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Study of *Fusarium* Head Blight of Wheat in Khuzestan Province in Iran and Reporting of *Fusarium xylaroides* as a New Causal Agents for Disease

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Abstract: In order to determine *Fusarium* diversity associated with spikes and grains of wheat, this study was conducted. Samples were taken from plants showing head scab symptoms and wheat grains that had been collected from different growing areas in Khuzestan, during growing seasons in 2002-2004. After being surface sterilized, samples from head and grains were placed into petri plates containing common media, selective media and blotter test. Isolated fungi were purified using single spore method and identified at the species level using different identification keys. Among the identified fungi, there were 10 different species of *Fusarium* including: *F. equiseti*, *F. semitectum*, *F. sambucinum*, *F. culmorum*, *F. verticilioides*, *F. proliferatum*, *F. lateritium*, *F. subglutinans*, *F. xylaroides* and *F. camptoceras* and one species of *Microdochium nivale* (= *F. nivale)*. Based on the available references, all *Fusarium* species that were recovered in this study, are reported from wheat head and grains for the first time from Khuzestan province. Pathogenicity test of these species using point-inoculation method indicated that all species were pathogenic to wheat. Thus is the first report indicating the *F. xylaroides* as the causal agent of *Fusarium* head blight of wheat for the first time.

Key words: *Fusarium* diversity, wheat, Khuzestan province, causal agents

INTRODUCTION

Fusarium Head Blight (FHB), also known as scab or tombstone, is a disease of wheat, barley, oats and other small cereal grains and corn. Generally disease is more important in warm and humid areas (Dubin *et al*., 1996). In the field, FHB is recognized by the premature bleaching of infected spikelets and the production of orange, spore-bearing structures called sporodochia at the base of the glumes. During wet weather, there may be whitish, occasionally pinkish, fluffy fungal growth on the infected heads in the field. It has been reported that yield lost by FHB in wheat is about 0.25-5% (Jennings and Turner, 2004; Parry *et al*., 1995).

FHB is caused by several species of *Fusarium*; however, *Fusarium graminearum* Schw., with perfect stage of *Gibberella zeae* (Schw.) Petch. is the primary species involved (Alizadeh and Saidi, 1996; Dubin *et al*., 1996; McMullen *et al*., 1997). Other *Fusarium* species that have been reported as the causal agents of FHB, including *F. equiseti*, *F. equiseti* var. *compactum*, *F. sulphureum*, *F. semitectum*, *F. poae*, *F. avenaceum*, *F. acuminatum*, *F. verticilioides*, *F. verticilioides* var. *subglutinans*, *F. verticilioides* var. *anthophilum*, *F. oxysporum*, *F. solani*, *F. culmorum*, *F. camptoceras*, *F. tricinctum*, *F. heterosporum*, *F. proliferatum*, *F. sporotrichioides* and *Microdochium nivale* (DeGalich, 1996; Dubin *et al*., 1996; Wang, 1996), but there is not any report indicating that *F. xylaroides* could be cause of FHB of wheat.

F. xylaroides is causal agent of coffee wilt disease (tracheomycosis) which was first observed on the *Coffea excelsa* in 1927 in the Central African Republic (Nelson *et al*., 1983; Geiser *et al*., 2005; Anonymous, 2006). In the 1940s and after that, the disease spread to Côte D'Ivoire and Ethiopia which disease was observed on the *Coffea canephora* and the *Coffea arabica* respectively. By 1993, coffee wilt was reported from South Western Uganda and Zaire (Floor, 2001). As it was mentioned before, this species has not been reported as a pathogen of wheat in the world and has not been recorded is in the fungal list of Iran.

Mycotoxins are secondary metabolites produced by some of the FHB pathogens. It is thought that in some cases the role of the toxin may be to aid plant infection of the plant by the pathogen. Mycotoxins are of concern due to their potentially harmful effect to both humans and animals (Jennings and Turner, 2004). High concentration of mycotoxins in some specimens of wheat collected from Mazandaran province, Iran was detected (Alizadeh and Saidi, 1999; Safaie *et al*., 2005; Zamani-Zadeh *et al*., 1995).

