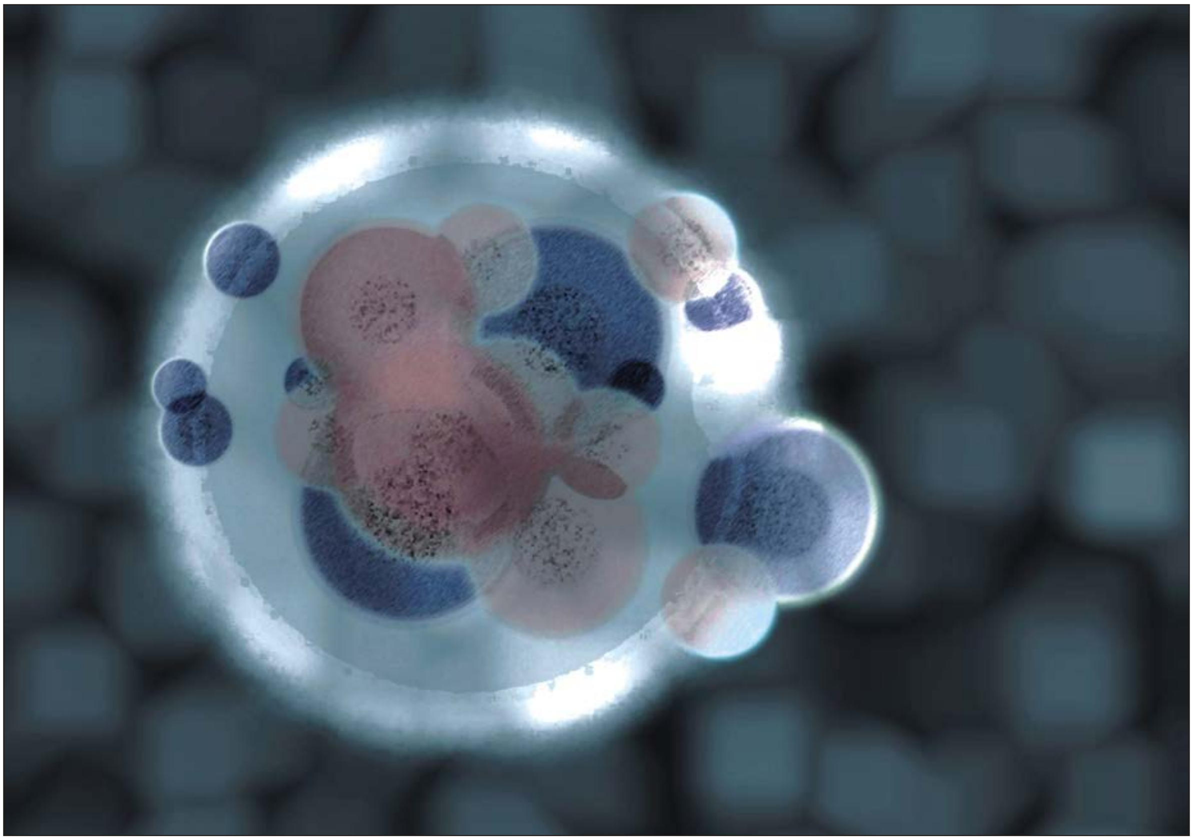

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Natural Occurrence of *Fusarium* Species Associated with Root and Stalk Rot of Maize in Kermanshah Province, Iran

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Abstract: The objective of this study was to identify *Fusarium* species associated with root and stalk rot of maize and their pathogenicity on root and stalk of maize in Kermanshah Province, Iran. In this survey 480 *Fusarium* strains were isolated and identified from maize ears collected from different geographic regions in Kermanshah province, Iran during 2006-2009. All these 480 strains belonged to 22 *Fusarium* species. This *Fusarium* strains were evaluated for their pathogenicity on maize and observed that *F. verticillioides*, *F. subglutinans* and *F. pseudoanthophilum* are the most pathogenic on stalk. Foot rot assessment revealed that *F. verticillioides* as the most damaging species. This is the first comprehensive report on identity and distribution of *Fusarium* spp. from maize in west of Iran while *F. pseudonygamai* was reported for the first time from west of Iran.

