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IDENTIFICATION AND PATHOGENICITY OF *FUSARIUM* SPECIES ASSOCIATED WITH HEAD BLIGHT OF WHEAT IN IRAN

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Abstract

In order to determine *Fusarium* species associated with wheat heads, 95 spike samples were collected from different geographic regions and wheat growing areas in the southwest, west, northwest and north of Iran during 2006-2008. Samples were collected from plants showing head scab symptoms. A total of 280 *Fusarium* strains were isolated and identified. All strains belonged to 19 *Fusarium* species according to morphological characters. All *Fusarium* strains were evaluated to test their pathogenicity on wheat which stands that *F. graminearum, F. culmorum, F. crookwellense, F. trichothecioides* and *F. chlamydosporum* were the most pathogenic to wheat's head. This report is the first in-depth study to identify of *Fusarium* spp. from wheat in Iran.

Introduction

Wheat is one of the basic agriculture crops which provide the food requirement in Iran (Farshadfar *et al*., 2008). *Fusarium* species are responsible for many economical important plant diseases such as head blight on cereals (Niaz & Dawar, 2009). *Fusarium* head blight (FHB), known as scab, is a disease of different cereals can leads to the decrease quality and quantity of wheat's grains (Bottalico & Perrone, 2002). In the field, FHB is recognized by the premature bleaching of infected spikelets and during wet weather there is whitish or pinkish fluffy fungal mat on the infected heads. Several *Fusarium* spp. cause scab in wheat head, including *F. graminearum, F. crookwellense, F. culmorum, F. equiseti, F. chlamydosporum, F. avenaceum, F. semitectum, F. verticillioides* and *F. camptoceras* (McMullen *et al.,* 1997). *Fusarium* spp. can also produce mycotoxins which have impact on animal and human health (Gamanya & Sibanda, 2001). Among these mycotoxins, zearalenone, fumonisins and trichothecenes potentially could occur in a variety of foodstuffs and feedstuffs, which are responsible for variety of toxic effects including infertility and reproductive problems, mutagenic and carcinogenic (Nelson *et al.,* 1991; Thiel *et al.,* 1991; Shephard *et al.,* 2005). Wheat is one of the most significant sources of mycotoxin contamination in human and animal diets. The incidence of mycotoxin in maize has been investigated from main production areas and a region of high cancer prevalence in Iran (Ghiasian *et al*., 2006). So it was required to identification of *Fusarium* spp. from other crops such as wheat.

Materials and methods

Collection of glume samples and isolation of *Fusarium* spp.: Ninety five spike samples showing disease symptoms were collected from various fields at spike stage, from wheat growing areas in the southwest, west, northwest and north of Iran during 2006-2008 (Table 1). The glumes were surface sterilized with 0.05% Sodium hypochlorite solution for 3 min, rinsed twice with sterilized distilled water and placed on Pepton-Pentachloronitrobenzene agar (PPA) plates that was proposed by Nash and Snyder (1962) and water agar (WA). All the plates were incubated under specify standard incubation conditions (Salleh & Sulaiman, 1984) for 48 h. The resulting single-spored *Fusarium* colonies were transferred onto potato dextrose agar (PDA) plates for further studies.

Identification of *Fusarium* spp. through morphological characterization: To study the growth rates and pigment production of *Fusarium* spp. all the strains were transferred onto PDA plates and incubated at ambient temperature. Ten replications were maintained for each *Fusarium* strain. For microscopic observations, all the strains of *Fusarium* were transferred to carnation leaf agar (CLA) (Fisher *et al.,* 1982) and potassium chloride agar (KClA) (Fisher *et al.,* 1983) mediums. The species were identified on the basis of macroscopic characteristics such as pigment production and growth rates on PDA plates, as well as their microscopic features including size of macroconidia, presence of microconidia and its production in chains or false heads, type of conidiogenous cells (monophialidies and polyplialidies conidiophores) and also absence or presence of chlamydospores (Gerlach & Nirenberg, 1982). Identification of species was based on species description of Gerlach & Nirenberg (1982) and Leslie & Summerell (2006).

