Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Virulence factors of *Fusarium* spp., causing wheat crown and root rot in Iran

Saeedeh DEHGHANPOUR-FARASHAH, Parissa TAHERI*, Mahrokh FALAHATI-RASTEGAR

Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O.Box: 91775-1163, Mashhad, Iran
*Corresponding author: p-taheri@um.ac.ir

Abstract. Crown and root rot of wheat, caused by *Fusarium* spp., limit crop yields worldwide, from rotting of seeds, seedlings, crowns, roots and basal plant stems. Virulence factors and virulence and aggressiveness of *Fusarium* spp. were investigated for isolates from Iran, obtained from wheat plants with crown and root rot symptoms. Forty isolates of *Fusarium* were used in this research. Among the isolates, nivalenol (NIV) was detected as the dominant trichothecene chemotype produced. Production of trichothecenes and zearalenone (ZEA) in autoclaved rice cultures of *Fusarium* isolates was analyzed using high performance liquid chromatography. The levels of NIV ranged from 258 to 1246 μg kg^{-1}, of deoxynivalenol (DON) from 45 to 1411 μg kg^{-1}, and of ZEA from 53 to 3220 μg kg^{-1}. All *Fusarium* isolates produced cellulase and pectinase enzymes. Positive correlation was observed between activity of cell wall degrading enzymes (CWDEs) produced by the isolates and their pathogenicity on wheat leaf segments. Virulence of trichothecene-producing isolates was greater than that of non-trichothecene-producing isolates. Considerable association was detected between the capability of *Fusarium* spp. isolates to produce virulence factors (such as mycotoxins and CWDEs) and their pathogenicity on wheat.

Keywords. Cell wall degrading enzymes, mycotoxins.

OPEN ACCESS

Citation: Dehghanpour-Farashah S., Taheri P., Falahati-Rastegar M. (2019) Virulence factors of *Fusarium* spp., causing wheat crown and root rot in Iran. *Phytopathologia Mediterranea* 58(1): 115-125. doi: 10.14601/Phytopathol_Mediterr-23860

Accepted: November 14, 2018

Published: May 15, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Dimitrios Tsitsigiannis, Agricultural University of Athens, Greece.

INTRODUCTION

Crown and root rot of wheat, caused by several *Fsarium* species, occur in most cereal producing regions of the world, including Europe, Australia, North America, South America, West Asia, South Africa and North Africa (Smiley *et al*., 1996; Paulitz *et al*., 2002; Smiley *et al*., 2005). In Iran, several *Fusarium* spp. have been isolated from crown and root rot symptoms in wheat growing regions (Besharati Fard *et al*., 2017). Fusaria produce a diverse array of toxic secondary metabolites (mycotoxins), which are involved in pathogenicity of these fungi to host plants. The most important *Fusarium* mycotoxins are trichothecenes and zearalenone, which can contaminate agricultural products, making them unsuitable for food or feed (Ma *et al*., 2013).

Trichothecenes are sesquiterpenoid molecules of which many variants are known. These have been chategorized as type A, including T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), and type B, including deoxynivalenol (DON, vomitoxin), nivalenol (NIV) and their mono- and di-acetylated derivatives (Yli-Mattila, 2010).

The ability of *Fusarium* spp. to produce particular mycotoxins can be investigated using biochemical and molecular techniques. Molecular techniques are based on detection of different gene clusters involved in production of mycotoxins. Chemotype determination can be performed with primers for several genes involved in trichothecene biosynthesis such as *Tri3*, *Tri5, Tri7*, *Tri12* and *Tri13* (Mahmoud and Shehata, 2017). The type of trichothecene produced by an isolate can be predicted based on genetic markers derived from the *Tri* gene cluster, containing the genes involved in tricothecene biosynthesis. Different mycotoxins have different toxicological properties. NIV is more toxic than DON to humans and domestic animals. Trichothecenes such as DON are potent inhibitors of eukaryotic protein biosynthesis (Van der Lee *et al*., 2015). Trichothecenes also play important roles as virulence factors in fungal pathogenesis.

Based on several previous reports, different types of trichothecene chemotypes are produced by *F. graminearum* and *F. culmorum*, and possibly by other *Fusarium* species (Khaledi et al. 2017, Desjardins et al. 1993; Sarver et al. 2011; Li et al. 2016). DON is one of the factors influencing virulence and aggressiveness of *Fusarium* spp. Some studies have reported that chemotype diversity depends on geographical distribution. Both DON and NIV chemotypes are reported from several countries in Asia, Africa, Europe, South and North America. The DON chemotype is reported as the major trichothecene chemotype present in North America, while the NIV chemotype has not been detected in this region. The NIV chemotype was most frequently isolated from some Asian and European countries (Gilbert *et al*., 2002; Zeller *et al*., 2003; Panthi *et al*., 2014).

Among the *Fusarium* isolates, NIV, 3-ADON and 15-ADON chemotypes were detected from different fields of Mazandaran and Golestan provinces in the northern region of Iran (Haratian *et al*., 2008; Malihipour *et al*., 2012), while 15-ADON was the only chemotype detected among the isolates collected from fields of Ardabil province in the North West of Iran (Malihipour *et al*., 2012; Davari *et al*., 2013). Among *Fusarium* isolates from fields of Golestan province in the North of Iran, the NIV genotype occurred more frequently, followed by 3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON) genotypes (Khaledi *et al*., 2016).

DON is the most predominant mycotoxin associated with progress of disease in host plants (Mirocha et al. 2003; Taheri 2018). DON is more phytotoxic on cereals than NIV (Desjardins 2006), disrupting normal cell function by inhibiting protein biosynthesis, and is a major virulence factor of *Fusarium* spp. pathogenic on cereals (Khaledi et al. 2017; Yu et al. 2008; Zhang et al. 2010).

Zearalenone (ZEA), a polyketide mycotoxin, has chronic estrogenic effects on mammals, causing reproductive problems in farm-raised pigs, experimental animals, livestock and humans (Gaffoor and Trail, 2006). ZEA can be produced pre- or post-harvest in maize and other cereals. Contaminations to ZEA by *Fusarium* spp. in maize, wheat and barley were reported from North, West and South of Iran (Karami-Osboo and Mirabolfathy, 2008; Ehsani *et al*., 2014). Production of ZEA by *F. acuminatum*, *F. crookwellense*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. sporotrichioides* and *F. semitectum* have been reported (Jiménez *et al*., 1996; Ezekiel *et al*., 2008).

The other mechanism involved in aggressiveness and virulence of *Fusarium* spp. is production of extracellular enzymes, which degrade host plant cell walls. These cell wall degrading enzymes (CWDEs), such as cellulases and pectinases, are crucial in the processes of colonization and disease establishment (Wanyoike *et al*., 2002; Kikot *et al*., 2010). The CWDEs are involved in softening the cell walls, increasing accessibility of cell wall components for degradation by other enzymes, which enables success of further infection steps and spread of fungal mycelia into the inner host plant tissues (Roncero *et al*., 2003; Ortega *et al*., 2013).