Key words: *Zea mays*, *Fusarium* spp., *Fusarium pseudonygamai*, pathogenicity, Western Iran

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops being cultivated in Iran. Worldwide reports have been shown that maize is one of the most prevalent crops contaminated by *Fusarium* (Fandohan *et al*., 2003). *Fusarium* spp. can cause a variety of diseases in many agricultural, horticultural and forestry crops. Due to their significant annual losses, studies on different aspects of its life cycle, biology, ecology, taxonomy and its pathogenicity has been regarded by plant pathologists. *Fusarium* species are a serious threat to susceptible crops worldwide, particularly maize (Nicolaisen *et al*., 2009). They cause root, stalk and ear rot, with severe reductions in crop yield, often estimated between 10 and 30% annually (Logrieco *et al*., 2002). There are two types of maize ear rot, called red ear rot and pink ear rot. Red ear rot universally caused by *Discolor* section and pink ear rot caused by *Liseola* section (Aliakbari *et al*., 2007). *Fusarium* spp. can also produce mycotoxins which relevant to animal and human health (Woloshuk and Shim, 2000; Velluti *et al*., 2001). *F. graminearum, F. culmorum, F. cerealis, F. poae* are the major producers of trichothecenes (Logrieco *et al*., 2002). Maize grains may be contaminated with different *Fusarium* toxins such as trichothecenes, zearalenone, fumonisins and moniliformin in grains that are harmful to both human and animals (IARC, 2002). Some of the strains of *F. verticillioides* do not produce disease in maize and are an endophyte, because it does not cause visible damage to the plant (Stone *et al*., 2000). The aim of this study is to identification of *Fusarium* species associated with maize and their pathogenicity assay on root and stalk of maize in Kermanshah province of Iran.

MATERIALS AND METHODS

Isolation and Identification of *Fusarium* spp.: During 2006-2009, diseased maize tissue sample were collected from 120 maize fields from different regions of Kermanshah province from West of Iran. Roots and stalks of diseased samples were washed under tap water. Then the samples were surface sterilized with 70% ethanol and cut into small blocks. The blocks were soaked in 1% sodium hypochlorite solution for 3 min and rinsed in several changes of sterile distilled water. All the sterilized samples were placed on Peptone-Pentachloronitrobenzene Agar (PPA) plates, a selective medium for *Fusarium* (Nash and Snyder, 1962). The plates were incubated under standard incubation conditions (Salleh and Sulaiman, 1984) for 24 h. The resulting single-spore *Fusarium* colonies were transferred to fresh Potato Dextrose

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Agar (PDA) plates and maintained at 4°C for further studies. 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), Spezieller Nahrstoffarmer Agar (SNA) (Nirenberg, 1976) and Potassium Chloride Agar (KClA) (Fisher *et al*., 1983) medium. 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 and Nirenberg, 1982). Identification of species was based on species description of Gerlach and Nirenberg (1982), Nelson *et al*. (1983) and Leslie and Summerell (2006).

Pathogenicity assay: All of the identified *Fusarium* species were tested for their pathogenicity on apparently healthy maize and individual plants were used for inoculation tests on root and stalk. The root and stalk of the maize were washed and surface sterilized before inoculation. For inoculation, each strain of *Fusarium* species were grown on PDA plates as described by Salleh and Sulaiman (1984). Conidial suspension of each strain was prepared by scrapping the mycelium with sterile distilled water onto 7 day-old cultures, shaken thoroughly and the concentration was adjusted to 2×10^6 conidia mL^{-1} using haemocytometer. Twenty milliliter of the spore suspension of each *Fusarium* species was sprayed on the root and stalk of the plants. Control plants were sprayed with 20 mL of sterile distilled water. Three replications were maintained for each strain and the experiment was repeated twice.

RESULTS AND DISCUSSION

In this study, 480 *Fusarium* isolates were isolated from diseased plants and based on morphological characteristics, these isolates were identified as 22 *Fusarium* species including *F. avenaceum*, *F. acuminatum*, *F. anthophilum*, *F. culmorum*, *F. chlamydosporum*, *F. equiseti*, *F. globosum*, *F. graminearum*, *F. lateritium*, *F. nygamai*, *F. oxysporum*, *F. poae*, *F. proliferatum*, *F. pseudoanthophilum*, *F. subglutinans*, *F. pseudonygamai*, *F. solani*, *F. semitectum*, *F. sambucinum*, *F. sporotrichioides*, *F. tricinctum* and *F. verticillioides* (Table 1, 2). Of the *Fusarium* isolates collected in Kermanshah province, Iran, *F. verticillioides* was the most prevalent with a frequency of 51.04 % (245 of 480), followed by *F. proliferatum* with a frequency of 30.42% (146 of 480). Among isolates, 25% from root and 75% from stalk tissue were recovered. All of the species except *F. oxysporum* and *F. lateritium* were recovered from stalk rot of maize. The species identified from root rot of maize were *F. verticillioides*, *F. oxysporum* and *F. lateritium*. In stalk tissues *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, *F. chlamydosporum*, *F. pseudoanthophilum*, *F. poae* and in root tissue *F. verticillioides* were predominant species. In this survey *F. pseudonygamai* is reported for the first time from Iran.