Pathogenicity Test: All of the identified *Fusarium* species were tested for their pathogenicity on healthy wheat seedlings variety Zarrin. The healthy wheat's were inoculated with each isolate 10-14 days after heading. Prior to inoculation, 10 spikes per pot were randomly selected. For inoculation, all of the isolates were grown on PDA plates and conidial suspension of each individual strain was prepared by scrapping the mycelium with sterile distilled water onto 7 day-old cultures, shaken thoroughly, and the concentration was adjusted to 1×10^6 conidia mL^{-1} using a haemocytometer. Plants were inoculated at anthesis stage by injection of 5 uL of macroconidial suspension in each spiklets using point-inoculation method by hypodermic syringe (Dubin *et al.,* 1997).

After 30 min of the inoculation, the experiments were carried out in the polyethylene humidity chamber conditions maintained at 25°C, 100% RH for 48 h. After incubation, plants were transferred to the greenhouse. The negative control plants were injected with 5 uL sterile distilled water per spike and for positive control; plants

were injected with 5 uL of aggressive *F. graminearum* isolate. In the greenhouse, symptoms of FHB were observed at 28 days after inoculation and rated for disease severity according to the method of Xue *et al.* (2004). Also percentage of infected spikelet's (IS) was estimated at 4 weeks after inoculation, when plants were at the soft dough stage. Disease severity was estimated visually *In Situ* for each inoculated spike on a 0 to 9 (no visible FHB symptoms to severely diseased, spike dead) scale described by Xue *et al*. (2004). Disease severities and percentages of IS from all plants in each pot were averaged and the means per pot of percent IS were used in the statistical analysis.

Table 1. *Fusarium* spp., identified on wheat samples

Place of sample collection	Total No. of spike samples	No. of spike samples infected with *Fusarium* spp.	*Fusarium* spp. identified
Ahvaz	5	5	*F.ve, F.se, F.pr, F.ny, F.an, F.sc, F.eq, F.gr, F.ve, F.ch*
Ahar	3	3	*F.eq, F.ox, F.pr, F.an, F.sc, F.ve, F.ny, F.gr, F.ch, F.se*
Andimeshk	4	4	*F.ch, F.an, F.sc, F.pr, F.ny, F.gr, F.ve, F.so*
Amol	3	3	*F.gr, F.pr, F.ve, F.ny*
Babol	2	2	*F.gr, F.ve*
Bahar	3	3	*F.gr, F.an, F.so, F.ve, F.pr, F.ny, F.eq, F.cr*
Babolsar	2	2	*F.gr, F.pr, F.ve, F.ny*
Behshahr	2	2	*F.gr, F.pr*
Bijar	3	3	*F.eq, F.so, F.ny, F.se, F.ox, F.av, F.gr, F.ve, F.cr*
Bisotun	5	5	*F.sc, F.ve, F.pr, F.ny, F.gr, F.se, F.av, F.eq, F.so, F.ox, F.co, F.an, F.sa, F.tr, F.ch, F.cu, F.sub, F.la*
Eilam	4	4	*F.ve, F.ny, F.gr, F.se, F.so, F.eq, F.av*
Hamadan	4	4	*F.gr, F.an, F.so, F.ox, F.sc, F.ve, F.pr, F.ny, F.eq, F.cr*
Kermanshah	3	3	*F.eq, F.so, F.sc, F.ve, F.pr, F.ny, F.gr, F.se, F.ox, F.av,*
Khoy	2	2	*F.sc, F.gr, F.se, F.eq, F.ve, F.pr, F.ox, F.av*
Lahijan	4	4	*F.gr, F.sc, F.pr, F.ny, F.se*
Langaroud	3	3	*F.sc, F.ve, F.pr, F.ny, F.gr*
Mahabad	3	3	*F.av, F.cu, F.gr, F.eq, F.so, F.sa, F.ve, F.se, F.ox, F.cr*
Myaneh	3	3	*F.av, F.cu, F.gr, F.eq, F.ve, F.se, F.ox, F.cr*
Nour	2	2	*F.pr, F.ny*
Sanandaj	4	4	*F.gr, F.sc, F.pr, F.ny, F.se, F.eq, F.ve, F.av*
Ramsar	3	3	*F.gr, F.pr, F.ve*
Rasht	5	5	*F.sc, F.ve, F.pr, F.ch, F.sub, F.se, F.av, F.eq, F.ny, F.gr, F.so, F.ox, F.co, F.an, F.sa, F.la*
Salmas	3	3	*F.pr, F.ny, F.gr, F.se, F.co, F.an, F.sa, F.tr*
Sari	5	5	*F.pr, F.ny, F.gr, F.se, F.co, F.an, F.sa, F.tr, F.ch, F.sub, F.la*
Takab	5	5	*F.eq, F.ox, F.se, F.av, F.gr, F.ve, F.sa, F.cu, F.sp, F.cr*
Tabriz	5	5	*F.ve, F.gr, F.sa, F.av, F.se, F.eq,, F.cu*
Urmia	5	5	*F.ve, F.gr, F.co, F.se, F.pr, F.eq, F.su*