The objectives of the present study were: (i) to investigate the capability of *Fusarium* spp. isolates (obtained from wheat plants with crown and root rot symptoms) for producing various virulence factors such as trichothecenes, ZEA and CWDEs such as cellulase and pectinase; and (ii) to examine pathogenicity and aggressiveness of *Fusarium* isolates on wheat, and determine relationships between ability of the isolates to produce virulence factors and their pathogenicity.

MATERIALS AND METHODS

Fungal inoculum preparation

Forty isolates, belonging to eight species of *Fusarium*, were obtained from wheat plants showing symptoms of crown and root rot in Yazd province of Iran. The isolates were deposited in the fungal collection in Ferdowsi University of Mashhad, Iran (Table 1). Fun-

Table 1. Capability of *Fusarium* spp. isolates obtained from wheat plants for production of pectinase and cellulase, based on quantitative (μg mL^{-1}) and qualitative (mm) analyses, together with virulence and aggressiveness traits of *Fusarium* spp. isolates on inoculated wheat leaf segments. Virulence was evaluated on the leaf segments at 7 d post inoculation.

Isolate code	Maximum of enzyme activity (μg mL^{-1})		Zone diameter (mm)		Virulence on leaf segments (LL[a], mm)	Aggressiveness on leaf segments (hpi[b])
	Pectinase±SE	Cellulase±SE	Pectinase on pectin agar media±SE	Cellulase on CMC agar media±SE		
Ab1	4028±89l mn [c]	1129.5±20.5 abc	6.6±0.8 efg	7.3±0.3 fg	11±2.3 def	24
Ab2	3865±1 no	674±28 kl	5±0.1 ij	3.6±0.3 lm	6±0.5 j	96
Ab3	3727±10.5 rst	735.5±23.5 ij	5±1.1 ij	4.6±0.8 jkl	7.6±0.6 gh	96
Ar1	4309±86 gh	1087.5±27.5 abcde	8.3±0.3 bcde	8.3±0.3 cd	12.6±2.4 de	36
Ar2	4264±49 ghijkl	1010±10 bc	7.3±0.8 de	6±0.5 hi	10±0.1 def	48
As1	4221±207.5 hi	954±31 efg	7.6±0.8 cd	7±0.5 gh	9±2 ef	36
As2	5218±28 bc	1139.5±4.5 abc	12.3±1.3 a	10.3±0.3 ab	25±1.7 a	12
As3	4135±42.5 jk	965±15 efg	6.6±0.8 efg	6±1.5 hi	9.6±1.4 ef	72
Ba1	3770±182.5 qr	787.5±7.5 hi	6±0.1 gh	4.3±0.3 jkl	8.3±2 gh	96
Ba2	4008±3 lmn	690.5±3.5 jkl	7±0.5 ef	5±0.5 jk	8.6±0.8 fg	72
Ba3	3982±17 lmn	967.5±7.5 defg	7±0.5 ef	5.6±0.6 ij	9.3±1.2 ef	48
Ba4	4460±182 ghij	1068±61.5 abcde	10±0.5 abcd	10±0.1 abc	17±1.7 bc	24
Ba5	3938±66.5 mn	514.5±31.5 no	4.6±0.3 jk	3±0.5 m	6±0.1 j	120
Ba6	3725±30 rst	845±25 ghi	6.3±0.6 fg	5.6±0.8 ij	9±1.5 ef	72
Ba7	5031±28.5 cd	1082±22 abcde	10.6±0.6 ab	9.6±0.3 abcd	20.6±1.8 ab	24
Ba8	4021±84 lmn	750±23 hi	6±1.5 gh	6±0.1 hi	8.3±1.6 gh	72
De1	5445±130 ab	1145±12 abc	12.3±1.4 a	11.3±0.8 a	25.6±2 a	12
De2	4089±11 lm	956±4 efg	8±0.1 bcde	4.6±0.3 jkl	8.3±1.6 gh	96
Kh1	3733±108.5 rst	543±133 mno	4.6±1.2 jk	4±0.1 kl	6.6±0.3 ij	120
Kh3	3790±60 pq	700±10 jkl	6.3±0.8 fg	5±0.1 jk	8.3±0.8 gh	96
Kh4	4864±112 def	971±36 defg	9±0.5 bc	9±0.5 bcd	15.6±1.6 bcde	12
Kh5	4171±58.5 ij	810±20 hi	5.6±1.2 hi	4±0.5 kl	7±1.1 hij	120
Kh6	4487±160 ghi	1122.5±12.5 abc	9.3±0.3 b	8.6±0.3 bcde	12±2.6 def	24
Meh1	3831±54 op	476±32 no	4.6±0.3 jk	3±0.1 m	5.6±0.3 j	120
Meh2	4392±75.5 ghijk	1085±50 abcde	6±1.5 gh	7.3±0.3 fg	10±0.5 def	48
Meh3	4080.5±30.5 kl	1009±36 bc	7.3±0.3 de	6±1 hi	9.3±2.3 ef	48
Mey1	3872±62.5 no	1167± 7.5 a	10.3±0.6 abc	9.3±0.3 bc	16±3.7 bcd	24
Mey2	3942±47 mn	599±14 lm	6±1 gh	4.6±0.3 jkl	7.6±0.6 gh	96
Mey3	3679±26 st	498±127 no	4.3±0.8 kl	3.3±0.8 lm	6±0.5 j	120
Mey4	5017±7.5 cd	1102.5 ±2.5 abcde	7.3±1.2 de	8±0.1 de	11±2.8 def	24
Ta1	3671±81 st	443.5±94.5 o	4±0.5 l	3±0.5 m	5±0.1 j	120
Ta2	3763±77 qr	1117.5±2.5 abcd	7±0.1 ef	5±1.1 jk	10.6±0.6 def	72
Ta4	3777±99 pq	1045±95 abcde	7.6±0.3 cd	7±0.5 gh	11.3±0.3 def	36
Ta5	5174±39 bcd	1159.5±15.5 ab	10.3±0.8 abc	10±0.5 abc	20.3±1.6 abc	24
Ta6	3546.5±106.5 t	829±6 ghij	5.6±1.4 hi	5±0.5 jk	8±2 gh	72
Ya1	4098.5±91.5 kl	890±20 fgh	6.6±1.3 ef	5.6±0.3 ij	9.3±0.3 ef	72
Ya2	4903±27 cde	1057 ±67.5 abcdef	8.3±0.3 bcde	7.6±0.3 ef	11.6±1.6 def	36
Ya3	4604±209.5 efg	1003±2 cd	8.6±0.3 bcd	9±0.1 bcd	14.3±3.9 bcdef	24
Ya4	5641.5±195.5 a	1151±8 abc	9±0.1 bc	9.3±0.3 bc	14±5.6 cd	24
Ya5	4552±289.5 fgh	1062.5±52.5 abcde	7.6±0.6 cd	8±0.5 de	12±3.5 def	36

[a] LL, Lesion length.

