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Fungi associated with root and crown rot of wheat in Khuzestan province, Iran

Mohammad Reza Eslahi

Khuzestan Agricultural and Natural Resources Research Center, Ahvaz, Iran.

Abstract: To identify the fungi associated with foot and root rot of wheat in the Khuzestan province, diseased samples were collected at all growth stages in three growing seasons of 2004-2007. Pieces of infected parts of the root and foot were surface sterilized and cultured on acidic and non acidic PDA media. One hundred and fifteen isolates were obtained and on the basis of macroscopic and microscopic characters and valid keys were identified as *Fusarium solani, F. equiseti, F. moniliforme, F. subglutinans, F. sambucinum, F. culmorum, F. proliferatum, F. pseudograminearum, F. longipes, F. avenacium, F. nygamai, F. semitectum, F. lateritium, Rhizoctonia solani, Bipolaris sorokiniana* and *Pythium* sp*. F. equiseti, F. solani* and *F. culmorum* with occurrence frequencies of 16.07, 16.07 and 12.5 % respectively were more frequent than all other species. Results of Pathogenicity tests indicated that *F. pseudograminearum* and *F.culmorum* were the main fungi associated with wheat root and crown rot disease in khuzestan while other *Fusarium* spp. such as *F. equiseti* are probably aggravated by moisture stress at different growth stages of crop due to poor irrigation management. *Rhizoctonia solani* with occurrence frequency of 8.03 % in some regions such as Ahvaz, Shoush, Shoushtar and Baghmalek was important agent of crown and root rot of wheat second to *Fusarium* species. *Bipolaris sorokiniana* and *Pythium* sp also caused crown and root rot but with less frequency percent.

Keywords: *Fusarium* spp., wheat, frequency, water stress, Khuzestan

Introduction

Wheat *(Triticum aestivum L.*) is the most important crop in Khuzestan province. Root and crown rot diseases are one of the most common and destructive diseases of wheat which occur every growing season. They cause early maturity and result in white heads and incomplete grain fill (Wallwork, 2000). Whole plants or individual tillers may be stunted. Necrotic brown lesions on seminal roots, crown, subcrown internodes and stem tissues are observed. Several different fungi cause root and crown rot of wheat, including *Fusarium, Rhizoctonia, Gaeumannomyces, Drecheslera, Exserohilum,* and *Bipolaris* spp. (Smiley *et al*., 2005; Cook *et al*., 2002; Freeman and Ward, 2004). *Fusarium* is one of the most important fungi of soil microflora (Leslie and Summerell, 2006). Some *Fusarium* species cause crown and root rot of wheat in temperate and subtropical regions (Windles and Holen, 1989). In recent years several researches were done in many wheat producing areas of Iran in this regard. For instance *Rhizoctonia solani, R. cerealis, F. graminearum* and *Gaeumannomyces graminis* were isolated from Mzandaran (Foroutan *et al*., 1995). Pathogenicity of *F. culmorum, F. avenaceum* and *F. acuminatum* were confirmed in

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* **Corresponding author**, e-mail: mr_eslahi@yahoo.com
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Fars province (Ravanlou and Banihashemi, 1999). The predominant pathogen causing common root and crown rot in Moghan area, northwest of Iran was identified to be *Bipolaris sorokiniana* (Hajigharari, 2009). *Rhizoctonia solani* is more severe in sandy soils where rainfall is lower (Gill *et al*., 2001). Take-all caused by *Gaeumannomyces graminis* var *tritici* is the least common agent of root rot but may be the most damaging, taking all of the yield. Take-all is typically a greater problem on winter wheat growing in wet soils (Cook, 2001).

This research was conducted to identify and to evaluate the fungi associated with root and crown rot of wheat in Khuzestan province.

Materials and Methods

Sample collection

In this study the Khuzestan province was divided into four regions on the basis of geographical position; north and northwest, south and southwest, northeast and southeast. Wheat plants showing symptoms of stunting, chlorosis and necrosis on sub-crown internodes and crown at the seedling to maturity stages were collected (5 to 20 fields were selected on the basis of the area under cultivation). Ten symptomatic plants were collected from each field and each sample was removed with as much of the root system as possible. Samples were stored in a dry and cold place. All of the samples were collected between November till April during 2004-2007.

