. Infection and colonization of melon by Monosporascus cannonballus in Arizona and California. Plant Pathology **53,** 54-57.