[b] hpi, hours post inoculation

[c] Different letters indicate significant differences ($P = 0.05$), according to Duncan analysis. Each experiment was repeated twice, with similar results.

gal inocula were produced in Mung Bean Broth (MBB) using the method of Zhang *et al.* (2013). Conidium suspensions were diluted in water containing 0.05% (v/v) Tween 20, to final concentration of 1×10^5 conidia mL^{-1}.

Virulence assays

Spring wheat cultivar (cv. Falat), obtained from Agricultural Research Center of Khorassan Razavi province in Iran, was grown in a greenhouse, with 12 h photoperiod, RH of 75%, and a day:night temperature regime of 18°C:12 °C. After 14 d, 7 cm segments from the mid-section of the first leaf of plants were harvested, and placed adaxial surface up on the surfaces of 0.5 % water agar in Petri plates, as described by Browne and Cooke (2004). Leaf segments were each inoculated at the center of the adaxial surface with 5 µL of conidium suspension. Sterile distilled water containing 0.05% (v/v) Tween 20 was applied on the control (non-inoculated) leaf segments. Petri dishes were incubated at 25°C with a 12 h:12 h light:dark cycle. After 7 d, the lesion length at the point of inoculation on each leaf was determined. The experiment was replicated three times for each isolate, and repeated twice.

Assessment of aggressiveness

Aggressiveness, as another quantitative component of pathogenicity, was investigated for each fungal isolate on detached leaves of wheat plants (cv. Falat) in laboratory conditions using the methods described by Malihipour *et al.* (2012) and Pariaud *et al.* (2009). Analysis of aggressiveness was determined based on hours post inoculation (hpi) for disease symptom appearance.

Qualitative analyses of cell wall degrading enzymes

For determining cellulase activity**,** Glucose Yeast Extract Peptone Agar containing 0.5% carboxy-methyl-cellulose in Petri plates was used for qualitative investigation of cellulase activity. After 3 to 5 d of fungal colony growth, plates were flooded with 0.2% aqueous Congo red solution and de-stained with 1M NaCl for 15 min. Appearance of yellow areas around fungal colonies in red medium indicated cellulase activity (Hankin *et al.*, 1971).

Pectinolytic activity was determined by growing the fungi in Petri plates containing Pectin Agar (containing 5 g L^{-1} pectin, 1 g L^{-1} yeast extract, and 15 g L^{-1} agar in distilled water; pH 5.0). After incubation of 3 to 7 d at 28°C, pectin utilization was detected by flooding the culture plates with freshly prepared iodine-potassium iodide solution (1.0 g iodine + 5.0 g potassium iodide in 330 mL distilled water) (Hankin *et al.*, 1971). Clear zones formed around fungal colonies indicated pectinolytic activity.

In each assay, Petri plates each inoculated with a PDA plug without fungus were used as as negative controls.

Quantitative analysis of cell wall degrading enzymes

For cellulase assays, fungal cultures were prepared in 500 mL capacity Erlenmeyer flasks each containing 250 mL of culture medium, as described by Abdel-Razik (1970). After inoculation, incubation was carried out under shaking (150 rpm) at 27°C and darkness for 10 d. Cellulase activity was assessed using the method of Wood and Bhat (1988). Absorbance was measured at 550 nm, and the amount of reducing sugar released was calculated from the standard curve for glucose. One unit of cellulase activity was defined as the amount of enzyme that catalyzed glucose at 1.0 µ mol min^{-1} during the hydrolysis reaction.

For pectinase assays, the fungal cultures were prepared in 500 mL capacity Erlenmeyer flasks each containing 250 mL culture medium, as described by Mac-Millan and Voughin (1964). Pectinase activity was determined based on the amount of reducing sugar (D-galacturonic acid) released into the culture supernatant. The amount of D-galacturonic acid was determined using the dinitrosalicylic acid colorimetric method of Colowich (1995), and absorbance was measured at 540 nm. The unit of enzyme activity was defined as the amount of enzyme that released galacturonic acid at 1 µ mol min^{-1}, according to the standard curve. The standard curve was developed based on the absorbance for different concentrations of D-galacturonic acid.

Detection of the genes responsible for production of NIV, DON and zearalenone

For detection of DON, NIV and zearalenone genes, the *Tri5*, *Tri13* and *PKS4* (polyketide synthase) genes were amplified by PCR, using the primers pairs *Tri5F* (5′-AGCGACTACAGGCTTCCCTC-3′) and *Tri5R* (5′-AAACCATCCAGTTCTCCATCTG-3′) for *Tri5*, *Tri13F* (5′-TACGTGAAACATTGTTGGC-3′) and *Tri13R* (5′-GGTGTCCCAGGATCTGCG-3′) for *Tri13*, and *PKS4F* (5′-CGTCTTCGAGAAGATGACAT-3′) and *PKS4R* (5′-TGTTCTGCAAGCACTCCGA-3′) for *PKS4* (Doohan *et al.*, 1999; Waalwijk *et al.*, 2003; Meng *et al.*,

2010). The PCR cycles consisted of an initial denaturation step at 94°C for 2 min, followed by 30 cycles of denaturation (95°C for 35 s), annealing (60°C for 30 s), extension (72°C for 30 s), and final extension at 72°C for 7 min. The PCR products were detected on 1% agarose gels.

Mycotoxin analyses in laboratory cultures

Mycotoxin production by the *Fusarium* isolates in laboratory cultures was investigated using the method of Alvarez *et al.* (2009). Briefly, 25 g of rice grains (*Oryza sativa* L.) were soaked in 100 mL of sterile distilled water for 6 h in 500 mL capacity flasks. Water was then drained and the rice grain was autoclaved twice. Five mL of inoculum suspension (1×10^5 conidia mL^{-1}) of each isolate was added to each flask and incubated at 26 ± 1°C in darkness for 3 weeks. The rice-fungus mixtures were each ground in a mortar and then dispensed in an Erlenmeyer flask with 75 mL of acetonitrile:methanol:water (80:5:15, v/v/v). HPLC analysess was carried out on a Waters Alliance 2695 separations module coupled to a Waters 474 scanning fluorescence detector (Waters Corporation) that was set at 365 nm excitation and 440 nm emission. To perform chromatographic separations, 500 µL of water:methanol (86:14, v/v) was added to each extract and cleaned with a C18 Spherisorb 5 µm (250 × 4.6 mm; Merck). The mobile phase was water:acetonitrile:methanol (78:12:10 v/v/v) with a flow rate of 2.5 mL min^{-1}. NIV, DON and zearalenone production were measured in µg per kg of sample. Standards of the DON, NIV and ZEA were used to construct a five-point calibration curve of peak areas versus concentrations. The injection volume was 50 µL for both the standard solutions and sample extracts.