Fungal isolation

Isolation of fungi associated with root and crown rot were made after surface sterilization. Each sample was rinsed under running water for 15 min. Five tissue pieces from root, crown and internodes (5mm) were surface sterilized with 1.5 % sodium hypochlorite for 1-2 min and washed two times in sterile distilled water. These pieces were plated onto Potato Dextrose Agar (PDA) supplemented with 1 drop lactic acid. Cultures were incubated at 20 ^{o}C for three days (Singleton *et al*., 1990). *Fusarium* spp. from infected tissue were isolated by Nash & Snyder medium and we used Rifampicin - amended PDA for isolation of *Gaeumannomyces graminis* var *tritici* (Duffy & Weller 1994) from suspected plants. Pure cultures of all fungi were prepared by hyphal tip and single spore culture methods (Nelson *et al*., 1983) and were subcultured on PDA for inoculum production.

Identification of fungi

Fusarium species were transferred onto Carnation-Leaf Agar (CLA), incubated under a combination of white and near-ultraviolet fluorescent lights with a 12 h photoperiod and 23/25 ^{o}C night/day temperature cycle. The cultures were identified based on general colony morphology, morphology of microconidia, macroconidia, conidiophores, false heads, sporodochia, chlamydospore formation, and using *Fusarium* diagnostic keys (Leslie and Summerell, 2006). Other fungi except *Pythium* sp. were identified according to their descriptions (Windels & Holen, 1989; Sneh *et al*., 1991).

Pathogenicity test

Pathogenicity tests were conducted on seedlings of cv. Chamran. One flask was filled with 200g of moistened wheat seeds. Seeds were autoclaved twice at 121 ^{o}C for 2 h. A plug of PDA culture, 5mm in diameter, was put in each flask. The flasks were incubated at 25 ^{o}C for 15 days and were shaken occasionally by hand. Then the colonized seeds were ground in a blender and mixed with pasteurized soil (1:5 v/v). Pots (30 cm in diameter) were filled with soil. Ten surface sterilized seeds were sown per pot. The experiment was conducted in greenhouse using complete randomized design with three replications. The pots were divided into two groups on the basis of irrigation. One group was irrigated normally using 100 ml of water/day. The second group was irrigated normally in the early stages of growth but two periods (10 days) of water stress were imposed afterwards (Rice and Geraldj, 1981). Seedlings in each pot were checked after four weeks for disease symptoms and were graded according to a 2 - digit pathogenicity scale (0 and 1) as recommended by Rice and Geraldj, 1981; whereby 0 indicates without symptoms, 1 = with symptom.

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Results

A total of 112 fungal isolates were collected from wheat growing fields (Tables 1, 2) and identified on the basis of *Fusarium* diagnostic keys and other relevant keys (figure 1).

Table 1 Wheat root - and crown rot - associated fungi isolated from different regions in Khuzestan.

Region	Species	No. of Isolates	Frequency %
Ahvaz	*Fusarium solani*	5	38.46
	F. equiseti	3	23.07
	F. moniliforme	2	15.38
	F. subglutinans	1	7.69
	Rhizoctonia solani	2	15.38
Dasht azadegan	*F. solani*	3	23.07
	F. equiseti	2	15.38
	F. sambucinum	2	15.38
	F. culmorum	6	46.15
Shoush	*F. proliferatum*	2	28.57
	F. pseudograminearum	1	14.28
	Pythium sp.	2	28.57
	R. solani	2	28.57
Andimeshk	*F. solani*	3	37.5
	F. moniliforme	2	25
	F. longipes	2	25
	B. sorokiniana	1	12.5
Dezful	*F. equiseti*	3	42.85
	F. avenaceum	1	14.28
	F. pseudograminearum	2	28.57
	B. sorokiniana	1	14.28
Shoushtar	*F. culmorum*	3	25
	F. solani	3	25
	F. sambucinum	2	16.66
	F. nygamai	1	8.33
	R. solani	3	25
Masjedsoleyman	*F. moniliforme*	2	25
	F. equiseti	3	37.5
	F. longipes	3	37.5
Izeh	*F. proliferatum*	2	22.22
	F. moniliforme	2	22.22
	F. culmorum	5	55.55
Baghmalek	*R. solani*	2	40
	F. solani	1	20
	F. semitectum	2	40
Mahshahr	*F. subglutinans*	1	25
	F. avenaceum	3	75
Hendijan	*F. equiseti*	3	30
	F. pseudograminearum	4	40
	F. solani	2	20
	F. lateritium	1	10
Ramhormoz	*F. solani*	1	25
	F. proliferatum	3	75
	F. subglutinans	2	66.66
	F. nygamai	1	33.33
Haftkel	*F. avenaceum*	2	18.18
	F. longipes	2	18.18
	F. lateritium	3	27.27
	F. equiseti	4	36.36

Table 2 Total number and frequency % of fungal isolates from root and crown rot of wheat in Khuzestan province.