Eight days after inoculation, disease symptoms were observed on the roots. Initially, the roots illustrated brownish discoloration and eventually turned to dark color, which indicates rotting of the root tissues. Twelve days after inoculation, the first symptoms on maize stalks on yellowish and then degrees of rotting were observed on the stalk base. The results in pathogenicity tests indicated that *F. verticillioides*, *F. subglutinans* and *F. pseudoanthophilum* are the most pathogenic on stalk and that only *F. verticillioides* is pathogenic on the root.

In this study, the association was observed between maize plant and the species of *Gibberella fujikuroi* species complex (Nirenberg and O'Donnell, 1998). In the pathogenicity test, *F. verticillioides* was recovered from the inoculated root and stalk which indicated that this species was able to cause root and stalk rot of maize. *F. verticillioides*, *F. proliferatum* and *F. subglutinans* are the common pathogens of maize in different climates (Lew *et al*., 1991; Miller, 2001; Logrieco *et al*., 2002; Munkvold, 2003). The results in this study are in accordance with other findings in Iran (Ghiasian *et al*., 2004), Dawodee (2002), Mohammadi *et al*. (2002) and Naderpour (2004) had reported that *F. verticillioides* and *F. proliferatum* as the most comon species isolated from maize in Iran. This fungus can be seed borne internally in symptomless, apparently healthy maize kernels (Thomas and Buddenhagen, 1980). Also some strains of this fungus can produce potent mycotoxins associated with serious animal and human diseases (Wilson *et al*., 1985). Mycotoxin-producing *Fusarium* species such as *F. verticillioides*, *F. graminearum* and *F. poae* were isolated at high levels from disease samples in this study. This indicates that the presence of high infections of stalk rot on the ears thus supporting the farmers information on the presence of the disease in their fields. Thus, identification of the different species of *Fusarium*, including saprobe, pathogenic and

Table 1: Morphological characters of *Fusarium* spp. associated with maize

		Conidial arrangement			Microconidia shape					Types of conidigenious cells		
Name of species	Microconidia	F	M	S	P	G	C	Ob	Ova	Poly	Mono	Chlamydospores
F. avenaceum	+	-	-	-	-	-	-	-	+	-	+	-
F. acuminatum	-	-	-	-	-	-	-	-	-	-	+	+
F. anthophilum	+	+	-	-	+	-	-	-	+	+	-	-
F. culmorum	-	-	-	-	-	-	-	-	-	-	+	+
F. chlamydosporum	+	-	-	-	-	-	-	-	+	+	-	+
F. equiseti	-	-	-	-	-	-	-	-	-	-	+	+
F. globosum	+	-	+	+	-	+	+	+	-	+	-	-
F. graminearum	-	-	-	-	-	-	-	-	-	-	+	+
F. lateritium	+	-	-	-	-	-	-	-	+	-	+	+
F. nygamai	+	-	-	+	-	-	+	+	-	+	-	+
F. oxysporum	+	+	-	-	-	-	-	+	+	-	+	+
F. poae	+	+	-	-	+	+	-	-	-	-	+	+
F. proliferatum	+	-	-	+	+	-	+	-	-	+	-	-
F. pseudoanthophilum	+	-	-	+	+	-	+	+	+	+	-	+
F. subglutinans	+	+	-	-	-	-	-	-	+	+	-	+
F. pseudonygamai	+	+	-	+	-	-	+	+	+	+	-	+
F. solani	+	+	-	-	-	-	-	+	+	-	+	
F. semitectum	+	-	-	-	-	-	+	-	-	+	-	+
F. sambucinum	-	-	-	-	-	-	-	-	-	-	+	+
F. sporotrichioides	+	+	-	-	+	+	-	-	+	-	+	+
F. tricinctum	+	+	-	-	+	-	-	+	+	-	+	+
F. verticillioides	+	-	-	+	-	-	+	-	-	-	+	-