Statistical analyses

All experiments were set up in completely randomized designs. The data were analyzed by one-way analysis of variance (ANOVA), and comparison of means was carried out using the Duncan's Multiple Range Test ($P \leq 0.05$. Statistical analyses and correlation tests were performed using software Statistical Package for the Social Sciences (SPSS; version 22).

RESULTS

Virulence and aggressiveness assays

Comparison of the data obtained from inoculation of *Fusarium* spp. isolates on wheat leaf segments revealed that different isolates had different virulence capabilities (Table 1). Significant differences in disease index were recorded among the isolates tested. Leaf assays revealed that the greatest lesion length was produced by *F. solani* isolate De1 and *F. flocciferum* isolate As2. The least disease was observed for the *F. equiseti* isolate Ta1 and *F. oxysporum* isoklate Meh1. Other isolates tested fell between these isolates with various levels of virulence on wheat leaf segments (Table 1, Figure 1). The results of the aggressiveness test on detached leaves showed more rapid development of disease symptoms by *F. solani* isolate De1 and *F. flocciferum* isolate As2, compared to the other isolates tested (Table 1).

Analysis of cell wall degrading enzymes

Qualitative and quantitative analysis of CWDEs showed that all the *Fusarium* isolates were capable of producing pectinase and cellulose enzymes (Table 1). In the quantitative assays, the amounts of CWDE activity among isolates varied from 443.5 to 1167 µg mL^{-1} for cellulase and 3546.5 to 5641.5 µg mL^{-1} for pectinase. Quantitative results of CWDE assays agreed with the qualitative results. The *F. equiseti* isolate Mey1 and *F. pseudograminearum* isolate Ta5 had the greatest *in vitro*

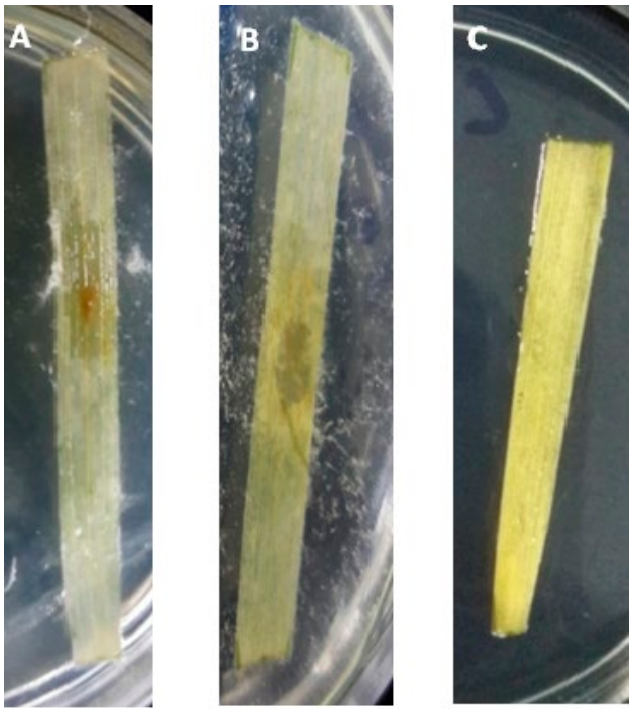

Figure 1. Disease symptoms caused by *Fusarium solani* isolate De1 and *F. flocciferum* isolate As2 on wheat leaves (A and B) and negative control (C).

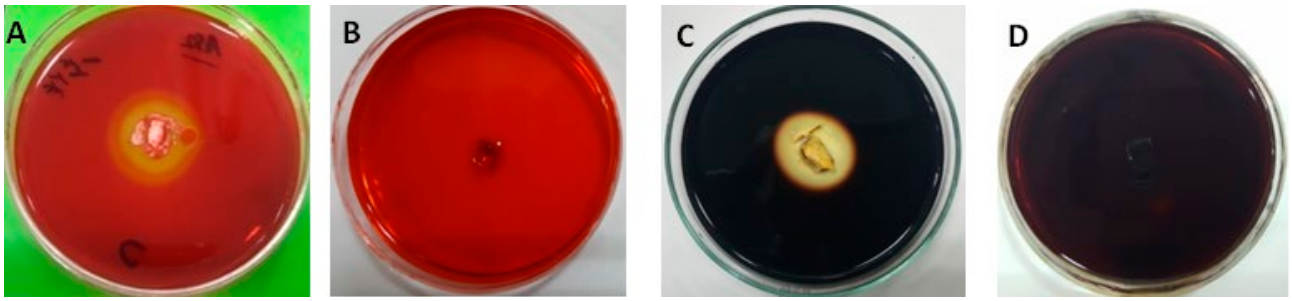

Figure 2. Cellulolytic activity of *Fusarium flocciferum* isolate As2 on GYP medium (A) and its negative control (B). Pectinolytic activity of this isolate on pectin agar medium (C) and its negative control (D).

cellulase activity of all the isolates. The least cellulase activity was measured for *F. equiseti* Ta1 and *F. oxysporum* Meh1. The *Fusarium oxysporum* Ya4 and *F. solani* De1 had the greatest pectinase activities. Least pectinase was measured for *F. flocciferum* Ta6 and *F. equiseti* Ta1. Based on the size of clear culture medium zones for cellulase activity, maximum activity was observed for *F. solani* De1 and *F. flocciferum* As2, while least activity was measured for *F. equiseti* Ta1, *F. oxysporum* Meh1 and *F. equiseti* Ba5.The least pectinase activity was measured for *F. equiseti* Ta1 and *F. pseudograminearum* Mey3, and *F. solani* isolate De1 and *F. flocciferum* isolate As2 gave the greatest pectinase activities (Figure 2).

Detection of trichothecene and zearalenone genotypes by PCR

The *Tri5* gene encodes trichodiene synthase, which catalyses the first step in trichothecene biosynthesis. This gene was detected using the primer set TRI5 (F)/ TRI5 (R), which produced a unique PCR product of 544 bp for the isolates which contained the *Tri5* gene (Figure 3A, Table 2) (Doohan *et al.*, 1999; Covarelli *et al.*, 2015). The results obtained from PCR of the *Tri5* gene showed amplification of this gene in 43% of the isolates, which produced either NIV or DON.

Also, the Tri13F/Tri13R primers for amplification of *Tri13* gene amplified a fragment in the range of 200 to 300 bp from DON producers and 400 to 450 bp from NIV producing isolates of *Fusarium* (Figure 3B, Table 2). Among the isolates producing trichothecenes, results obtained from PCR of the *Tri13* gene showed amplification of this gene for 35% of DON-producing isolates, and 65% of NIV- producing isolates.