Species	Total Number	Total Frequency (%)
F. solani	18	16.07
F. equiseti	18	16.07
F. culmorum	14	12.5
Rhizoctonia solani	9	8.03
F. moniliforme	8	7.14
F. proliferatum	7	6.25
F. pseudograminearum	7	6.25
F. longipes	7	6.25
F.avenaceum	6	5.35
F. subglutinans	4	3.57
F. sambucinum	4	3.57
F. lateritium	4	3.57
F. nygamai	2	1.78
F. semitectum	2	1.78
Pythium sp.	2	1.78
B. sorokiniana	2	1.78

Pathogenicity test

Results showed that *Fusarium pseudograminearum* and *F. culmorum* caused root and crown rot under normal irrigation regime, whereas all of the other *F.* spp. caused disease only after water stress. *R. solani* and *Bipolaris sorokiniana* also produced visible symptoms. In all replications of pathogenicity experiments with visible symptoms, each casual agent was re-isolated from infected tissues.

Frequencies and distribution of species

F. solani and *F. equiseti* were the most frequent species while the *F. nygamani*, *F. semitectum*, *Pythium* sp. and *B. sorokiniana* were the least frequent ones (Table 2).

The two most common species contained about 32 % of the isolates. The distribution of the prevailing species with relation to different regions was calculated as 92 % for *F. solani* and 69 % for *F. eqiseti* (Fig. 2).

Discussion

Samples were taken from north and northwest, south and southwest, northeast and southeast of Khuzestan. Drought is a serious problem in south and south east such as Ahvaz and Dashte Azadegan and also in some regions of northeast such as Masjedsoleyman.

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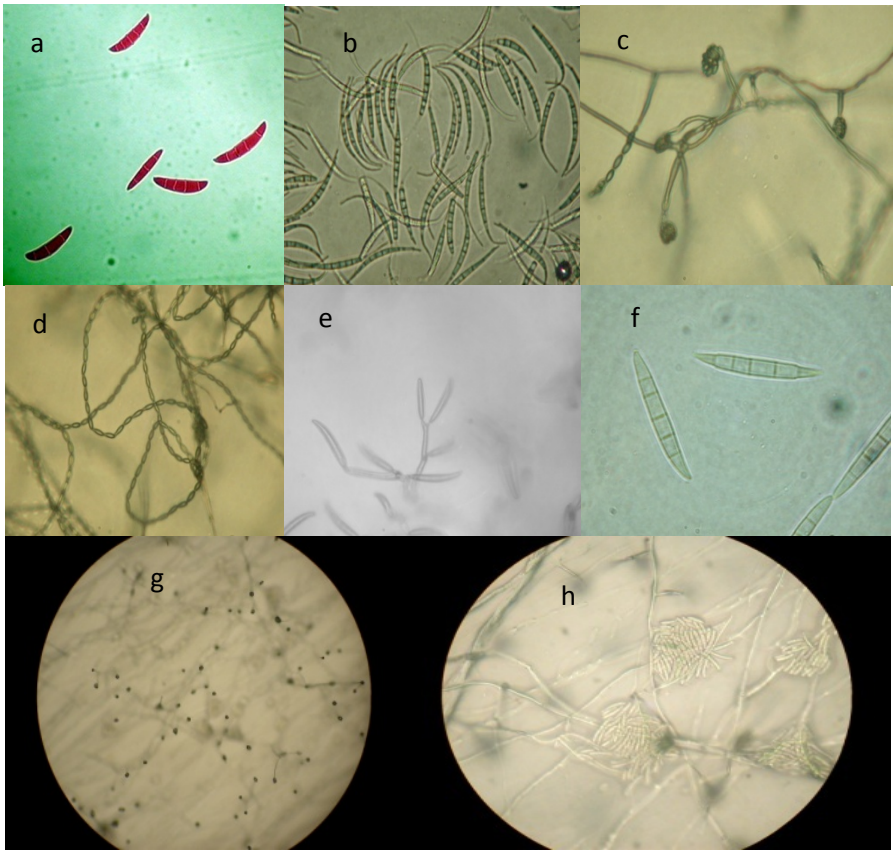

