Identity and Pathogenicity of Fungi Associated with Crown and Root Rot of Dryland Winter Wheat in Azerbaijan

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Abstract

A comprehensive survey was performed to assess fungal populations associated with crown and root rot of wheat throughout the main wheat-growing areas of Azerbaijan. Samples were taken from 76 fields; 630 fungal strains were isolated, identified, and evaluated for pathogenicity. The identification was conducted with morphological and molecular tools such as species-specific PCR and DNA sequencing of the internal transcribed spacer (ITS) and *translation elongation factor 1-α* (*EF1-α*) loci. The fungus found in the greatest number of fields (44) was *Fusarium culmorum* with 192 isolates, followed by *F. acuminatum*. Other *Fusarium* spp. isolates were identified: *F. equiseti*, *F. pseudograminearum*, *F. graminearum*, *F. incarnatum*, *F. avenaceum*, *F. hostae*, *F. oxysporum*, *F. proliferatum*, *F. algeriense*, and *F. brachygibbosum*. *Bipolaris sorokiniana*, *Curvularia spicifera*, *Exserohilum pedicellatum*, *Nigrospora oryzae*, and *Rhizoctonia* spp. isolates were also identified, associated with underground parts of wheat. Phylogenetic analyses based

on ITS and *EF1-α* sequences of the isolates showed that the isolates belonging to the same species were clearly separated in the dendrogram. Pathogenicity assays revealed that *F. culmorum*, *F. pseudograminearum*, and *F. graminearum* were most aggressive; *F. avenaceum*, *F. hostae*, *F. algeriense*, *B. sorokiniana*, *C. spicifera*, and *R. solani* isolates were moderately aggressive; *C. inaequalis*, *E. pedicellatum*, and *N. oryzae* were weakly aggressive; and others were nonpathogenic. The result of this study exhibited the existence of a wide range of species associated with crown and root rot of wheat in Azerbaijan. Additionally, this is the first report of *F. hostae*, *F. algeriense*, *C. spicifera*, *C. inaequalis*, and *N. oryzae* as pathogens on wheat in Azerbaijan. Azerbaijan is the second country after Algeria in which *F. algeriense* was detected.

Keywords: *Bipolaris*, common root rot, *Fusarium*, *Fusarium* crown rot, pathogenicity, soilborne diseases, *Triticum* spp., wheat diseases,

Wheat (*Triticum* spp.) is the third most important crop in terms of global production, with an average annual production of almost 772 million tons in 2017, after maize and rice (FAOSTAT 2019). Bread wheat (*Triticum aestivum* L.) and durum wheat (*T. durum* Desf.) provide a major contribution to the diets of humans and livestock in Azerbaijan, with an average annual production of about 1.77 million tons in a planted area of about 596,000 ha. Much of the production is under irrigation, with winter wheat planted from October to November, with an average yield of 3.0 to 3.5 t/ha. Rotation crops under irrigation include cotton, maize, and forage crops. Dryland production in areas with precipitation of <400 to 450 mm/year yield only 1.3 to 1.9 t/ha. Average minimum temperature in January in the rainfed wheat production region of Gobustan is –11.4°C while average maximum temperature in June is 24.8°C. Average annual precipitation ranges from 380 to 420 mm.

A complex of fungi attacking the crown and root tissues of wheat causes a serious problem and significant yield reductions in the major

wheat-producing regions of the world (Bockus et al. 2010; Gonzalez and Trevathan 2000). Although most prevailing species in this complex can change yearly and regionally in dryland winter wheat production areas, several fungi have been reported in the species complex: *Bipolaris sorokiniana* Shoemaker (teleomorph: *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur, syn. *Helminthosporium sativum* Pammel, C. M. King & Bakke), *Fusarium pseudograminearum* O'Donnell & T. Aoki (teleomorph: *Gibberella coronicola* T. Aoki & O'Donnell), *F. graminearum* Schwabe (teleomorph: *G. zae* (Schwein.) Petch), *F. culmorum* (Wm. G. Sm.) Sacc., *F. avenaceum* (Fr.) Sacc. (teleomorph: *G. avenacea* R. J. Cook), *F. equiseti* (Corda) Sacc. (teleomorph: *G. intricans* Wollenw.), *F. oxysporum* Schltdl., *F. acuminatum* Ellis & Everh. (teleomorph: *G. acuminata* Wollenw.), *Microdochium nivale* (Fr.) Samuels & I. C. Hallett (syn. *F. nivale* (Fr.) Sorauer), *F. algeriense* I. Laraba & K. O'Donnell, and *Rhizoctonia solani* J. G. Kühn (teleomorph: *Thanaophorus cucumeris* (A. B. Frank) Donk) (Cook and Veseth 1991; Gonzalez and Trevathan 2000; Hill et al. 1983; Laraba et al. 2017; Moya-Elizondo et al. 2011; Paulitz et al. 2002; Smiley and Patterson 1996; Smiley et al. 2005; Tunali et al. 2008). These pathogens may exist one by one; however, they generally coincide in the same areas and even in individual plants (Paulitz et al. 2002).

B. sorokiniana, causing common root rot (CRR), is the most common species of anamorphs of *Cochliobolus* as a root pathogen of wheat. In addition to causing dark discoloration on lower leaf sheaths, crown, subcrown internode, and root tissues, *B. sorokiniana* causes spot blotch, seed black point, seedling blight, and head blight (Bakonyi et al. 1997). The annual yield losses caused by CRR in wheat were estimated at 5.7% in Canadian prairies and varied between 13.9 and 23.9% in Australia for a susceptible wheat cultivar (Ledingham et al. 1973; Wildermuth et al. 1992). CRR symptoms on cereals also have been determined to be caused by different species of *Bipolaris* and *Curvularia* (anamorph of *Cochliobolus*) such as *Curvularia spicifera* (Bainier) Boedijn,

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C. inaequalis (Shear) Boedijn, and *B. bicolor* (Mitra) Shoemaker, but less severe (Bach et al. 2003; Gonzalez and Trevathan 2000; Morejon et al. 2006).

Fusarium crown rot (FCR) is caused by numerous *Fusarium* spp.; however, several surveys conducted to investigate the species causing FCR have revealed that the most common and devastating species are *F. culmorum* and *F. pseudograminearum* in many wheat-growing regions of the world (Backhouse et al. 2004; Cook 1981; Moya-Elizondo et al. 2011; Shikur Gebremariam et al. 2018; Smiley and Patterson 1996; Tunali et al. 2008). Although *F. culmorum* prefers lower temperatures than *F. pseudograminearum*, summer temperatures in the dryland area increase the prevalence of both pathogens (Cook 2010; Dyer et al. 2009). These species have been frequently isolated from infected plants but a complex etiology of FCR has been demonstrated by several studies (Backhouse et al. 2004; Fedel-Moen and Harris 1987; Laraba et al. 2017; Smiley and Patterson 1996).

Rhizoctonia spp. are less frequently reported as the cause of damaging cereal crown and root tissues. *Rhizoctonia* root rot and bare patch are caused by different anastomosis groups (AGs) of *R. solani*—AG-2-1, AG-3, AG-4, AG-5, AG-8, and AG-11 isolates—which cause lesions on crown and seminal roots of wheat plants in Canada, Turkey, and the United States (Broders et al. 2014; Jaaffar et al. 2016; Ogoshi et al. 1990; Ünal et al. 2015). A decrease in the severity and decline of *Rhizoctonia* root rot to negligible levels has been found in long-term cereal monocrop production (Lucas et al. 1993; MacNish 1988; Yin et al. 2013).