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In Iran, FHB was first reported in 1977 from Dasht-e-Naz region of Sari (Ershad, 1995). It is an important disease of wheat in different areas of Iran, such as Mazandaran, Gorgan, Gonbad and Moghan regions, but had not been observed in Khuzestan province. In 2001-2002, because of the favorable weather conditions for disease development and heavy rainfalls at booting and anthesis stages, the disease was observed in Khuzestan (Moosawi- Jorf, 2003). During April 2002, field surveys revealed heavy infection of spikes in many wheat fields in Shoosh (Moosawi- Jorf, 2003; Moosawi- Jorf and Farrokhi-Nejad, 2005). The purpose of this study was to confirm the existence of the FHB of wheat in Khuzestan province and determine it's the causal agent (s) in this region.

MATERIALS AND METHODS

Isolation and identification: To isolate and identify the causal agent (s) of FHB, samples of spikes and grains with disease symptoms were collected from various fields, at spike stage, in Khuzestan province in the south-west of Iran. Fungi were isolated using common and selective media and blotter test routinely (Dhingra and Sinclair, 1995). To do so, the glumes and grains were surface disinfected with 0.05% sodium hypochlorite, rinsed with sterile distilled water three times and plated into Water Agar (WA) and selective Nash Snyder media and or on the sterilized wet filter papers in petri dishes. Isolated fungi were purified by single spore method. Morphological characteristics of the isolates were studied on Potato Dextrose Agar (PDA) and carnation-leaf agar (CLA) at 25°C day/20°C night and 12 h. photoperiod. Keys of Booth (1971), Burgess (1988, 1994) and Nelson *et al.* (1983) were used for identification. Dimensions were measured by OLYSIA BioReport software.

Pathogenicity test

Preparation of plants: For the pathogenicity test, grains of main cultivated variety, Chamran were disinfected using 0.05% sodium hypochlorite for 2 min, rinsed with sterile distilled water three times and planted in plastic pots (Dhingra and Sinclair, 1995). Pots were kept in a greenhouse at 17-25°C under natural light (about 14 h per day) until the appearance of the spike stage.

Preparation of inoculum and inoculation: Inoculum of each *Fusarium* species was prepared via plating of each isolate on PDA amended with yeast extract using Freeman and Rodriguez (1993) method. Plates were incubated in an incubator at 23°C (12 h photoperiod). Conidia were collected from 5 days old cultures with a small amount of sterile distilled water by scratching the surface of cultures (Freeman and Rodriguez, 1993). The collected conidial suspension was filtered through two layers of cheesecloth and its concentration was adjusted to 1×10^6 spores mL^{-1}. Plates were arranged in a completely randomized design with six replicates. Plants were inoculated at anthesis stage by injection of 5 µL of macroconidial suspension at concentration of 10^6 conidium mL^{-1} in each spiklets using point-inoculation method by a hypodermic syringe (Dubin *et al.*, 1996). Check plants were injected with the same amount of sterile distilled water. Inoculated spikes were sprayed daily with distilled water and covered with transparent nylons. Pots were incubated for a week at 23°C (16 h light period), then the spiklets were surveyed for the occurrence of infection and reisolation.

RESULTS AND DISCUSSION

Regarding to the isolate characteristics on CLA, PDA and using different keys of Nelson *et al.* (1983) and Burgess (1994), fungal isolates were identified at the species level. *Microdochium nivale* (= *Fusarium nivale)* and the following *Fusarium* species were among the identified fungi: *F. equiseti*, *F. semitectum*, *F. sambucinum*, *F. culmorum*, *F. verticilioides*, *F. proliferatum*, *F. lateritium*, *F. subglutinans*, *F. xylaroides* and *F. camptoceras* (Table 1). According to the available references, all *Fusarium* species that were obtained in this study, are reported from wheat head and grains from Khuzestan province for the first time.

This is the first report about the association of *F. xylaroides* with the FHB of wheat. *F. xylaroides* were isolated from spikes and grains of wheat which were collected from Shoosh and Shooshtar regions.