The *PKS4* (polyketide synthase) gene of *F. graminearum* has been reported to be essential in production of ZEA (Lysøe *et al.*, 2006). The size of PCR products obtained in detecting this gene was approx. 280 bp (Fig-

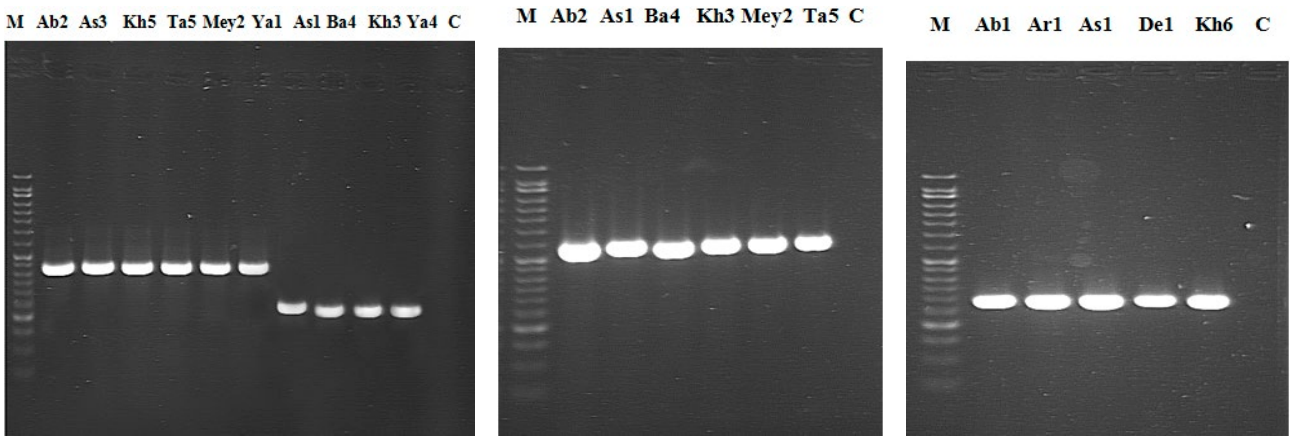

Figure 3. Amplification products using the primer pairs *Tri5F*/*Tri5R* (A), *Tri13F*/*Tri13R* (B), *PKS4F*/*PKS4R* (C), M: marker (1,500 bp). The primer set *Tri5F*/*Tri5R* produced a unique PCR product with the size of 544 bp for the isolates which contain the *Tri5* gene. The *Tri13F*/*Tri13R* primers amplified a fragment in the range of 200 to 300 bp from DON-producing *Fusarium* isolates and 400 to 450 bp from NIV-producing isolates. The *PKS4F*/*PKS4R* primer set produced a fragment of 280 bp for the zearalenone-producing isolates.

Table 2. Origin and species of *Fusarium* isolates obtained from wheat plants in Yazd province of Iran, together with the presence of trichothecene- (NIV and DON) and zearalenone- (ZEA) specific markers detected in the isolates by PCR assays, and their capability for mycotoxin production analyzed by HPLC.

Isolate code	Sample site	Species	Trichotecene genotype	PCR assay results			Toxin production *in vitro* (μg kg−1)		
				Tri5	Tri13	PKS4	NIV	DON	ZEA
Ab1	Abarkuh	*F. acuminatum*	-	-	-	+	-	-	ND
Ab2	Abarkuh	*F. culmorum*	NIV	+	+	+	298	ND	186
Ab3	Abarkuh	*F. culmorum*	DON	+	+	+	ND	1411	252
Ar1	Ardakan	*F. pseudograminearum*	NIV	+	+	+	1007	ND	3075
Ar2	Ardakan	*F. flocciferum*	-	-	-	-	-	-	-
As1	Ashkezar	*F. acuminatum*	DON	+	+	+	ND	379	ND
As2	Ashkezar	*F. flocciferum*	-	-	-	+	-	-	ND
As3	Ashkezar	*F. equiseti*	NIV	+	+	+	850	ND	ND
Ba1	Bafq	*F. solani*	-	-	-	+	-	-	ND
Ba2	Bafq	*F. acuminatum*	-	-	-	+	-	-	ND
Ba3	Bafq	*F. proliferatum*	-	-	-	+	-	-	ND
Ba4	Bafq	*F. culmorum*	DON	+	+	+	ND	1093	114
Ba5	Bafq	*F. equiseti*	-	-	-	-	-	-	-
Ba6	Bafq	*F. proliferatum*	-	-	-	+	-	-	67
Ba7	Bafq	*F. pseudograminearum*	DON	+	+	+	ND	65	3220
Ba8	Bafq	*F. equiseti*	-	-	-	+	-	-	ND
De1	Taft	*F. solani*	-	-	-	+	-	-	ND
De2	Taft	*F. flocciferum*	-	-	-	-	-	-	-
Kh1	Khatam	*F. flocciferum*	-	-	-	-	-	-	-
Kh3	Khatam	*F. equiseti*	DON	+	+	+	ND	103	ND
Kh4	Khatam	*F. culmorum*	NIV	+	+	+	1135	ND	230
Kh5	Khatam	*F. acuminatum*	NIV	+	+	+	350	ND	73
Kh6	Khatam	*F. pseudograminearum*	NIV	+	+	+	1083	ND	1760
Meh1	Mehriz	*F. oxysporum*	-	-	-	+	-	-	53
Meh2	Mehriz	*F. equiseti*	-	-	-	+	-	-	ND
Meh3	Mehriz	*F. solani*	-	-	-	-	-	-	-
Mey1	Meybod	*F. equiseti*	-	-	-	+	-	-	105
Mey2	Meybod	*F. equiseti*	NIV	+	+	+	480	ND	ND
Mey3	Meybod	*F. pseudograminearum*	NIV	+	+	+	258	ND	1385
Mey4	Meybod	*F. oxysporum*	-	-	-	+	-	-	ND
Ta1	Taft	*F. equiseti*	-	-	-	+	-	-	ND
Ta2	Taft	*F. equiseti*	-	-	-	+	-	-	96
Ta4	Taft	*F. pseudograminearum*	NIV	+	+	+	954	ND	1670
Ta5	Taft	*F. pseudograminearum*	NIV	+	-	+	1246	ND	2525
Ta6	Taft	*F. flocciferum*	-	-	-	-	-	-	-
Ya1	Yazd	*F. culmorum*	NIV	+	+	+	743	ND	365
Ya2	Yazd	*F. equiseti*	-	-	-	+	-	-	ND
Ya3	Yazd	*F. equiseti*	-	-	-	+	-	-	78
Ya4	Yazd	*F. oxysporum*	DON	+	+	+	ND	45	ND
Ya5	Yazd	*F. oxysporum*	-	-	-	-	-	-	-

ure 3C, Table 2). Overall, 83% of the *Fusarium* isolates produced this amplicon in the PCR assays.