Little is known about the distribution and prevalence of pathogens of underground parts of winter wheat in Azerbaijan. One report showed a prevalence of 17.5 to 21.4% of root rot of wheat crops, mainly *Fusarium* spp. and *B. sorokiniana* in the wheat-growing area of Shaki City (Mehdiyev 2014). Therefore, a detailed survey was carried out with the objective of identifying the fungal pathogens responsible for crown and root rot in the major wheat production areas of the country and testing pathogenicity of isolates belonging to determined species on a susceptible wheat cultivar.

Materials and Methods

Sampling. An extensive survey was conducted to collect samples showing typical symptoms of crown and root rot from 76 winter wheat fields in the main wheat-growing regions of Azerbaijan in June 2017 (Fig. 1). Samples were taken from the wheat plants collected at the stages of near maturity and maturity and after harvest. Sample sites were selected arbitrarily with a separation distance of 7 to 10 km. For each field, 20 to 30 tillers of wheat in a 5-ha area were randomly sampled, put in paper bags, labeled with relevant sample information, transported to the laboratory, and stored at room temperature.

Isolation and maintenance of cultures. Plants were cut about 20 cm above the stem base; the crown, root, and stem base tissues of each plant were rinsed with tap water for at least 15 min to remove soil particles adhering to the surface; and stems were examined for lesions. Small 3-cm sections of the necrotic or discolored crown, sub-crown, and root tissues of the plants were surface disinfested with 1% sodium hypochlorite solution for 1 min, triple rinsed with sterile

Fig. 1. Map of the Republic of Azerbaijan showing the different agro-geographical regions and sampling sites during June 2017.

distilled water, and dried on sterilized filter paper in a laminar flow hood. Dried sections were chopped into 1-cm lengths and placed on 1/5-strength potato dextrose agar (PDA) amended with streptomycin (0.1 g/liter) and chloramphenicol (0.05 g/liter) to eliminate bacterial contamination. Following 5 days of incubation in the dark at 20°C, fungal colonies recovered from the sections were subcultured on new PDA plates and purified using the hyphal-tip or single-spore isolation method. To evaluate for conidia and chlamydospores, *Fusarium*-like colonies were also transferred to Spezieller Nährstoffarmer agar (SNA) medium and incubated at 20°C for 10 days (Leslie and Summerell 2006). Fungi were routinely grown on PDA at 20°C in the dark. All isolates were stored at 4°C on PDA stock plates, on filter papers in the microcentrifuge tubes at –20°C, and in vials containing 15% glycerol at –80°C for long-term storage.

DNA extraction. A modified version of the cetyltrimethylammonium bromide (CTAB)-based method as described in the diversity arrays technology protocol (<https://www.diversityarrays.com>) was used to extract genomic DNA. Approximately 100 mg of mycelia and spores of each isolate was harvested by gently scraping the surface of the culture and treated with preheated (65°C) extraction-lysis buffer (2% CTAB, 0.8 M NaCl, 125 mM Tris-HCl [pH 8.0], 25 mM EDTA [pH 8.0], 2% PVP-40, 1% sarcosyl, and 0.5% sodium disulfite) for DNA extraction. The resultant DNA was dissolved in 200 µl of sterile Milli-Q water. The concentration of DNA was measured by a DS-11 FX Series Spectrophotometer (Denovix Inc.) and adjusted to 50 ng/µl with sterile Milli-Q water for PCR assays.

Species identification. Morphological identifications of *Fusarium* spp. isolates based on microscopic characteristics, colony appearance, and pigmentation were performed according to Leslie and Summerell (2006) while the dematiaceous fungal isolates were identified by following the key of Ellis (1971) and Sivanesan (1987).

Species-specific PCR assays were performed to identify species using primers sets of FcOIF/FcOIR, Fp1-1/Fp1-2 (Nicholson et al. 1998), Fg16F/Fg16R (Aoki and O'Donnell 1999), and COSA_F/COSA_R (Matusinsky et al. 2010) for *F. culmorum*, *F. graminearum*, *F. pseudograminearum*, and *B. sorokiniana*, respectively (Table 1).

To amplify the translation elongation factor 1-α (*EF1-α*) gene region for *Fusarium* spp. and the internal transcribed spacer (ITS) region, including 5.8S of ribosomal DNA (rDNA) for other fungi, primer pair EF1 (5'-ATGGGTAAGGARGACAAGAC-3') and EF2 (5'-GGARGTACCAGTSATCATG-3') described by O'Donnell et al. (1998) and primer pair ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') described by White et al. (1990) were used, respectively. Amplification of DNA fragments was carried out in the Arktik Thermal Cycler (Thermo Scientific) and PCRs were conducted in a 50-µl reaction mixture containing 5 µl of 10× PCR buffer, 0.4 µM each primer, 50 ng of template DNA, 0.2 mM each dNTP, and 1.25 U of *Taq* DNA Polymerase (New England BioLabs). The thermal cycler program for amplifying the ITS region was 3 min for an initial denaturation step at 94°C; followed by 35 cycles with 30 s at 94°C, 30 s at 52°C, and 1 min at 72°C; and a 10-min extension at 72°C. To amplify the *EF1-α* locus, amplification conditions included an initial denaturation step of 3 min at 94°C; followed by 35 cycles of 1 min at 94°C, 45 s at 52°C, and 1 min at 72°C; with a final extension of

10 min at 72°C. The amplified DNA fragments were purified with Wizard SV Gel and PCR Clean-Up System (Promega Corp., Madison, WI, U.S.A.) according to the manufacturer's instructions and subjected to bidirectional direct sequencing (Macrogen, Inc., Seoul, Korea).

The resultant sequences were aligned with ClustalW, which is a multiple sequence alignment method (Thompson et al. 1994). The sequences were analyzed, and BLAST searched against GenBank (<https://blast.ncbi.nlm.nih.gov/>) to identify the closest available reference sequences in the complete NCBI nucleotide collection (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic analyses of the isolates and reference isolates available in the GenBank database was performed with MEGA X software (Kumar et al. 2018) for the ITS region and *EF1-α* gene, separately. A neighbor-joining tree was constructed using the Tamura and Nei (1993) model with 1,000 bootstrap replicates.

