Black root rot caused by *Curvularia inaequalis* on *Pyrus communis* in Iran

Samad Jamali¹ · Hadi Khateri¹ · Nasim Rezaei¹

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Abstract

Pyrus communis seedlings showing symptoms of leaf blight and black root rot were collected in a commercial production area in Kermanshah province, West Iran. Based on morphology and phylogeny of DNA sequence data for the internal transcribed spacer (ITS) rDNA region, the isolated pathogen was identified as *Curvularia inaequalis*. Pathogenicity tests in vivo were made on seedlings. Inoculated seedlings produced characteristic symptoms, and subsequent pathogen recovery confirmed pathogenicity. This study provides the first report of the occurrence of *Curvularia inaequalis* on *P. communis* causing black root rot symptoms in Iran.

Keywords *Curvularia inaequalis* · Phylogenetic analysis · Black root rot · Iran

There are about 20 species in the genus *Pyrus* (Bailey and Bailey 1976). The most commercially grown species throughout the temperate zones of the world and Iran is *Pyrus communis*, also called the European pear. This species is scattered over a large area in Iran, from north to northwest, west, and south central regions (Najafzadeh and Arzani 2015). In Iran, the total area under this crop is estimated to be 19,219 ha with a total production of 180,000 t and a productivity of 2.3 t/ha. Diseases of pear include, fire blight caused by *Erwinia amylovora*, anthracnose caused by *Colletotrichum acutatum*, and scab caused by *Venturia pyrina* (van der Zwet and Keil 1979; Mousakhah and Khodaparast 2011). In May 2019, yellowing, leaf blight, and black root rot were found in seedlings of commercial production area in the Kermanshah province (West Iran) (Fig. 1 d-f). For isolation of the causal agent, the root systems were washed, surface disinfested in 0.5% w/v sodium hypochlorite solution for 3 min, rinsed in sterile distilled water, dried in a laminar flow hood, and plated on potato dextrose agar (PDA) amended with chloramphenicol (25 µg/ml). Plates were incubated at 25 °C for 5 days to allow the fungi to grow. Only a fungus was isolated from the roots and crowns of the infected plants.

Fungal colonies on potato dextrose agar at 25 °C were fast growing (3 cm after one week), velvety and brown to blackish brown. Conidiophores were mostly straight, simple or branched, pale to brown, septate, with 21.7–105.5 (average = 52.6) µm length and 4.7–8.3 (average = 6.7) µm width ($n = 50$), with integrated conidiogenous cells (Fig. 1 a–b). The conidia ($n = 50$) were mostly slightly curved, pale brown to black at maturity, smooth, with three to four septa, and measured 19.5–37.5 × 7.5–13.8 µm (average 25.2 × 9.6 µm) (Fig. 1c). On the basis of the above characteristics, the pathogen was identified as *Curvularia* (Holliday 1980). The voucher specimen was deposited in the Fungal Reference Collection of the Ministry of Jihad-e Agriculture (IRAN 3870C and IRAN 3871C) at the Iranian Research Institute of Plant Protection, Tehran, Iran. Molecular characterization of two fungal isolates (IRAN 3870C and IRAN 3871C) was performed by sequencing nuclear ribosomal DNA internal transcribed spacer (ITS) region. Fungal genomic DNA was extracted using a genomic DNA purification Kit (Sinagen, Iran) according to the manufacturer's instructions, and the ITS region was amplified using the universal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCCTCC GCT TAT TGA TAT G-3') (White et al. 1990). Two sequences with 540 bp and 550 bp were deposited in GenBank (accession nos. MN720350 and MN720351). Results of BLAST analysis (<https://blast.ncbi.nlm.nih.gov/>) of the two isolates showed 100% identity with valid sequences HE861822, HE861821 and FN397304 of *C. inaequalis* deposited in GenBank. Phylogenetic analysis was

✉ Samad Jamali
S.jamali@razi.ac.ir

¹ Department of Plant Protection, College of Agriculture, Razi University, Kermanshah, Iran

Fig. 1 *Curvularia inaequalis*; A. Conidiophores, B. Conidium attached to conidiophores, C. Conidia, D-F. Typical symptoms of pear disease on leaves and roots collected from the field, G-I. Symptoms observed in

pathogenicity test; leaf blight and black root rot symptoms on pear inoculated with *Curvularia inaequalis*, J. Control (bar = 5 μm)

performed using the ITS sequences generated in this study with sequences available in GenBank (Table 1). DNA sequences were edited with BioEdit version 7.2.5 and aligned with ClustalW (Thompson et al. 1994). Poorly aligned positions and gaps of the sequences were excluded from the final alignment of each dataset using Gblocks software version 0.91b (Castresana 2000) under the less stringent parameters selected. The phylogenetic tree was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura 1980). Phylogenetic analysis was conducted in MEGA Ver. X program (Kumar et al. 2018). The sequence of *Alternaria alternata* (AY751456) was used as the outgroup. Phylogenetic tree of ITS region grouped together isolates of this study with other isolates of *C. inaequalis* including ex-type isolate CBS 102.42 (Manamgoda et al. 2014) (Fig. 2). IRAN 3870C and 3871C isolates were used to perform pathogenicity tests. Inocula was prepared by