Mycotoxin analyses using HPLC

Data of detection of trichothecenes produced by *Fusarium* isolates on rice grain showed that among 17 isolates which amplified the *Tri5* gene, 657% produced NIV and 35% produced DON. The levels of NIV ranged from 258 to 1,246 μg kg^{-1}, of DON from 45 to 1,411 μg kg^{-1}, and of ZEA from 53 to 3,220 μg kg^{-1} (Table 2).

DISCUSSION

This study was a detailed investigation of trichothecene genotypes and quantification of NIV, DON and ZEA by HPLC from *Fusarium* species associated to wheat crown and root rot in Iran. Activities of CWDEs and their relationships with virulence factors, aggressiveness and pathogenicity of *Fusarium* spp. isolates to wheat leaf tissues were also evaluated.

Different types of virulence factors can be produced by different *Fusarium* species pathogenic on cereals (Taheri 2018). Mycotoxins, such as trichthecenes and ZEA, and CWDEs, are among the main factors involved in virulence and aggressiveness of *Fusarium* spp. on host plants. We conclude that the two most virulent *Fusarium* isolates in this study (*F. solani* isolate De1 and *F. flocciferum* AS2), which do not produce tricothecenes, may produce other types of virulence factors, such as CWDEs as demonstrated here. Involvement of other virulence factors, such as lipases, xylanases, protein kinases, other proteins and various transcription factors (reviewed by Taheri, 2018), in pathogenicity of these fungi, needs to be investigated in the future studies.

Quantitative and qualitative activities of CWDEs, including cellulase and pectinase which are involved in the infection processes of *Fusarium* spp. on wheat, were investigated here. Aggressiveness of *Fusarium* spp. involves different mechanisms or components, such as production and release of extracellular enzymes which degrade host plant cell walls. The CWDEs are crucial in the processes of pathogen colonization and establishment of disease. Once infection is established, mycotoxins are released and these interfere with the metabolism, physiological processes and structural integrity of host cells (Ortega *et al*., 2013). These enzymes are particularly important for phytopathogenic fungi without specialized penetration structures (Gibson *et al*., 2011). The activities of CWDEs produced by the *Fusarium* isolates, which caused maximum or minimum virulence on wheat leaves, were compared for possible associations between the CWDEs and virulence. The isolates *F. solani* De1 () and *F. flocciferum* As2, which showed the greatest virulence on wheat leaves, had greater enzyme activities at different time points investigated. In contrast, the isolates Ta1 and Meh1 had the least virulence capability, and the lowest levels of CWDE activity. Correlation analysis revealed high levels of direct association between the capability of *Fusarium* spp. in producing CWDEs and their virulence on the wheat leaves (Table 3). Similarly, Khaledi *et al*. (2016) demonstrated the association of aggressiveness and virulence of *Fusarium* spp. isolates causing head blight of wheat with the levels of CWDEactivity.

Table 3. Correlation analyses between activity of cell wall degrading enzymes (in quantitative and qualitative assays) produced by *Fusarium* spp. isolates and their virulence on wheat leaves.

Correlation	Virulence on leaf segments	*P* value (two-tailed)
Pectinase (quantitative)	0.78231	< .0001
Pectinase (qualitative)	0.94502	< .0001
Cellulase (quantitative)	0.71337	< .0001
Cellulase (qualitative)	0.90916	< .0001

In the present study, trichothecene genotype detection revealed 50% amplification of the *Tri5* gene for *F. acuminatum* isolates, 27% amplification for *F. equiseti* isolates, and 25% amplification for those of *F. oxysporum*. *Fusarium pseudograminearum* and *F. culmorum* isolates had 100% amplification of the *Tri5* gene. However, this gene was not detected in isolates of *F. solani*, *F. proliferatum* and *F. flocciferum*. In accordance with our data, Tan and Niessen (2003) showed that *F. solani* was not capable of producing trichothecenes and ZEA, and this species lacked the *Tri5* gene required for biosynthesis of trichothecenes. Khaledi *et al*. (2016) reported that some *F. proliferatum* isolates causing wheat head blight amplified the *Tri5* gene and this species has the ability of trichothecenes biosynthesis, which is in agreement with the findings from the present study.

The *Tri13* gene from the *Fusarium* trichothecene biosynthetic gene cluster is responsible for conversion of DON to NIV (Lee *et al*., 2001). Our results showed that the NIV was produced by 65% of the isolates, whereas 35% of the isolates produced DON. There are few reports on the geographical distribution of trichothecene chemotypes produced by *Fusarium* spp. in different regions of Iran. Our data showed that the distribution of DON and NIV was not equal in different parts of the studied province, and that NIV was the dominant chemo-

type. Other investigations in Iran showed dominance of the NIV chemotype in Mazandaran (Haratian *et al*., 2008) and Golestan provinces (Abedi-Tizaki *et al*., 2013; Khaledi *et al*., 2016). Other studies in different regions of the world such as Africa, Asia and Europe have confirmed the presence of NIV and DON chemotypes, but only the DON type has been detected in North America (Miedaner *et al*., 2000). Both NIV and DON chemotypes have been identified together, in Europe and South America, and the DON chemotype was dominant in these regions. In Asian countries such as Korea and Japan, the NIV chemotype had the greatest distribution (Gale *et al*., 2011; Lee *et al*., 2002).

The results of the present study relating to virulence of *Fusarium* isolates on wheat leaf segments showed that all isolates were pathogenic to wheat (cv. Falat), and differences in virulence were observed. Some reports have showned that trichothecenes are virulence factors in plants, and they may contribute to colonization of wheat crowns by the pathogen (Mudge *et al*., 2006). Maier *et al*., (2006) showed that NIV and DON act as virulence factors on wheat, while only the NIV chemotype is virulent on maize (. In general in the present study, the isolates with NIV chemotype were more aggressive than the other chemotypes of trichothecenes produced by *Fusarium* spp. This is in agreement with the observations of other researchers (Cumagun *et al*., 2004; Khaledi *et al*., 2016).

The polyketide synthase gene *PKS4*, which is involved in ZEA biosynthesis, was used in our study for developing a PCR-based assay to detect ZEA-producing *Fusarium* isolates (Meng *et al*., 2010). The isolates of *F. flocciferum* and *F. solani* did not produce ZEA. The results of our study showed that the levels of ZEA were not correlated with virulence and aggressiveness of *Fusarium* spp. isolates. This is similar to results of Kuhnem *et al*. (2015), who found that the level of ZEA produced by *F. graminearum* was not related to severity of the disease caused by this species on maize.

Finding novel and effective ways to prevent or decrease production of different types of virulence factors by *Fusarium* spp. may be helpful in management of destructive diseases caused by these important and commonly occurring phytopathogenic fungi.

ACKNOWLEDGMENTS

The Ministry of Science, Research and Technology, and Ferdowsi University of Mashhad, Iran, financially supported this research (project number 3/41181 approved on 2/6/2016). The authors have no conflicts of interest to declare.