Pathogenicity tests. To test pathogenicity, seed of cultivar Seri 82 (*Triticum aestivum*, bread wheat) were treated for 5 min in 0.5% NaOCl and placed in plates containing a piece of sterile filter paper saturated with water to germinate for 3 days. Plastic pots (9 cm in diameter and 17 cm long) were filled with a mixture substrate of peat (KTS 1; Klasmann-Deilmann), sterile vermiculite, and sterile soil (1:1:1, vol/vol/vol) up to 5 cm below the top of the pots. Five seedlings were placed on the soil surface for each pot. For *Fusarium* spp. isolates, the inoculation was conducted with mycelial plugs removed from the margin of the actively growing PDA plate of each isolate with a sterile cork borer (10 mm in diameter). Plugs of each isolate were put around the wheat seedlings and covered with the mixture substrate. For assessing the pathogenicity of dematiaceous fungal isolates, the mixture substrate for covering the seedlings was inoculated with a conidial suspension of each isolate to obtain a density of 250 conidia/g (Duczek et al. 1985). The colonized wheat kernels method adapted from Demirci (1998) was performed to evaluate the pathogenicity of *Rhizoctonia* spp. isolates. The inoculation was carried out immediately by placing 10 colonized wheat kernels in contact with wheat seedlings and covering them with the mixture. Sterile agar plugs were used for the control treatment. There were five seeds per pot with three replications (each pot represented one replicate). Six weeks after incubation at a 12-h photoperiod and 24°C, plants were harvested, washed, and investigated for discoloration or lesions on crown, subcrown internode, and root tissues. The symptoms were assessed by using the index system, where 1 = no discoloration (clean), 2 = slight discoloration (<25% of the surface area discolored), 3 = moderate discoloration (25 to 50% discolored), and 4 = severe discoloration (at least 50% and surrounding the circumference of tissues discolored), as described by Ledingham et al. (1973). The study was repeated two times. The mean disease ratings of five isolates for each species were determined on 15 replicated seeds (5 seeds per pots). Scores of 1 to 2 were considered non-pathogenic or weakly pathogenic if there was a significant difference between treatment and control, scores of 2 to 3 were considered moderately pathogenic, and scores higher than 3 were considered aggressively pathogenic.

Disease severity scores obtained from the pathogenicity were analyzed for significance by analysis of variance, followed by Fisher's least significant difference test at *P* = 0.05, with the Statistical Analysis System (SAS version 9.0; SAS Institute Inc.; Cary, NC, U.S.A.).

Table 1. Primers used for species-specific PCR in this study

Species	Primers	Sequences (5'–3')	Product size (bp)	References
<i>Fusarium culmorum</i>	FcOIF	ATGGTGAACCTCGTCGTGGC	570	Nicholson et al. 1998
	FcOIR	CCCTTCTTACGCCAATCTCG	...	...
<i>F. pseudograminearum</i>	Fp1-1	CGGGGTAGTTTCACATTTCCG	520	Aoki and O'Donnell 1999
	Fp1-2	GAGAATGTGATGACGACAATA	...	...
<i>F. graminearum</i>	Fg16F	CTCCGGATATGTTGCGTCAA	420	Nicholson et al. 1998
	Fg16R	GGTAGGTATCCGACATGGCAA	...	...
<i>B. sorokiniana</i>	COSA_F	TCAAGCTGACCAAAATCACCTTC	520	Matusinsky et al. 2010
	COSA_R	CTTCTCACCAGCATCTGAATATATGA	...	...

Results

In total, 630 fungal strains were isolated from symptomatic wheat samples collected from 76 fields in the wheat-growing regions of Azerbaijan during the 2017 growing season. As a result of the classification of species based on morphological and molecular tools, 443 isolates of *Fusarium* spp., 96 of *B. sorokiniana*, 13 of *Exserohilum pedicellatum* (A. W. Henry) K. J. Leonard & Suggs, 45 of *Nigrospora oryzae* (Berk. & Broome) Petch, 13 of *Rhizoctonia* spp., 14 of *C. spicifera*, and 6 of *C. inaequalis* were identified (Table 2).

Among all strains, *Fusarium* spp. strains were found at a frequency of about 70.3%. The *EF1-α* gene sequences of 64 representative *Fusarium* spp. isolates ranged from 589 to 727 bp long, with identity matches of 99 to 100% with those of corresponding *Fusarium* spp. in the GenBank database. The sequences were deposited in the NCBI and accession numbers are shown in Figure 2. *F. culmorum* and *F. acuminatum* were predominant *Fusarium* spp., with frequencies of 43.3 and 28.2%, respectively, among *Fusarium* spp. isolates, followed by *F. pseudograminearum* and *F. equiseti*, with frequencies of 9.7 and 7.2%, respectively. *F. hostae* Geiser & Juba, *F. proliferatum* (Matsush.) Nirenberg, *F. avenaceum*, *F. oxysporum*, *F. graminearum*, *F. incarnatum* Subraman. & Rao, and *F. algeriense* were found less commonly, with frequencies of 2.5, 2.0, 1.6, 1.6, 1.4, 1.1, and 0.9%, respectively, whereas *F. brachygibbosum* Padwick was the least predominant (two isolates could be identified in one field from Kurdamir).

Anamorphs of *Cochliobolus* and the other dematiaceous fungi *E. pedicellatum* and *N. oryzae* were isolated from 36.8, 17.1, and 28.9% of the fields examined, respectively. BLASTn queries based on the ITS of isolates showed that the sequences of isolates were 99 to 100% identical to those of the corresponding species in the GenBank database. The sequences were deposited in the GenBank with accession numbers shown in Figure 3. Phylogenetic analyses based on ITS sequences of the isolates study and reference sequences of *B. sorokiniana*, *C. spicifera*, *C. inaequalis*, *E. pedicellatum*, and *N. oryzae* available in GenBank indicated that the isolates belonging to the same species were clearly separated in the dendrogram.

Thirteen isolates morphologically like *Rhizoctonia* spp. were isolated from samples representing six fields. The AG of isolates representing each field was identified by the sequencing of the ITS region of rDNA. Two isolates were determined as AG-4 HGII whereas AG-2-1, AG-Fa, AG-K, and AG-G were represented by one isolate. The result of analyzing the ITS sequences of isolates using the BLAST algorithm showed that sequences were 99 to 100% identical to corresponding AG sequences in GenBank. All *Rhizoctonia* spp. isolates were clustered in the dendrogram with the isolates belonging the same AGs (Fig. 3).

Species-specific PCR with primers sets FcOIF/FcOIR, Fp1-1/Fp1-2, Fg16F/Fg16R, and COSA_F/COSA_R amplified 570, 520, 420, and 520 bp for all strains of *F. culmorum*, *F. graminearum*, *F. pseudograminearum*, and *B. sorokiniana*, respectively (data not shown).

The pathogenicity of 73 isolates belonging to 18 fungal species and binucleate *Rhizoctonia* spp. isolated from winter wheat in

Azerbaijan were tested for their ability to cause disease. *F. culmorum*, *F. pseudograminearum*, and *F. graminearum* isolates caused severe disease on crown and root of wheat plants with means of 3.52, 3.49, and 3.23 disease severity scores, respectively, and were considered to be highly virulent species (Table 3). Some isolates of these species caused severe damage and resulted in the death of whole plants in some pots. *B. sorokiniana*, *F. avenaceum*, *F. algeriense*, multinucleate isolates of *R. solani*, *F. hostae*, and *C. spicifera* produced moderate disease severities, with mean scores of 2.81, 2.72, 2.45, 2.38, 2.20, and 2.03, respectively. There was no difference in scoring of the mean of rot severity between three of the five isolates of *N. oryzae* and control plants ($P < 0.05$); however, two *N. oryzae* isolates were weakly virulent, causing slight discoloration on a few crowns and seminal roots of inoculated plants. Means of disease severity scores for *C. inaequalis* and *E. pedicellatum* isolates measured less than 2 (weakly virulent) but there were significant differences at $P = 0.05$ from noninoculated control plants. *F. acuminatum*, *F. incarnatum*, *F. equiseti*, *F. oxysporum*, *F. brachygibbosum*, *F. proliferatum*, and binucleate *Rhizoctonia* spp. isolates exhibited no significant differences from control plants and were determined to be nonpathogenic.