colonizing fungal isolates on sterile wheat grain (Westerlund et al. 1974). Pear seeds were disinfected with sodium hypochlorite 10% w/v, and then washed three times with sterile distilled water. Ten grams of inoculum was placed under seeds and covered with a thin soil in each pot with a 0.5 kg capacity containing sterilized vermiculite: perlite: soil (2:1:1 v:v:v). Four seeds used in each pot and the inoculated plants were placed in a greenhouse at 24 °C for 35–42 days in a completely randomized design with four replications. Three weeks after inoculation with *C. inaequalis*, symptoms developed as foliar yellowing and black cankers that extended upward and downward of roots of all emerged seedlings (Fig. 1 g-i). Reisolation from all inoculated plants after observation of symptoms were performed, and isolates were compared to original cultures providing evidence for fulfilling Koch's postulates. *Curvularia inaequalis* have been reported as phytopathogenic fungus from zoysiagrass (*Zoysia japonica*),

Table 1 GenBank accession numbers of internal transcribed spacer sequences of rDNA (ITS) of isolates used in this study. The isolates in this study are shown in bold

Species	Strain no.	Locality	Host	GenBank number
<i>Bipolaris chloridis</i>	CBS 242.77	China	Rhodes grass (<i>Chloris gayana</i>)	JN192372
<i>Bipolaris melinidis</i>	BRIP 12898	China	–	JN601035
<i>Curvularia hawaiiensis</i>	BRIP 15933	Australia	Rhodes grass (<i>Chloris gayana</i>)	JN601028
<i>Curvularia hawaiiensis</i>	MFLUCC 10–0730	Thailand	Panigrass (<i>Panicum</i> sp.)	JX256427
<i>Curvularia inaequalis</i>	FMR 11544	USA	–	KP131954
<i>Curvularia inaequalis</i>	C8–1	Iran	Strawberry (<i>Fragaria</i> × <i>ananassa</i>)	JQ585672
<i>Curvularia inaequalis</i>	4Lss1	USA	Buffalo grass (<i>Buchloe dactyloides</i>)	KC897660
<i>Curvularia inaequalis</i>	5BLss4	USA	Buffalo grass (<i>Buchloe dactyloides</i>)	KC897661
<i>Curvularia inaequalis</i>	CBS 102.42	France	Dune Sand soil	KJ922375
<i>Curvularia inaequalis</i>	IRAN 3870C	Iran	<i>Pyrus communis</i>	MN720350
<i>Curvularia inaequalis</i>	IRAN 3871C	Iran	<i>Pyrus communis</i>	MN720351
<i>Curvularia ischaemi</i>	CBS 630.82	Solomon Islands	<i>Ischaemum indicum</i>	JX256428
<i>Curvularia lunata</i>	CBS 730.96	USA	Human lung biopsy	JX256429
<i>Curvularia perotidis</i>	Alcorn 7846–2	Canada	–	AF071320
<i>Curvularia portulacae</i>	CBS239.48	USA	<i>Portulaca oleracea</i>	KJ909775
<i>Curvularia protuberata</i>	CBS 376.65	UK	wavy Hair-grass (<i>Deschampsia flexuosa</i>)	KJ922376
<i>Curvularia spicifera</i>	L1	China	Sugarcane (<i>Saccharum officinarum</i>)	JN695634
<i>Curvularia spicifera</i>	145,392	India	Pui (<i>Basella alba</i>)	AB678224
<i>Curvularia spicifera</i>	CBS 246.62	USA	Buffalo grass (<i>Buchloe dactyloides</i>)	HF934914
<i>Curvularia spicifera</i>	CBS 274.52	Spain	soil	JN192387

bermudagrass (*Cynodon dactylon*) and bentgrass (*Agrostis stolonifera*) in Korea (Kim et al. 2000), peanut (*Arachis hypogaea*) and buffalograss (*Buchloe dactyloides*) in USA

(Amaradasa and Amundsen 2014), velvetgrass (*Holcus lanatus*) in Spain (Herrero et al. 2009; Sanchez Marquez et al. 2010; Manamgoda et al. 2011), and maize plant (*Zea*

Fig. 2 Phylogram of maximum likelihood analysis of two *Curvularia inaequalis* isolates from this study (indicated with triangle shape), together with other isolates of *Curvularia* spp. based on the internal transcribed spacer region of rDNA (ITS). *Alternaria alternata* is included as the outgroup

mays) in Northern China (Xin-hua et al. 2019). Also, it has been reported that *C. inaequalis* is among the most frequent human pathogen within the genus (da Cunha et al. 2013). Several species of the genus *Curvularia* have been reported to infect different field crops such as rice, barley and maize in Iran (Ershad 2009). In Iran, Ayoubi et al. (2017) reported *C. inaequalis* as a pathogen on strawberry causing leaf blight and fruit rot (Table 1).

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