LITERATURE CITED

Abdel-Razik A.A., 1970. The parasitism of white *Sclerotium cepivorum* Berk.the incitant of white rot of onion. PhD thesis, Faculty of Agriculture, Assiut University, Assiut, Egypt

Abedi-Tizaki M., Sabbagh S.K., Mazaheri Naeini S.K., Sepehrikia S., 2013. Chemotyping of *Fusarium graminearum* using *Tri13* trichothecene biosynthetic gene. *Journal of Plant Protection* 2: 487-500.

Alvarez C.A., Azcarate M.P., Fernandez P.V., 2009. Toxigenic potential of *Fusarium graminearum sensu stricto* isolates from wheat in Argentina. *International Journal of Food Microbiology* 135: 131–135.

Besharati Fard M., Mohammadi A., Darvishnia M., 2017. *Fusarium* species associated with Wheat crown and root tissues in the Eastern Iran. *Archives of Phytopathology and Plant Protection* 50: 123-133.

Browne R.A., Cooke B.M., 2004. Development and evaluation of an *in vitro* detached leaf assay for pre-screening resistance to *Fusarium* head blight in wheat. *European Journal of Plant Pathology* 110: 91–102.

Colowich S.P., 1995. Methods in Enzymology. London: Academic Prees INC.

Covarelli L., Beccari G., Prodi A., Generotti S., Etruschi F.J.C., … Mañesc J., 2015. *Fusarium* species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy. *Journal of the Science of Food and Agriculture* 95: 540–551.

Cumagun C.J., Bodwen R.L., Jurgenson J.E., Leslie J.F., Miedaner T., 2004. Genetic mapping of pathogenicity and aggressiveness of *Gibberella zeae* (*Fusarium graminearum*) toward wheat. *Phytopathology* 94: 520–526.

Davari M., Wei S.H., Babay-Ahari A., Arzanlou M., Waalwijk C., … van Diepeningen A.D., 2013. Geographic differences in trichothecene chemotypes of *Fusarium graminearum* in the North-West and North of Iran. *World Mycotoxin Journal* 6: 137–150.

Desjardins A.E., Hohn T.M., McCormick S.P., 1993. Trichothecene biosynthesis in Fusarium species: chemistry, genetics, and significance. *Microbiology Review* 57: 595-604.

Desjardin A. E, 2006. *Fusarium mycotoxins: Chemistry. Genetics and Biology*. APS Press, St. Paul, MN, USA.

Doohan F.M., Weston G., Rezanoor H.N., Parry D.W., Nicholson P., 1999. Development and use of a reverse transcription-PCR assay to study expression of Tri5 *in vitro* and *in planta*. *Applied and Environmental Microbiology* 65: 3850–3854.

Ehsani M., Chaichi Nosrati A., Shirazi-Beheshtiha S.H., 2014. Measuring toxin Zearalenone in wheat harvest-

ed from three regions of North, West and South of Iran. *Bulletin of Environment, Pharmacology and Life Sciences* 3: 69-76.

Ezekiel C.N., Odebod A.C., Fapohunda S.O., 2008. Zearalenone production by naturally occurring *Fusarium* species on maize, wheat and soybeans from Nigeria. *Journal of Biodiversity and Environmental sciences* 2: 77-82.

Gaffoor I., Trail F., 2006. Characterization of two polyketide synthase genes involved in zearalenone biosynthesis in *Gibberella zeae*. *Applied and Environmental Microbiology* 72: 1793–1799.

Gale L.R., Harrison S.A., Ward T.J., O'Donnel K., Milus E.A., … Kistler H.C., 2011. Nivalenol type populations of *Fusarium graminearum* and *F. asiaticum* are prevalent wheat in southern Louisiana. *Phytopathology* 101: 124–134.

Gibson D.M., King B.C., Hayes M.L., G.C. Bergstrom, 2011. Plant pathogens as a source of diverse enzymes for lignocellulose digestion. *Current Opinion in Microbiology* 14, 264–270.

Gilbert J., Abramson D., McCallum B., Clear R., 2002. Comparison of Canadian *Fusarium graminearum* isolates for aggressiveness, vegetative compatibility, and production of ergosterol and mycotoxins. *Mycopathologia* 153: 209-215.

Hankin L., Zucker M., Sands D.C., 1971. Improved solid medium for the detection and enumeration of pectolytic bacteria. *Journal of Applied Microbiology* 22: 205-209.

Haratian M., Sharifnabi B., Alizadeh A., Safaie N., 2008. PCR analysis of the *Tri13* gene to determine the genetic potential of *Fusarium graminearum* isolates from Iran to produce Nivalenol and Deoxynivalenol. *Mycopathologia* 166: 109–116.

Jiménez M., Máñez M., Hernández E., 1996. Influence of water activity and temperature on the production of zearalenone in corn by three *Fusarium* species. *International Journal of Food Microbiology* 29: 417-421.

Karami-Osboo R., Mirabolfathy M., 2008. Natural zearalenone contamination of wheat from golestan province, northern Iran. *Iranian Journal of Plant Pathology* 44: 60-65.

Khaledi N., Taheri P., Falahati Rastegar M., 2017. Identification, virulence factors characterization, pathogenicity and aggressiveness analysis of *Fusarium* spp., causing wheat head blight in Iran. *European Journal of Plant Pathology* 147: 897-918.

Kikot G.E., Hours R.A., Alconada T.M., 2010. Extracellular enzymes of *Fusarium graminearum* isolates. *Brazilian Archives of Biology and Technology* 53: 779–783.

Kuhnem P.R., Del Ponte E.M., Dong Y., Bergstrom G.C., 2015. *Fusarium graminearum* isolates from wheat and maize in New York show similar range of aggressiveness and toxigenicity in cross- species pathogenicity test. *Phytopathology* 105: 441-448.

Lee T., Oh D.W., Kim H.S., Lee J., Kim Y.H., … Lee Y.W., 2001. Identification of deoxynivalenol and nivalenol producing chemotypes of *Gibberella zeae* by using PCR. *Applied and Environmental Microbiology* l67: 2966–2972.

Lee T., Han Y.K., Kim K.H., Yun S.H., Lee Y.W., 2002. *Tri13* and *Tri7* determine deoxynivalenol and nivalenol producing chemotypes of *Gibberella zeae*. *Applied and Environmental Microbiology* 68: 2148-2154.

Li Z.F., He C.L., Wang Y., Li M.J., Dai Y.J., … Lin W., 2016. Enhancement of trichothecene mycotoxins of *Fusarium oxysporum* by ferulic acid aggravates oxidative damage in *Rehmannia glutinosa* Libosch. *Scientific Reports* 6: 33962.

Lysøe E., Klemsdal S.S., Bone K.R., Frandsen R.J.N., Johansen T., … Giese H., 2006. The *PKS4* gene of *Fusarium graminearum* is essential for zearalenone production. *Applied and Environmental Microbiology* 72: 3924–3932.