Discussion

The present study identified 18 fungal species and binucleate *Rhizoctonia* spp. isolates that were associated with diseased tissues of winter wheat. This is the first detailed survey on pathogens of underground parts of wheat in Azerbaijan. The identification of species was conducted with morphological and molecular techniques. Pathogenic characterization was employed with Seri 82 to reveal the aggressiveness of the pathogens to wheat.

Accurate identification of isolates and analyses of pathogenic variabilities within the pathogen populations provide critical information for control strategies and breeding schemes. Species identification of genera *Fusarium*, *Bipolaris*, *Curvularia*, *Exserohilum*, and *Nigrospora* and subspecies discrimination of *Rhizoctonia* spp. based on morphological characteristics are time consuming and laborious and pose difficulties such as high morphological similarities, difficulty to induce sporulation, and requirement of tester isolates to identify *Rhizoctonia* spp. To overcome these bottlenecks, molecular phylogenetic analyses based on ITS and *EF1-α* sequences have been widely used for the identification of different species in these genera (Berbee et al. 1999; Geiser et al. 2004; Hernández-Restrepo et al. 2018; Manamgoda et al. 2012; Sharon et al. 2008; Wang et al. 2017). In this study, the sequences of ITS and *EF1-α* loci were successfully used for discrimination of the species and AGs of *Rhizoctonia* spp. obtained from surveyed fields. The *EF1-α* locus was used to identify *Fusarium* spp. obtained from diseased wheat plants in Turkey and China (Shikur Gebremariam et al. 2018; Xu et al. 2018). The sequences of ITS and the largest subunit of RNA polymerase (RPB1) sequences were necessary for the precise identification of *F. algeriense* isolates. Species-specific PCR was used for the identification of three *Fusarium* spp. and *B. sorokiniana* obtained

Table 2. Fungi associated with crown and root rot of dryland winter wheat in Azerbaijan

GPS location approximated				Fungus ^z																	
City	N ^y	Latitude (N)	Longitude (E)	Fc	Fpg	Fg	Fac	Fi	Fav	Fh	Fe	Fo	Fb	Fp	Fal	Bs	Cs	Ci	Ep	R	No
Qobustan	1	40°30′	48°59′	0/0	0/0	0/0	1/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/7	0/0	0/0	0/0	0/0	0/0
Ismailli	9	40°40′–40°47′	47°57′–48°32′	0/0	0/0	2/6	7/19	0/0	0/0	3/6	0/0	0/0	0/0	0/0	1/2	4/19	0/0	0/0	1/1	1/3	3/5
Oguz	5	40°56′–41°02′	47°26′–47°34′	1/5	0/0	0/0	3/9	0/0	2/5	3/5	0/0	0/0	0/0	0/0	1/2	3/17	1/2	0/0	1/3	1/4	0/0
Shaki	20	40°37′–41°37′	47°07′–47°26′	5/18	1/5	0/0	15/46	0/0	0/0	0/0	5/15	2/5	0/0	0/0	0/0	6/33	4/10	1/2	3/5	0/0	3/7
Barda	10	40°15′–40°19′	47°07′–47°15′	10/43	1/3	0/0	2/7	2/4	0/0	0/0	0/0	1/2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/4	8/16
Agdash	2	40°16′	47°12′	2/8	0/0	0/0	1/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/6	0/0	2/4	0/0	0/0	1/1
Ujar	5	40°24′–40°30′	47°32′–47°58′	2/9	4/13	0/0	1/4	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/4
Kurdamir	24	40°05′–40°26′	48°08′–48°31′	24/109	5/22	0/0	12/34	1/1	2/2	0/0	7/17	0/0	1/2	4/9	0/0	4/14	1/2	0/0	2/4	2/2	5/12
Total	76	...	...	44/192	11/43	2/6	42/125	3/5	4/7	6/11	12/32	3/7	1/2	4/9	2/4	19/96	6/14	3/6	7/13	6/13	22/45

^y Total number of fields.

^z Number of fields from which the species was obtained/number of isolates obtained. Abbreviations: Fc = *Fusarium culmorum*, Fpg = *F. pseudograminearum*, Fg = *F. graminearum*, Fac = *F. acuminatum*, Fi = *F. incarnatum*, Fav = *F. avenaceum*, Fh = *F. hostae*, Fe = *F. equiseti*, Fo = *F. oxysporum*, Fb = *F. brachygibbosum*, Fp = *F. proliferatum*, Fal = *F. algeriense*, Bs = *Bipolaris sorokiniana*, Cs = *Curvularia spicifera*, Ci = *C. inaequalis*, Ep = *Exserohilum pedicellatum*, R = *Rhizoctonia* spp., and No = *Nigrospora oryzae*.

in this study. The PCR with the primer sets provided definite identification for these species, which was consistent with previous reports (Abdallah-Nekache et al. 2019; Matusinsky et al. 2010; Nicholson et al. 1998).

In all, 11 of 17 fungal species determined in this study were identified as *Fusarium* spp. *F. culmorum* was found to be the most predominant species occurring on crown and root tissues of wheat with 192 isolates and was detected in 57.9% of the fields surveyed. Among the complex of *Fusarium* spp. associated with FCR, *F. culmorum* has also been reported as the most frequently isolated species in the United States (Moya-Elizondo et al. 2011; Strausbaugh et al. 2004), New Zealand (Bentley et al. 2006), eastern Australia (Burgess et al. 1981), Turkey (Tunali et al. 2008), Norway (Kosiak et al. 2003), Latvia (Bankina et al. 2013), Chile (Moya-Elizondo et al. 2015), and Algeria (Abdallah-Nekache et al. 2019). This present study was also consistent with previous observations that *F. culmorum* prefers cooler areas. We observed that *F. acuminatum* was the second most

commonly isolated *Fusarium* sp. from below ground, with 125 isolates representing about 55.3% of the fields surveyed. The ratio of *F. acuminatum* with 125 isolates to total isolates was 19.8%. This agreed with the result of a survey conducted in Turkey that *F. acuminatum* had close isolation frequency with *F. culmorum* at 14% (Shikur Gebremariam et al. 2018). *F. acuminatum* fungus has been found in association with *B. sorokiniana* in the root-disease complex, especially in dryland wheat production areas of Colorado and Wyoming (Fernandez et al. 1985; Gonzalez and Trevathan 2000; Hill et al. 1983; Specht and Rush 1988). *F. acuminatum* isolates were isolated mostly with *B. sorokiniana* in this study. Similar to *F. culmorum*, there are many reports that this fungus prefers cold weather, which is a common climatic condition in the survey area (Hall and Sutton 1998; Pettitt et al. 2003). *F. pseudograminearum* and *F. equiseti* were represented with 43 and 32 isolates, respectively. *F. pseudograminearum* (formerly known as *F. graminearum* group I) was isolated from 14.5% of the fields surveyed in the wheat-growing area of the

Fig. 2. Phylogeny of *Fusarium* spp. isolates associated with crown and root rot of winter wheat in Azerbaijan based on the translation elongation factor 1- α gene region (neighbor-joining). Bootstrap values (percentage, based on 1,000 replications) are shown on the branches. Some representative isolates of *Fusarium* spp. from this study were selected for the phylogenetic tree. The remaining isolates were retrieved from NCBI database.

country. The prevalence of this fungus has been reported to increase especially in arid and low-rainfall areas and years (Poole et al. 2013; Smiley and Patterson 1996), which explains why fungi were mostly obtained from Ujar and Kurdamir. *F. equiseti* has been reported as a dominant fungus associated with crown and root rot of wheat in Saskatchewan (Fernandez and Jefferson 2004), Mississippi (Gonzalez and Trevathan 2000), Italy (Rossi et al. 1995), Canada (Fernandez et al. 2014), and Turkey (Shikur Gebremariam et al. 2018), which confirms our results.