+: Presence, -: Absence, F: False heads (chains absent), M: Medium to long chains (>15), S: Short chains (<15), P: Pyriform, G: Globose, C: Clavate, Ob: Obovoid, Ova: Oval to allantoids/fusiod, Poly: Polyphialidic, Mono: Monophialidic

Table 2: Macroconidia characters of *Fusarium* spp. associated with maize

			General morphology			
Name of species	Sporodochia colour	No. of septa	Apial cell	Basal cell	Pigmentation on PDA	Macroconidia size (μm)
F. avenaceum	Pale orange	3-5	Tapered to pointed	Nfs	Yellow	45-66×3.0-4.0
F. acuminatum	Pale orange	3-5	Tapered to pointed	Fs	Red	35-65×3.2-5.4
F. anthophilum	Orange	3-4	Curved	Pdfs	Violet	29-64×2.6-4.5
F. culmorum	Brown	3-4	Rounded	Notch	Red	30-61×4.0-7.0
F. *chlamydosporum*	Not observed	3-5	Curved and pointed	Nfs	Red	30-42×3.0-4.5
F. equiseti	Orange	5-7	Tapered	Fs	Brown	44-77×3.2-5.7
F. globosum	Not observed	3-5	Slightly curved	Fs	Violet	24-74×2.0-4.0
F. graminearum	Not observed	5-7	Tapered	Fs	Red	38-71×4.0-6.5
F. lateritium	Orange	4-7	Hook or break	Nfs	Beige	38-71×3.6-6.0
F. nygamai	Orange	3-5	Short and tapered	Nfs	Violet	24-54×2.0-5.0
F. oxysporum	Pale orange	3	Curved	Fs	Violet	33-61×3.0-6.0
F. poae	Not observed	3-5	Curved	Fs	Red	35-48×3.0-5.4
F. proliferatum	Pale orange	3-5	Curved	Pdfs	Violet	19-59×3.0-5.0
F. pseudonygamai	Not observed	3-5	Tapered	Pdfs	Violet	24-64×2.0-4.2
F. pseudoanthophilum	Not observed	3-5	Bent or pointed	Fs	Violet	39-74×3.0-3.9
F. subglutinans	Orange	3-5	Curved	Pdfs	Dark purple	26-68×3.0-5.0
F. solani	Cream	3-5	Rounded	Nfs	Cream	32-71×3.5-6.0
F. semitectum	Orange	3-5	Curved and tapered	Fs	Brown	38-55×3.0-5.0
F. sambucinum	Orange	3-5	Pointed	Fs	Red	34-55×3.0-5.0
F. sporotrichioides	Orange	3-5	Curved and tapered	Nfs	Red	23-54×3.0-5.5
F. tricinctum	Pale orange	3-5	Curved	Fs	Red	24-51×3.0-5.0
F. verticillioides	Orange	3-5	Curved	Nfs	Violet	30-58×2.3-4.3

Pdfs: Poorly developed foot shape, Nfs: Notch or foot shape, Fs: Foot shape

toxin producing species, is of vital importance (Nelson *et al*., 1994). Our results indicated that a section *Liseola* or complex of *Gibberella fujikuroi* species (Nirenberg and O'Donnell, 1998) could be pathogenic to the maize and suggested that *F. verticillioides* could be the main causal agent of stalk and root rots. However, strains of *F. subglutinans* and *F. pseudoanthophilum* were the main causal agents of stalk rot as well. In this survey, the widespread of species especially in *Gibberella fujikuroi* species complex offers the opportunity for relatively easy collection of large numbers of species by phylogenetically-based classification (O'Donnell, 1996). The identification of the *Fusarium* species contaminating maize in different areas is evidence for a need, not only for studying the levels of the interaction between *Fusarium* pathogens and maize, but also to obtain a precise picture of the toxicological risks related to the maize consumption by humans and animals (Logrieco *et al*., 2002). The present study demonstrates the importance of *Fusarium* stalk and root rot of maize in Iran. We believe that this study will serve as a foundation for the further identification of *Fusarium* species, particularly molecular level from Iran. This is the first comprehensive report on identification of many *Fusarium* spp. from maize in west of Iran.

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