Figure 1 Microscopic characteristics of some *Fusarium* isolates: a) macroconidia of *Fusarium culmorum* 40x, b) macroconidia of *Fusarium equeseti* 40x, c) false heads of *Fusarium proliferatum* 40x, d) spore chain of *Fusarium proliferatum* 40x, e) macroconidia of *Fusarium semitectum* 40x, f) macroconidia of *Fusarium semitectum* 40x, g) false heads of *Fusarium solani* 10x and h) macroconidia of *Fusarium solani* 10x.

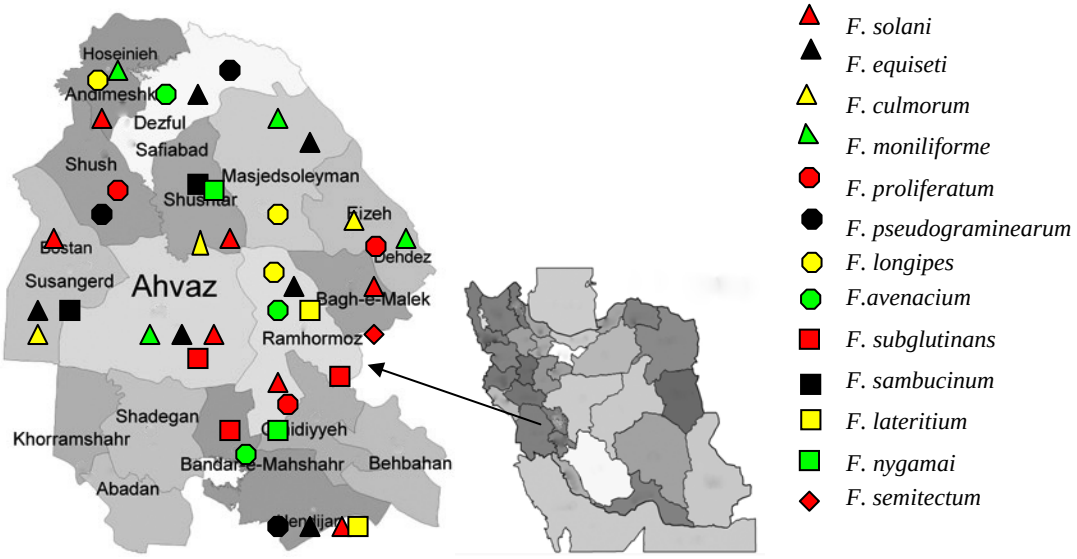

Figure 2 Distribution map of *Fusarium* species in Khuzestan province Iran.

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This study showed that *Fusarium* species were the predominant pathogens causing root and crown rot of wheat in Khuzestan province, while other fungi such as *B. sorokiniana, R. solani* and *Pythium* sp. were observed as well. Some of *Fusarium* species are commonly associated with warm dry condition and are greatly influenced by temperature and rainfall (Chekali *et al*., 2011). *Fusairum pseudograminearum* and *F. culmorum* were pathogenic and infected the seedlings without any water stress while the other *Fusarium* spp. caused disease symptoms in roots of plants only under water stress condition. Among the identified *Fusarium* species *F. equiseti, F. solani* and *F. culmorum* were more frequent. *F. equiseti* was observed in all areas including regions with drought and water stress.

The three species of *F. equiseti, F. solani* and *F. culmorum* have been collected from stubbles in South Africa (Klassen *et al*., 1991) as well. They were limiting factors for wheat production in Nigeria (Los, *et al*.,1994). *F. equiseti* has been reported as a potential infective agent (Burgees *et al*., 1995). It was collected from root and crown rot of wheat and was more frequent than other species in Lorestan province, Iran (Darvishnia *et al*., 2007). *F. solani* is cosmopolitan and causes crown and root rot on most plants (Burgess *et al*., 1995). *F. culmorum* is widespread seed borne pathogen and the causal agent of crown and root rot of wheat and barley (Burgess *et al*., 1994). This species was isolated from crown and root of wheat and barley in Iran (Vafaie *et al*., 2001) and its pathogenicity was confirmed on date palm (Mosavi-jorof *et al*., 1999) and faba bean in Khuzestan (Azimi *et al*., 2005). *F. avenacium* has been mostly isolated from barley and wheat (Nelson *et al*., 1983). *F. nygamai, F. lateritium, F. equiseti, F. solani, F. culmorum, F. moniliforme, F. proliferatum, F. longipes, F. sambucinum* and *F. avenaceum* have previously been reported from Iran (Zare and Ershad, 1997).