The remaining isolates belonging to seven *Fusarium* spp. were detected with a frequency of less than 8.1% among all isolates. They were identified in previous surveys conducted in wheat fields to determine crown and root rot pathogens (Fernandez et al. 2014; Moya-Elizondo et al. 2015; Shikur Gebremariam et al. 2018; Xu et al. 2018). *F. graminearum* and *F. avenaceum* were common pathogens associated with FCR after *F. culmorum* and *F. pseudograminearum*. In fact, in contrast to this study, they were exhibited as the most common species in some areas (Moya-Elizondo et al. 2015). The presence of *F. hostae* was determined at an isolate frequency of 1.75%

in the limited area of only 11 fields of Ismaili and Oguz, whereas Shikur Gebremariam et al. (2018) identified the pathogen in 8.6% of the wheat fields but more commonly in the surveyed areas of Turkey. *F. algeriense* has been newly described as a novel crown rot pathogen of wheat while previously described in the *F. burgessii* species complex (Laraba et al. 2017). There are no reports of the existence of this pathogen outside Algeria. In this study, we determined four isolates from two fields as *F. algeriense*. Azerbaijan is the second country after Algeria in which the pathogen was detected as a pathogen causing crown rot on wheat.

Percent incidence of *B. sorokiniana* isolates in all isolates was 15.2% in this study, which conforms previous observations of this fungus being frequently isolated from crown and root tissues of winter wheat (Chen et al. 1996; Fedel-Moen and Harris 1987). *C. spicifera* and *C. inaequalis*, although less frequently, also were isolated as associated with CRR, which was consistent with previous reports (Gonzalez and Trevathan 2000; Hill et al. 1983; Specht and Rush 1988).

Isolates of *Rhizoctonia* spp. were found in 7.9% of the fields. Rhizoctonia root rot was caused by *R. solani*, occurring as lesions on the

Fig. 3. Phylogeny of fungal isolates associated with crown and root rot of winter wheat in Azerbaijan based on the internal transcribed spacer region (Neighbor-joining). Bootstrap values (percentage, based on 1,000 replications) are shown on the branches. Some representative isolates from this study were selected for the phylogenetic tree. The remaining isolates were retrieved from NCBI database.

seminal and crown roots (Paulitz et al. 2002). AG-4 HGII and AG-2-1 of *R. solani* were determined in this study for the first time on wheat in Azerbaijan (Özer et al. 2019b); however, the reports have been verified as AGs related to crown and root rots in wheat-growing areas in England (Brown et al. 2014) and the United States (Jaaffar et al. 2016).

E. pedicellatum, first described from wheat roots in the United States, and *Nigrospora* spp. were also previously isolated from discolored subcrown internodes of wheat with a lower frequency (Fernandez et al. 2014; Moya-Elizondo et al. 2015; Sivanesan 1987). Surprisingly, in our study, *N. oryzae* and *E. pedicellatum* were found to be quite common in the area, with frequencies 28.9 and 17.1% of the fields surveyed, respectively. This was the first report of *E. pedicellatum* associated with crown and root rot of wheat in Azerbaijan (Özer et al. 2019a).

Results of our seedling pathogenicity tests generally conformed to previous reports on the fungal pathogenicity of crown or root tissues of wheat seedlings. *F. culmorum*, *F. pseudograminearum*, and *F. graminearum* were the most aggressive pathogens and caused severe FCR symptoms on wheat (damping-off of seedlings and rotted crown and stem bases that resulted in the death of seedlings) while *F. avenaceum* and *B. sorokiniana* isolates caused similar but less severe symptoms, without the death of seedlings (Fernandez and Chen 2005; Shikur Gebremariam et al. 2018; Smiley and Patterson 1996; Xu et al. 2018). *F. algeriense* isolates caused crown rot, with discoloration of seminal roots, similar to symptoms in the publication in which the pathogen was described (Laraba et al. 2017). *F. hostae* isolates exhibiting moderate symptoms in the tests were found to be moderately virulent on wheat in Turkey by Shikur Gebremariam et al. (2016). *F. acuminatum*, *F. incarnatum*, *F. equiseti*, *F. oxysporum*, *F. brachyglabrum*, and *F. proliferatum* isolates in pathogenicity tests were determined to be nonpathogens based on disease severity scores not statistically significant different ($P < 0.05$) compared with control plants, which was in agreement with previous findings from pathogenicity studies on wheat (Shikur Gebremariam et al. 2018; Tunali et al. 2008; Xu et al. 2018). However, contrary to our results, isolates of *F. acuminatum*, *F. equiseti*, and *F. oxysporum* exhibiting the pathogenic reaction to wheat at different levels have

been detected in some reports (Gonzalez and Trevathan 2000; Smiley and Patterson 1996).

C. spicifera strains were moderately or weakly virulent on wheat, with discoloration of the crown and seminal roots of seedlings but less severe than symptoms caused by *B. sorokiniana*, which was similar to the findings of Gonzalez and Trevathan (2000). Although no reports suggested that *C. inaequalis* caused significant damage to wheat, studies demonstrated that disease severity was considerably enhanced over single inoculations with *F. culmorum* and *B. sorokiniana* if combined with *C. inaequalis* (Fedel-Moen and Harris 1987).

E. pedicellatum strains and two *N. oryzae* strains were weakly pathogenic on wheat in this study while three *N. oryzae* isolates were nonpathogens. *Nigrospora* spp. isolates have been reported to be similar in this study: sometimes saprophytic and nonpathogenic and sometimes weakly pathogenic on wheat (Eken et al. 2016). *E. pedicellatum* was reported to be associated with dark-brown lesions on roots and root rot of wheat and maize (Sivanesan 1987).

R. solani isolates belonging to AG-4 and AG-2-1 were reported as pathogenic to wheat while binucleate strains of *Rhizoctonia* spp. were found to be nonpathogens (Demirci 1998). Our result supported these findings; AG-4 HGII strains caused rot, discoloration, and lesions on crown, subcrown internode, and root tissues while strain AG-2-1 was moderately virulent, causing only discoloration.