F.av=F. avenaceum, F.cu=F. culmorum, F.an=F. anthophilum, F.co=F. compactum, F.ch=F. chlamydosporum, F.eq=F. equiseti, F.gr=F. graminearum, F.pr=F. proliferatum, F.ox=F. oxysporum, F.sa=F. sambucinum, F.cr=F. crookwellense, F.sc=F. scripi, F.ny=F. nygamai, F.sp=F. sporotrichioides, F.so=F. solani, F.la=F. lateritium, F.tr=F. trichothecioides, F.se=F. semitectum, F.ve=F. verticillioides.

Results

Ninety five spike samples were collected and analyzed for the occurrence of *Fusarium* spp.. All the spike samples were found positive for *Fusarium* species. A total of 280 *Fusarium* strains were isolated and identified through morphological characters. According to morphological characters all these strains belonged to 19 *Fusarium* spp., were *F. avenaceum, F. compactum, F. culmorum, F. equiseti, F. graminearum, F. nygamai, F. anthophillum, F. oxysporum, F. proliferatum, F. trichothecioides, F. crookwellense, F. chlamydosporum, F. sambucinum, F. scripi, F. solani, F. sporotrichioides, F. semitectum, F. lateritium* and *F. verticillioides* (Table 1 & 2). Of the *Fusarium* isolates collected from the wheat growing areas in Iran, *F. graminearum* was the most prevalent with frequency of 28%, followed by *F. verticillioides* and *F. proliferatum* with frequency of 14% and 10% respectively (Table 3). Four days after inoculation, disease symptoms were observed on the heads. Initially, FHB symptoms appeared on water-soaked then on lose their chlorophyll and mycelium developed abundantly in the infected spikelets and the infection spreads to adjacent spikelets or through the entire head. The results in pathogenicity tests indicated that *F. graminearum* had the greatest IS (75%), followed by *F. culmorum* (65%) *F. crookwellense* (50%), *F. trichothecioides* (50%), *F. chlamydosporum* (50%), *F. avenaceum* (45%), *F. verticillioides* (45%) and *F. sporotrichioides* (45%) are the most pathogenic on the heads and caused severe blighting of wheat heads following inoculation in the greenhouse (Table 3). Therefore, these eight species were considered highly to moderately pathogenic. *F. proliferatum, F. equiseti* and *F. nygamai* did not show blight heads but sometimes caused damage to inoculated spikelets (Table 3).

Table 2. Morphological characters of *Fusarium* spp. associated with wheat head blight.

Name of species	Chlamydospores	Pigmentation on PDA	Number of septa	Microconidia	Types of conidigenious cells		General morphology		Macroconidia size (µm)
					Poly	Mono	Apical cell	Basal cell	
F. avenaceum	-	Yellow	3-5	+	-	+	Tapered to pointed	Nfs	40-69 × 3.2-4.1
F. anthophilum	-	Violet	3-4	+	+	+	curved	Pdfs	33-66 × 2.5-4.5
F. crookwellense	+	Red	5	-	-	+	Tapered to pointed	Fs	32-72 × 4.1-6.7
F. culmorum	+	Red	3-4	-	-	+	Rounded	Notch	32-54 × 4.0-7.0
F. chlamydosporum	+	Red	3-5	+	+	+	Curved and pointed	Nfs	34-44 × 3.0-4.5
F. compactum	+	Red	5	-	-	+	Tapered , elongate	fs	24-65 × 3.0-6.3
F. equiseti	+	Brown	5-7	-	-	+	Tapered ,elongate	Fs	45-85 × 3.2-5.6
F. graminearum	+	Red	5-7	-	-	+	Tapered	Fs	35-75 × 4.0-6.5
F. lateritium	+	Beige	4-7	+	-	+	Hook or break	Nfs	35-72 × 3.6-6.0
F. nygamai	+	Violet	3-5	+	+	+	Short and tapered	Nfs	27-57 × 2.1-5.0
F. oxysporum	+	Violet	3	+	-	+	Curved	Fs	30-58 × 3.0-5.8
F. proliferatum	-	Violet	3-5	+	+	+	Curved	Pdfs	23-60 × 3.0-5.0
F. solani	+	Ream	3-5	+	-	+	Rounded	Nfs	34-66 × 3.6-6.0
F. semitectum	+	Brown	3-5	-	+	+	Curved and Tapered	Fs	35-58 × 3.0-5.0
F. sambucinum	+	Red	3-5	-	-	+	pointed	Fs	35-56 × 3.0-5.1
F. sporotrichioides	+	Red	3-5	+	+	+	Curved and Tapered	Nfs	26-53 × 3.0-5.5
F. scirpi	+	Violet	6-7	+	+	+	Tapered and elongate	Fs	46-80 × 3.9-6.0
F. trichothecioides	+	Red	3-5	-	-	+	Tapered to pointed	Fs	36-56 × 3.3-5.5
F. verticillioides	-	Violet	3-5	+	-	+	Curved	Nfs	36-62 × 2.5-4.5