Ma L.J., Geiser D.M., Proctor R.H., Rooney A.P., O'Donnell K., … Kazan K., 2013. *Fusarium* pathogenomics. *Annual Review of Microbiology* 67: 399–416.

MacMillan J.D., Voughin R.H., 1964. Purification and properties of a polyglacturonic acid- transeliminase produced by *Clastridium multiformentans*. *Biochemistry* 3: 564–572.

Mahmoud M.A., Shehata S.M., 2017. Molecular identification and characterization of *Fusarium* spp. associated with wheat grains. *International Journal of Advanced Research in Biological Sciences* 4: 77-87.

Maier F.J., Miedaner T., Hadeler B., Felk A., Salomon S., … Schaefer W., 2006. Involvement of trichothecenes in fusarioses of wheat, barley and maize evaluated by gene disruption of the trichodiene synthase (*Tri5*) gene in three field isolates of different chemotype and virulence. *Molecular Plant Pathology* 7: 449–461.

Malihipour A., Gilbert J., Piercey-Normore M., Cloutier S., 2012. Molecular phylogenetic analysis, trichothecene chemotype patterns and variation in aggressiveness of *Fusarium* isolates causing head blight in wheat. *Plant Dis*ease 96: 1016–1025.

Meng K., Wang Y., Yang P., Luo H., Bai Y., … Yao B., 2010. Rapid detection and quantification of zearalenone-producing *Fusarium* species by targeting the zearalenone synthase gene PKS4. *Food Control* 21: 207–211.

Miedaner T., Reinbrecht C., Schilling A.G., 2000. Association among aggressiveness, fungal colonization, and mycotoxin production of 26 isolates of *Fusarium*

graminearum in winter rye head blight. *Journal of Plant Diseases and Protection* 107: 124-134.

Mirocha C. J., Xie W., Filho E.R., 2003. Chemistry and detection of fusarium mycotoxins. In fusarium head blight of wheat and barley. In K. J. Leonard & W. R. Bushnell (Eds.), the American Phytopathological society (Vol. 2003, pp. 144–164). Minnesota, USA: St. Paul.

Mudge A.M., Macky R.D., Dong Y.H., Gardiner D.M., White R.G., Manners J.M., 2006. A role for the mycotoxin deoxynivalenol in stem colonisation during crown rot disease of wheat caused by *Fusarium graminearum* and *Fusarium pseudograminearum*. *Physiological and Molecular Plant Pathology* 69: 73-85.

Ortega L.M., Kikot G.E., Astoreca A.L., Alconada T.M., 2013. Screening of *Fusarium graminearum* isolates for enzymes extracellular and deoxynivalenol production. *Journal of Mycology* 358140: 1–7.

Panthi A., Hallen-Adams H., Wegulo S.N., Nopsa J.H., Baenziger P.S., 2014. Chemotype and aggressiveness of isolates of *Fusarium graminearum* causing head blight of wheat in Nebraska. *Canadian Journal of Plant Pathology* 36: 447–455.

Pariaud B., Ravigné V., Halkett F., Goyeau H., Carlier J., Lannou C., 2009. Aggressiveness and its role in the adaptation of plant pathogens. *Plant Pathology* 58: 409–424.

Paulitz T.C., Smiley R.W., Cook R.J., 2002. Insights into the prevalence and management of soil-borne cereal pathogens under direct seeding in the Pacific Northwest, USA. *Canadian Journal of Plant Pathology* 24: 416-428.

Roncero M.I.G., Hera C., Ruiz-Rubio M., Maceira F.I., Madrid M.P., … Dipietro A., 2003. *Fusarium* as a model for studying virulence in soilborne plant pathogens. *Physiological and Molecular Plant Pathology* 62: 87–98.

Sarver B.A., Ward T.J., Gale L.R., Broz K., Kistler H.C., … O'Donnell K., 2011. Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. *Fungal Genetetics and Biology* 48: 1096-1097.

Smiley R.W., Collins H.P., Rasmussen P.E., 1996. Diseases of wheat in long-term agronomic experiments at Pendleton, Oregon. *Plant Disease* 80: 813-820.

Smiley R.W., Gourlie J., Easley S., Patterson L.M., Whittaker R., 2005. Crop damage estimates for crown rot of wheat and barley in the Pacific Northwest. *Plant Disease* 89: 595-604.

Taheri P. 2018. Cereal diseases caused by *Fusarium graminearum*: from biology of the pathogen to oxidative burst-related host defense responses. *European Journal of Plant Pathology* 152: 1–20.

Tan M.K., Niessen L.M., 2003. Analysis of rDNA ITS sequences to determine genetic relationships among, and provide a basis for simplified diagnosis of *Fusarium* species causing crown rot and head blight of cereals. *Mycological. Research* 107: 811-821.

Van der Lee T., Zhang H., van Diepeningen A., Waalwijk C., 2015. Biogeography of *Fusarium graminearum* species complex and chemotypes: a review. *Food Additives & Contaminants: Part A. Chem Anal Control Expo Risk Assess* 32: 453–460.

Waalwijk C., Kastelein P., de Vries I., Kerenyi Z., van der Lee T., Hesselink T., 2003. Major changes in *Fusarium* spp. in wheat in the Netherlands. *European Journal of Plant Pathology* 109: 743–754.

Wanyoike W.M., Kang Z., Buchenauer H., 2002. Importance of cell wall degrading enzymes produced by *Fusarium graminearum* during infection of wheat head. *European Journal of Plant Pathology* 108: 803–810.

Wood T.M., Bhat M., 1988. Methods for measuring cellulase activities. *Methods in Enzymology* 160: 87–112.

Yli-Mattila T., 2010. Ecology and evolution of toxigenic *Fusarium* species in cereals in northern Europe and Asia. *Journal of Plant Pathology* 92: 7-18.

Yu J.B., Bai G.H., Zhou W.C., Dong Y.H., Kolb F.L., 2008. Quantitative trait loci for fusarium head blight resistance in recombinant inbred population of Wangshuibai/Wheaton. *Phytopathology* 98: 87–94.

Zeller K.A., Bowden R.L., Leslie J.F., 2003. Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. *Phytopathology* 93: 874-880.

Zhang H., Zhang Z., van der Lee T., Chen W.Q., Xu J., … Feng J., 2010. Population genetic analyses of Fusarium asiaticum populations from barley suggest a recent shift favoring 3ADON producers in southern China. *Phytopathology* 100: 328–336.

Zhang J.B., Wang J.H., Gong A.D., Chen F.F., Song B., … Liao Y.C., 2013. Natural occurrence of *Fusarium* Head Blight, mycotoxins and mycotoxin producing isolates of *Fusarium* in commercial fields of wheat in Hubei. *Plant Pathology* 62: 92–102.