This is the first comprehensive survey to identify fungal species associated with crown and root rot of wheat conducted in the main wheat-growing regions of Azerbaijan. In the current study, the fungal species were assessed at the species level using morphological and molecular tools such as species-specific PCR and DNA sequencing of ITS and *EF-1α* loci. No similar extensive survey was performed on fungal populations associated with wheat previously done in Azerbaijan with which we could compare these results. The FCR pathogen *F. culmorum* was the most frequently isolated and the most aggressive fungus among all species. *F. pseudograminearum* and *F. graminearum*, found at relatively lower frequencies, caused serious FCR symptoms. *F. hostae* and *F. algeriense* were first reported as moderately pathogenic on wheat in Azerbaijan. *B. sorokiniana* and other *Cochliobolus* anamorphs were found to be related to CRR. Although *C. spicifera* and *C. inaequalis* caused less severe symptoms

Table 3. Pathogenicity of fungi isolated from winter wheat in Azerbaijan

Fungus ^w	Isolate ^x					Control	LSD _{0.05}	Mean
	1	2	3	4	5			
<i>Fc</i>	3.80 a	3.63 a	3.57 ab	3.33 bc	3.27 c	1.14 d	0.25	3.52
<i>Fpg</i>	3.77 a	3.70 a	3.40 b	3.33 b	3.27 b	1.14 c	0.28	3.49
<i>Fg</i>	3.33 a	3.13 a	...	...	...	1.14 b	0.22	3.23
<i>Fac</i> ^y	1.43	1.40	1.40	1.37	1.17	1.14	0.37	1.35
<i>Fi</i> ^y	1.23	1.10	1.06	...	...	1.14	0.31	1.13
<i>Fav</i>	2.97 a	2.87 a	2.67 ab	2.37 b	...	1.14 c	0.37	2.72
<i>Fh</i>	2.33 a	2.27 ab	2.23 ab	2.17 ab	2.00 b	1.14 c	0.27	2.20
<i>Fe</i> ^y	1.30	1.23	1.21	1.20	1.17	1.14	0.20	1.22
<i>Fo</i> ^y	1.33	1.30	1.20	...	...	1.14	0.27	1.28
<i>Fb</i> ^y	1.10	...	...	...	...	1.14	0.30	1.10
<i>Fp</i> ^y	1.40	1.40	1.36	1.33	...	1.14	0.35	1.38
<i>Fal</i>	2.53 a	2.37 a	...	...	...	1.14 b	0.41	2.45
<i>Bs</i>	3.15 a	3.13 a	2.93 ab	2.50 ab	2.36 b	1.14 c	0.61	2.81
<i>Cs</i>	2.29 a	2.15 ab	2.09 abc	1.91 bc	1.75 c	1.14 d	0.35	2.03
<i>Ci</i>	1.56 a	1.48 a	1.41 a	...	...	1.14 b	0.20	1.48
<i>Ep</i>	2.13 a	1.90 a	1.46 b	1.43 bc	1.40 bc	1.14 c	0.29	1.66
<i>R (bn)</i> ^y	1.03 (A) ^z	1.16 (Fa) ^z	1.20 (K) ^z	...	...	1.14	0.23	1.13
<i>R (mn)</i>	2.60 a (4) ^z	2.53 b (4) ^z	2.03 b (2) ^z	...	...	1.14 c	0.37	2.38
<i>No</i>	1.80 a	1.77 a	1.40 b	1.33 b	1.30 b	1.14 b	0.37	1.52

^w Abbreviations: *Fc* = *Fusarium culmorum*, *Fpg* = *F. pseudograminearum*, *Fg* = *F. graminearum*, *Fac* = *F. acuminatum*, *Fi* = *F. incarnatum*, *Fav* = *F. avenaceum*, *Fh* = *F. hostae*, *Fe* = *F. equiseti*, *Fo* = *F. oxysporum*, *Fb* = *F. brachyglabrum*, *Fp* = *F. proliferatum*, *Fal* = *F. algeriense*, *Bs* = *Bipolaris sorokiniana*, *Cs* = *Curvularia spicifera*, *Ci* = *C. inaequalis*, *Ep* = *Exserohilum pedicellatum*, *R* = *Rhizoctonia* spp. (*bn* = binucleate and *mn* = multinucleate), and *No* = *Nigrospora oryzae*.

^x Disease severity on 15 seedlings from pathogenicity assay based on a scale of 1 to 4. Means within a line followed by the same letter do not differ significantly at $P = 0.05$ according to Fisher's least significant difference (LSD) test.

^y There was no significant difference between all isolates in the line and control plants ($P < 0.05$).

^z Anastomosis group of *Rhizoctonia* sp. isolates.

than *B. sorokiniana*, both were determined to be pathogens on wheat for the first time for Azerbaijan in this study. *E. pedicellatum* and *N. oryzae* strains were found to be minor threats to winter wheat but at relatively higher frequencies compared with the other previous reports, highlighting the need for further work on these species if combined the other pathogens. The results of our study provide crucial and helpful information to select resistant varieties and improve disease management strategies against the major crown and root diseases of wheat in Azerbaijan. Further investigation is needed to understand their potential distribution and impact on the wheat crop.