+ = Presence, - = Absence, Poly= Polyphialidic, Mono= Monophialidic, Pdfs= Poorly developed foot shape, Nfs= Notch or foot shape, Fs= Foot shape

Table 3. Percentage of infected spikelets and frequency of different *Fusarium* species isolated from spike wheat samples from the largest area of wheat plantation in Iran

Name of Species	Infected spikelets (%)	Frequency (%)
F. avenaceum	45	8
F. anthophilum	0	2
F. crookwellense	50	6
F. culmorum	65	6
F. chlamydosporum	50	3
F. compactum	0	1
F. equiseti	10	3
F. graminearum	75	28
F. lateritium	0	2
F. nygamai	6	3
F. oxysporum	0	2
F. proliferatum	12	10
F. solani	0	1
F. semitectum	0	2
F. sambucinum	0	2
F. sporotrichioides	45	3
F. scirpi	0	1
F. trichothecioides	50	3
F. verticillioides	45	14

Discussion

In our study identification of *Fusarium* spp., and their pathogenicity on wheat was investigated in the largest area of wheat plantation in Iran. The fungal isolation assays made on spike samples collected throughout the Kermanshah, Kurdistan, Hamadan, Khuzestan, Western Azarbaijan, Estern Azarbaijan and Eilam provinces clearly indicate that the members of section *Discolor* could be pathogenic to the wheat and suggested that *F. graminearum* and *F. culmorum* could be the main causal agent of head blight. Several studies have shown that *Fusarium* spp., in section *Discolor* can be readily isolated from cereal grains and according to this, results obtained in this study are in agreement with the previous findings of Nicholson *et al.* , (2003) and Dawson *et al.,* (2004).

Investigations on the pathogenicity of these species indicated that *F. graminearum* and *F. culmorum* are the most important pathogens and have a main role to cause FHB on wheat in mentioned provinces. The results of this study are in agreement with the previous literatures from north and south of Iran (Zare & Ershad, 1997; Moosawi-Jorf *et al.,* 2007). In this survey all of the *Fusarium* species isolated from spike samples indicates that *Fusarium* species are capable contaminants of grains of wheat and may produce mycotoxins. On the other hands the occurrence of mycotoxins in wheat by *Fusarium* species is of great concern worldwide, and their presence in processed feeds and foods seems unavoidable (Bottalico & Perrone, 2002). Of the *Fusarium* isolates collected from mentioned provinces, *F. graminearum* was the most prevalent with a frequency of 28%, followed by *F. verticillioides* and *F. proliferatum* with a frequency of 22% and 18% respectively. Similar results were found by other researchers in north and south of Iran (Zare & Ershad, 1997; Moosawi-Jorf *et al.,* 2007; Kachuei *et al.,* 2009).

This study concludes that *Fusarium* spp., can cause FHB on wheat crop and may produce mycotoxins which have impact on human and animal health. This report is the first in-depth study to identity, pathogenicity and distribution of *Fusarium* spp. from wheat in Iran. We believe that this study will serve as a basis for further identification of *Fusarium* species using molecular techniques. Mycotoxin profiles produced by these species are under progress.

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