F. pseudograminearum and *F.culmorum* were mostly isolated in late tillering stage and early heading stage but in seedling stage other species of *Fusarium* along with *Pythium* and *Rhizoctonia* could be isolated. These results indicated that the host may be weakened by the mentioned fungi in early stages of growth and its susceptibility to other pathogens is thereby increased. Isolation of fungi in different growth stages showed that the greatest number of casual agents were different *Fusarium* species which probably become more aggressive by water stress at different growth stages of the crop. Distribution of *Fusarium* species was not uniform and depended on water stress so that a species that was not previously seen in a region could be observed after water stress.

Pathogenicity tests of *B. sorokiniana* and *R. solani* were positive but in the case of *Pythium* sp. despite being pathogenic, no distinguishable symptoms similar to those of *B. sorokiniana, R. solani* or *Fusarium* spp. were observed. *Gaumannomyces graminis* var. *graminis* was not observed in cultures. Most soils in Khuzestan are often calcareous and it seems this fungus can not grow in such soils.

Since the rotation of wheat, canola and corn is a common agricultural practice in Khuzestan province, the effect of rotation in combination with water stress on prevalence of wheat crown and root pathogenic fungi may be considered for future research.

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قارچ‌های همراه با پوسیدگی طوقه و ریشه گندم در استان خوزستان

محمدرضا اصلاحی

اهواز – بلوار گلستان - مرکز تحقیقات کشاورزی و منابع طبیعی خوزستان
پست الکترونیکی مسئول مکاتبه: mr_eslahi@yahoo.com

چکیده: برای شناسایی قارچ‌های همراه با طوقه و ریشه گندم در استان خوزستان، نمونه‌های بیمار در همه مراحل رشد در سه فصل کشت در سال‌های ۱۳۸۳ تا ۱۳۸۶ جمع‌آوری شدند. قطعاتی از قسمت‌های آلوده ریشه و طوقه ضدعفونی سطحی شد و روی محیط کشت سیب‌زمینی- دکستروز- آگار اسیدی و غیر اسیدی کشت گردید. یک‌صد و پنجاه جدایه از نمونه‌های کشت داده شده به‌دست آمد و براساس خصوصیات میکروسکوپی و ماکروسکوپی و کلیدهای معتبر گونه‌های *Fusarium solani*، *F. equiseti*، *F. moniliforme*، *F. subglutinans*، *F. proliferatum*، *F. longipes*، *F. nygamai*، *F. sambucinum*، *F.pseudograminearum*، *F. semitectum*، *Rhizoctonia solani*، *Bipolaris sorokiniana* و *Pythium* sp. شناسایی شدند. گونه‌های *F. equiseti*، *F.solani* و *F. culmorum* به‌ترتیب با ۱۶/۰۷، ۱۶/۰۷ و ۱۲/۵ درصد از سایر گونه‌ها فراوانی بیشتری داشتند. آزمون بیماری‌زایی برای همه گونه‌ها انجام گرفت. نتایج نشان داد که *F. pseudograminearum* و *F. culmorum* قارچ‌های اصلی مرتبط با بیماری پوسیدگی ریشه و طوقه گندم در استان خوزستان هستند در حالی‌که سایر گونه‌های فوزاریوم از قبیل *F. equiseti* ممکن است که در اثر تنش رطوبتی در مراحل مختلف رشد گیاه مهاجم شوند. *Rhizoctonia solani* با فراوانی ۸/۰۳ درصد در بعضی از مناطق از قبیل اهواز، شوش، شوشتر و باغملک بعد از گونه‌های فوزاریوم عامل اصلی پوسیدگی ریشه و طوقه گندم بود. همچنین *Bipolaris sorokiniana* و *Pythium* sp. با فراوانی کمتری موجب پوسیدگی ریشه و طوقه شدند.

واژگان کلیدی: *Fusarium pseudograminearum, F. culmorum, F. equiseti* و تنش آبی

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