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Literature Cited

- Abdallah-Nekache, N., Laraba, I., Ducos, C., Barreau, C., Bouznad, Z., and Bouregghda, H. 2019. Occurrence of Fusarium head blight and Fusarium crown rot in Algerian wheat: Identification of associated species and assessment of aggressiveness. *Eur. J. Plant Pathol.* 154:499-512.
- Aoki, T., and O'Donnell, K. 1999. Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the Group 1 population of *F. graminearum*. *Mycologia* 91:597-609.
- Bach, E. E., Barros, B. C., and Kimati, H. 2003. Induced resistance against *Bipolaris bicolor*, *Bipolaris sorokiniana* and *Drechslera tritici-repentis* in wheat leaves by xanthan gum and heat-inactivated conidial suspension. *J. Phytopathol.* 151:411-418.
- Backhouse, D., Abubakar, A. A., Burgess, L. W., Dennisc, J. I., Hollaway, G. J., Wildermuth, G. B., and Henry, F. J. 2004. Survey of *Fusarium* species associated with crown rot of wheat and barley in eastern Australia. *Australas. Plant Pathol.* 33:255-261.
- Bakonyi, J., Aponyi, I., and Fischl, G. 1997. Diseases caused by *Bipolaris sorokiniana* and *Drechslera tritici-repentis* in Hungary. Pages 80-88 in: *Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot*. E. Duveiller, H. J. Dubin, J. Reeves, and A. McNab, eds. CIMMYT, Mexico, DF, Mexico.
- Bankina, B., Bimšteine, G., Ruža, A., Priekule, I., Paura, L., Vaivade, I., and Fridmanis, D. 2013. Winter wheat crown and root rot are affected by soil tillage and crop rotation in Latvia. *Acta Agric. Scand. B Soil Plant Sci.* 63:723-730.
- Bentley, A. R., Cromey, M. G., Farrokhi-Nejad, R., Leslie, J. F., Summerell, B. A., and Burgess, L. W. 2006. Fusarium crown and root rot pathogens associated with wheat and grass stem bases on the South Island of New Zealand. *Australas. Plant Pathol.* 35:495-502.
- Berbee, M. L., Pirseyedi, M., and Hubbard, S. 1999. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* 91: 964-977.
- Bockus, W. W., Bowden, R. L., Hunger, R. M., Morrill, W. L., and Murray, T. D. 2010. *Compendium of Wheat Diseases and Pests*, 3rd ed. American Phytopathological Society, St. Paul, MN, U.S.A.
- Broders, K. D., Parker, M. L., Melzer, M. S., and Boland, G. J. 2014. Phylogenetic diversity of *Rhizoctonia solani* associated with canola and wheat in Alberta, Manitoba, and Saskatchewan. *Plant Dis.* 98:1695-1701.
- Brown, M. B., Woodhall, J., Mooney, S. J., and Ray, R. V. 2014. The occurrence and population dynamics of *Rhizoctonia solani* in soil of winter wheat. Pages 107-112 in: *The Dundee Conference. Crop Protection in Northern Britain 2014*, Dundee, U.K. 25-26 February 2014. The Association for Crop Protection in Northern Britain.
- Burgess, L. W., Dodman, R. L., Pont, W., and Mayers, P. 1981. Fusarium diseases of wheat, maize and grain sorghum in eastern Australia. Pages 64-76 in: *Fusarium: Diseases, Biology and Taxonomy*. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University Press, University Park, PA, U.S.A.
- Chen, C., Collins, D. J., and Morgan-Jones, G. 1996. Fungi associated with root rot of winter wheat in Alabama. *J. Phytopathol.* 144:193-196.
- Cook, R. J. 1981. Fusarium diseases of wheat and other small grains in North America. Pages 39-52 in: *Fusarium: Diseases, Biology, and Taxonomy*. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. The Pennsylvania State University Press, University Park, PA, U.S.A.
- Cook, R. J. 2010. Fusarium root, crown, and foot rots and associated seedling diseases. Pages 37-39 in: *Compendium of Wheat Diseases and Pests*, 3rd ed. W. W. Bockus, R. L. Bowden, R. M. Hunger, W. L. Morrill, T. D. Murray, and R. W. Smiley, eds. American Phytopathological Society, St. Paul, MN, U.S.A.
- Cook, R. J., and Veseth, R. J. 1991. *Wheat Health Management*. American Phytopathological Society, St. Paul, MN, U.S.A.
- Demirci, E. 1998. *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. *Plant Pathol.* 47:10-15.
- Duczek, L. J., Verma, P. R., and Spurr, D. T. 1985. Effect of inoculum density of *Cochliobolus sativus* on common root rot of wheat and barley. *Can. J. Plant Pathol.* 7:382-386.
- Dyer, A. T., Johnston, R. H., Hogg, A. C., and Johnston, J. A. 2009. Comparison of pathogenicity of the Fusarium crown rot (FCR) complex (*F. culmorum*, *F. pseudograminearum* and *F. graminearum*) on hard red spring and durum wheat. *Eur. J. Plant Pathol.* 125:387-395.
- Eken, C., Spanbayev, A., Tulegenova, Z., and Yechshzhanov, T. 2016. First report of *Nigrospora oryzae* on wheat in Kazakhstan. *Plant Dis.* 100:861.
- Ellis, M. B. 1971. *Dematiaceous Hyphomycetes*. CAB, Kew, Surrey, U.K.
- FAOSTAT. 2019. Food and Agriculture Organization Statistical Database. <http://www.fao.org/faostat/en/#data/QC>
- Fedel-Moen, R., and Harris, J. R. 1987. Stratified distribution of *Fusarium* and *Bipolaris* on wheat and barley with dryland root rot in South Australia. *Plant Pathol.* 36:447-454.
- Fernandez, J. A., Wofford, D. S., and Horton, J. L. 1985. Interactive effects of freezing and common root rot fungi on winter wheat. *Phytopathology* 75:845-847.
- Fernandez, M. R., and Chen, Y. 2005. Pathogenicity of *Fusarium* species on different plant parts of spring wheat under controlled conditions. *Plant Dis.* 89:164-169.
- Fernandez, M. R., Fox, S. L., Hucl, P., Singh, A. K., and Stevenson, F. C. 2014. Root rot severity and fungal populations in spring common, durum and spelt wheat, and Kamut grown under organic management in western Canada. *Can. J. Plant Sci.* 94:937-946.
- Fernandez, M. R., and Jefferson, P. G. 2004. Fungal populations in roots and crowns of common and durum wheat in Saskatchewan. *Can. J. Plant Pathol.* 26:325-334.
- Geiser, D. M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T. J., Zhang, N., Kuldau, G. A., and O'Donnell, K. 2004. FUSARIUM-ID v1.0: A DNA sequence database for identifying Fusarium. *Eur. J. Plant Pathol.* 110:473-479.
- Gonzalez, M. S., and Trevathan, L. E. 2000. Identity and pathogenicity of fungi associated with root and crown rot of soft red winter wheat grown on the upper coastal plain land resource area of Mississippi. *J. Phytopathol.* 148:77-85.
- Hall, R., and Sutton, J. C. 1998. Relation of weather, crop, and soil variables to the prevalence, incidence, and severity of basal infections of winter wheat in Ontario. *Can. J. Plant Pathol.* 20:69-80.
- Hernández-Restrepo, M., Madrid, H., Tan, Y. P., Da Cunha, K. C., Gene, J., Guarro, J., and Crous, P. W. 2018. Multi-locus phylogeny and taxonomy of *Exserohilum*. *Persoonia* 41:71-108.
- Hill, J. P., Fernandez, J. A., and McShane, M. S. 1983. Fungi associated with common root rot of winter wheat in Colorado and Wyoming. *Plant Dis.* 67:795-797.
- Jaaffar, A. K. M., Paulitz, T. C., Schroeder, K. L., Thomashow, L. S., and Weller, D. M. 2016. Molecular characterization, morphological characteristics, virulence, and geographic distribution of *Rhizoctonia* spp. in Washington State. *Phytopathology* 106:459-473.
- Kosiak, B., Torp, M., Skjerve, E., and Thrane, U. 2003. The prevalence and distribution of *Fusarium* species in Norwegian cereals: A survey. *Acta Agric. Scand. B* 53:168-176.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analyses across computing platforms. *Mol. Biol. Evol.* 35:1547-1549.
- Laraba, I., Keddad, A., Bouregghda, H., Abdallah, N., Vaughan, M. M., Proctor, R. H., Busman, M., and O'Donnell, K. 2017. *Fusarium algeriense*, sp. nov., a novel toxigenic crown rot pathogen of durum wheat from Algeria is nested in the *Fusarium burgessii* species complex. *Mycologia* 109:935-950.
- Ledingham, R. J., Atkinson, T. G., Horricks, J. S., Mills, J. T., Piening, L. J., and Tinline, R. D. 1973. Wheat losses due to common root rot in the prairie provinces of Canada, 1969-71. *Can. Plant Dis. Surv.* 53:113-122.
- Leslie, J. F., and Summerell, B. A. 2006. *The Fusarium Laboratory Manual*. Blackwell Publishing Ltd, Oxford, UK.
- Lucas, P., Smiley, R. W., and Collins, H. P. 1993. Decline of *Rhizoctonia* root rot on wheat in soils infested with *Rhizoctonia solani* AG-8. *Phytopathology* 83:260-265.
- MacNish, G. C. 1988. Changes in take-all (*Gaeumannomyces graminis* var. *tritici*), rhizoctonia root rot (*Rhizoctonia solani*) and soil pH in continuous wheat with annual applications of nitrogenous fertilizer in Western Australia. *Aust. J. Exp. Agric.* 28:333-341.
- Manamgoda, D. S., Cai, L., McKenzie, E. H. C., Crous, P. W., Madrid, H., Chukeatirote, E., Shivas, R. G., Tan, Y. P., and Hyde, K. D. 2012. A phylogenetic and taxonomic re-evaluation of the *Bipolaris* - *Cochliobolus* - *Curvularia* complex. *Fungal Divers.* 56:131-144.
- Matusinsky, P., Frei, P., Mikolasova, R., Svacinova, I., Tvaruzek, L., and Spitzer, T. 2010. Species-specific detection of *Bipolaris sorokiniana* from wheat and barley tissues. *Crop Prot.* 29:1325-1330.
- Mehdiyev, I. T. 2014. Identification of root rot diseases in winter wheat and investigation of their biological development characteristics. Pages 64-67 in: *Book of Abstracts in Scientific Works of ASAU, Ganca, Azerbaijan*.
- Morejon, K. R., Moraes, M. H. D., and Bach, E. E. 2006. Identification of *Bipolaris bicolor* and *Bipolaris sorokiniana* on wheat seeds (*Triticum aestivum* L.) in Brazil. *Braz. J. Microbiol.* 37:247-250.