Growth rate of *F. xylaroides* colonies was moderately fast and reached 5.8-6.5 cm diameter in 10 days at 25°C on PDA. Aerial mycelium was sparse, appressed arachnoid to villous and sometime floccose in the central part. The

Table 1: *Fusarium* species isolated from spicks and grains of wheat

Fusarium species	No. of isolates from grains	No. of isolates from spikes
Microdochium nivale (*syn. = Fusarium nivale)*	-	7
F. equiseti	-	27
F. semitectum	6	10
F. sambucinum	-	7
F. culmorum	-	4
F. verticilioides	11	4
F. proliferatum	12	3
F. lateritium	4	2
F. subglutinans	5	-
F. xylaroides	1	2
F. camptoceras	-	1

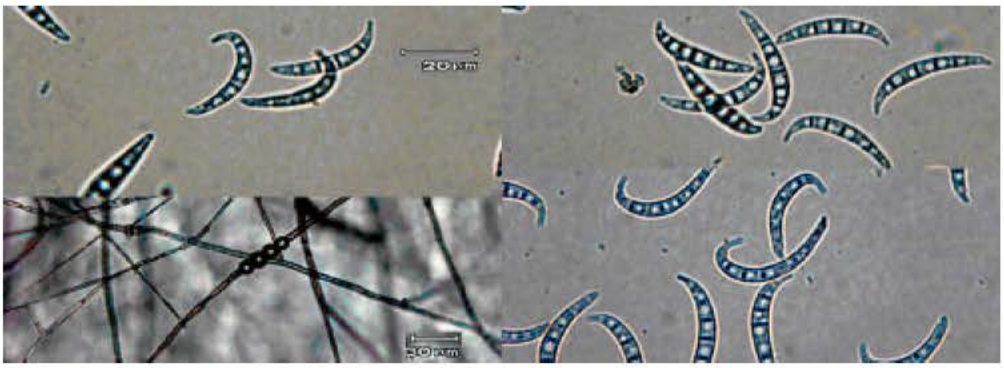

Fig. 1: *Fusarium xylaroides*, macroconidia and chlamidospores (original)

color of colonies were whitish, to greyish pink. Sporolation started within 5-7 days on CLA as unicellular to commonly 3-4 celled conidia on aerial mycelium. Culture sometimes had a slightly powdery appearance. After about 3 weeks, small flat or somewhat erumpent sporodochia or confluent pionnotal slimes developed on carnation leaf agar. Masses of macroconidia were cream to pink in sporodochia and pionnetes, respectively. Conidiophores were initially formed as a single lateral phialide on the hyphae, but there was another type of phialide that was produced in sporodochia or pionnetes was loosely branched, terminating in a group of 2 to 4 phialides. Only monophialids were observed. Macroconidia with 3-4 septum were very falcate to strongly curved, often with a very distinctly constricted, hooked apical cell and usually with a pedicellate basal cell. Macroconidia were measured 30.24 (23.85-37.60) X 3.75 (2.71-4.57) µm in diameter. In this study, all isolates of this species produced chlamydospores abundantly singly, in chain or clamps, intermediate or terminally on the mycelia. *F. xylaroides* does not produce microconidia in chain and does not produce polyphialides. These characters is clearly differentiate *F. xylaroides* from *F. annulatum* and *F. succisae*. Macroconidia are strongly curved and distinguished this species from *F. equiseti* (Fig. 1).

The pathogenicity of all *Fusarium* species listed above was approved using point-inoculation method. Infection was observed after 4-7 days and the pathogens were subsequently reisolated from the inoculated spikltes. The results indicated that all *Fusarium* species were pathogenic to wheat and produced the FHB symptom. In check treatments no infection were occurred. Pathogenicity of these species have not been reported previously from Khuzestan province, Iran and this is the first report about the pathoginicity of *F. xylaroides* on wheat as one of the causal agent of FHB.

In Iran 6 species of *Fusarium* including *F. graminearum*, *F. culmorum*, *F. proliferatum*, *F. semitectum*, *F. subglutinans* and *F.verticilioides* have been known as the causal agents of FHB (Ershad, 1995).

Investigations on the pathogenicity of these species indicated that *F. graminearum* and *F. culmorum* are the most important pathogens and distribution and the former one has a main role to introduced of FHB of wheat in Mazandaran, Gorgan, Gonbad and Moghan regions. *Giberella zeae* has been observed on the wheat, rice and maize debries of infected regions and produced in lab conditions (Golzar, 1993).

There are 5 major species of *Fusarium* responsible for FHB on wheat in the UK including *Fusarium avenaceum* (*Gibberella avenacea*), *Fusarium culmorum*, *Fusarium graminearum* (*Gibberella zeae*), *Fusarium poae* and *Microdochium nivale* (*Monographella nivalis* formerly *Fusarium nivale*). Each species is capable of infecting a crop on an individual basis or as part of a complex of species (Jennings and Turner, 2004).

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