- Moya-Elizondo, E., Arismendi, N., Castro, M. P., and Doussoulin, H. 2015. Distribution and prevalence of crown rot pathogens affecting wheat crops in southern Chile. *Chil. J. Agric. Res.* 75:78-84.
- Moya-Elizondo, E. A., Rew, L. J., Jacobsen, B. J., Hogg, A. C., and Dyer, A. T. 2011. Distribution and prevalence of *Fusarium* crown rot and common root rot pathogens of wheat in Montana. *Plant Dis.* 95:1099-1108.
- Nicholson, P., Simpson, D. R., Weston, G., Rezanoor, H. N., Lees, A. K., Parry, D. W., and Joyce, D. 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. *Physiol. Mol. Plant Pathol.* 53:17-37.
- O'Donnell, K., Cigelnik, E., and Nirenberg, H. I. 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia* 90:465-493.
- Ogoshi, A., Cook, R. J., and Bassett, E. N. 1990. *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. *Phytopathology* 80:784-788.
- Özer, G., Göre, M. E., Alkan, M., Yaman, T., and Dababat, A. A. 2019a. First report of *Exserohilum pedicellatum* causing root rot of wheat in Azerbaijan. *Plant Dis.* 103:1416.
- Özer, G., Göre, M. E., Mustafa, İ., Khalilova, S., Muminjanov, H., and Dababat, A. A. 2019b. First report of *Rhizoctonia solani* AG-4 HGII and AG-2-1 causing root rot of wheat in Azerbaijan. *Plant Dis.* 103:2132.
- Paulitz, T. C., Smiley, R. W., and Cook, R. J. 2002. Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, USA. *Can. J. Plant Pathol.* 24:416-428.
- Pettitt, T., Xu, X., and Parry, D. 2003. Association of *Fusarium* species in the wheat stem root complex. *Eur. J. Plant Pathol.* 109:769-774.
- Poole, G. J., Smiley, R. W., Walker, C., Huggins, D., Rupp, R., Abatzoglou, J., Garland-Campbell, K., and Paulitz, T. C. 2013. Effect of climate on the distribution of *Fusarium* spp. causing crown rot of wheat in the Pacific Northwest of the United States. *Phytopathology* 103:1130-1140.
- Rossi, V., Cervi, C., Chiusa, G., and Languasco, L. 1995. Fungi associated with foot rots on winter wheat in northwest Italy. *J. Phytopathol.* 143:115-119.
- Sharon, M., Sneh, B., Kunitz, S., Hyakumachi, M., and Naito, S. 2008. Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. *Mycoscience* 49:93-114.
- Shikur Gebremariam, E., Dababat, A., Erginbas-Orakci, G., Karakaya, A., Sharma-Poudyal, D., and Paulitz, T. C. 2016. First report of *Fusarium hostae* causing crown rot of wheat (*Triticum* spp.) in Turkey. *Plant Dis.* 100:216.
- Shikur Gebremariam, E., Sharma-Poudyal, D., Paulitz, T. C., Erginbas-Orakci, G., Karakaya, A., and Dababat, A. A. 2018. Identity and pathogenicity of *Fusarium* species associated with crown rot on wheat (*Triticum* spp.) in Turkey. *Eur. J. Plant Pathol.* 150:387-399.
- Sivanesan, A. 1987. Graminicolous Species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and Their Teleomorphs. *Mycological Papers* 158. CAB International, Wallingford, U.K.
- Smiley, R. W., Gourlie, J. A., Easley, S. A., Patterson, L. M., and Whittaker, R. G. 2005. Crop damage estimates for crown rot of wheat and barley in the Pacific Northwest. *Plant Dis.* 89:595-604.
- Smiley, R. W., and Patterson, L. M. 1996. Pathogenic fungi associated with *Fusarium* foot rot of winter wheat in the semiarid Pacific Northwest. *Plant Dis.* 80:944-949.
- Specht, L. P., and Rush, C. M. 1988. Fungi associated with root and foot rot of winter wheat and populations of *Cochliobolus sativus* in the Texas Panhandle. *Plant Dis.* 72:959-963.
- Strausbaugh, C. A., Bradley, C. A., Koehn, A. C., and Forster, R. L. 2004. Survey of root diseases of wheat and barley in southeastern Idaho. *Can. J. Plant Pathol.* 26:167-176.
- Tamura, K., and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10:512-526.
- Thompson, J. D., Higgins, D. G., and Gibson, T. J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22:4673-4680.
- Tunali, B., Nicol, J. M., Hodson, D., Uckun, Z., Büyüç, O., Erdurmuş, D., and Bağci, S. A. 2008. Root and crown rot fungi associated with spring, facultative, and winter wheat in Turkey. *Plant Dis.* 92:1299-1306.
- Ünal, F., Bayraktar, H., Yıldırım, A. F., Akan, K., and Dolar, F. S. 2015. Determination of *Rhizoctonia* species and anastomosis groups in wheat production areas in Kayseri, Kırşehir, Nevşehir and Aksaray provinces. *Plant Prot. Bull.* 55:107-122.
- Wang, M., Liu, F., Crous, P. W., and Cai, L. 2017. Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens. *Persoonia* 39: 118-142.
- White, T. T., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: *PCR Protocols: A Guide to Methods and Applications*. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, San Diego, CA, U.S.A.
- Wildermuth, G. B., Tinline, R. D., and McNamara, R. B. 1992. Assessment of yield loss caused by common root rot in wheat cultivars in Queensland. *Aust. J. Agric. Res.* 43:43-58.
- Xu, F., Yang, G., Wang, J., Song, Y., Liu, L., Zhao, K., and Han, Z. 2018. Spatial distribution of root and crown rot fungi associated with winter wheat in the North China Plain and its relationship with climate variables. *Front. Microbiol.* 9:1054.
- Yin, C., Hulbert, S. H., Schroeder, K. L., Mavrodi, O., Mavrodi, D., Dhingra, A., Schillinger, W. F., and Paulitz, T. C. 2013. Role of bacterial communities in the natural suppression of *Rhizoctonia solani* bare patch disease of wheat (*Triticum aestivum* L.). *Appl. Environ. Microbiol.* 79:7428-7438.