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Full Length Research Paper

Wheat crown and root rotting fungi in Moghan area, Northwest of Iran

Behzad Hajieghrari

Department of Plant Production, Moghan Junior College of Agriculture, University of Mohaghegh - Ardabili, Ardabil, Iran. E-mail: bhajieghrari@uma.ac.ir. Tel: +989143186861. Fax: +984527463417.

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Wheat root and crown rot (*Triticum aestivum* L.) is a disease problem in many wheat producing area as well as Moghan wheat growing area, Northwest of Iran. In this study, injured plants of wheat with blighting, stunting, and death of mature plants, also necrotic lesions on seminal crown roots, sub crown internodes and crown, as well as basal stem tissue symptoms were collected from different regions of Moghan wheat growing area. Fungi associated with wheat root and crown rot were isolated and identified based on general colony morphology, the morphology of mycelia, reproductive structure and taxonomic description. Also pathogenicity of 28 selected isolates from various identified species was determined on wheat (*T. aestivum* L. CV. Atila 4; a common grown cultivar of wheat in Moghan area) seedlings. The results indicated that the predominant pathogens implicated in causing common root and crown rot in this study was *Bipolaris sorokiniana*. In addition to *B. sorokiniana*, *Fusarium culmorum*, *Fusarium Pseudograminearum* and Gaeumannomyces/Phiallophora complex are the most widely distributed species on wheat growing belt in Moghan area. *Fusarium solani*, *Fusarium crookwellence*, *Fusarium clamidosporum*, *Fusarium proliferatum*, *Fusarium poa*, *Fusarium udum* and *Fusarium babinda* are wheat root associated species of *Fusarium* ascertained during our investigation. Pathogenicity test revealed that *B. sorokiniana*, *F. culmorum*, *F. graminearum*, *F. crookwellence*, *F. udum* and Gaeumannomyces/Phiallophora complex are active pathogens and others are saprophytes, which are also involved in the destruction of root tissues, without being the cause of injury.

Key words: Wheat, common root and crown rot, *Bipolaris sorokiniana*, *Fusarium* sp., Gaeumannomyces/ Phiallophora complex.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most strategically important crop globally, particularly in Iran, and has been widely grown for many years in Moghan area, Northwest of Iran. Root and crown rot of wheat occurs throughout the growing season of wheat; it is a disease problem in many producing areas of the world, and has been a serious problem in major wheat growing areas of Iran, particularly in Moghan wheat belt recently. It is associated with high economic losses. The term, "common root and crown rot" has been widely used to designnate a group of complex disease that is characterized by blighting, stunting and death of seedling, stunting of mature plants, necrotic lesions on seminal and crown roots, subcrown internodes, crown and stem tissue. Later in the season, other symptoms arise such as the premature death of tillers with little or no grain in wheat plants as well as other small grain cereals similar to but distinct from take-all disease (Sallans, 1965). Common root and crown rot is caused by several different soil borne pathogens, but it is demonstrated that the most important cause of the common root and crown rot complex is known as the fungus, *Bipolaris sorokiniana* (Telemorph; *Cochliobolus sativus* Ito and Kuribayashi). The telemorph stage is rarely found under natural condition (Mathre et al., 2003), and was first found in Zambia where two different mating types must appear together (Kumar et al., 2002; Raemarkers, 1988) and usually does not develop in culture unless in the presence of opposite mating types (Mathre et al., 2003; Kumar et al., 2002). It is said that in addition to *Crocus sativus*, several species of Fusarium are associated in the common root and crown rot complex (Windels and Holen, 1989; Wiese, 1987; Sallans, 1965). Fusarium is one of the most ubiquitous abundant and important genera of soil microflora (Leslie and

Summerell, 2006; Nirenberg, 1990; Nelson et al., 1983; Booth, 1971). Several Fusarium species are widespread pathogen causal agent of brown foot rot of cereals, especially in both temperate and semitropical areas (Windels and Holen, 1989; Wiese, 1987; Sallans, 1965). Also two species of Curvularia (*C. ramose* and *C. speciefera*) are included in common root and crown rot complex (Sallans, 1965).

Rhizoctonia solani Kuhn Anastomosis group eight (AG8) caused "bare patch", colonized the roots of wheat producing symptoms including stunted shoot growth, white heads, reduced blackened roots and premature grain ripening. It is more severe in sandy soils where rainfall is low (Gill et al., 2001; Gill et al., 2000).

Take-all of wheat is caused by the fungus, *Gaeumannomyces graminis* var. tritici. It is the most deadly root disease of wheat worldwide; it also affects triticale, barley and ray, but to a lesser extent (Freeman and Ward, 2004), causes stunting and nutrient-deficiency symptoms in the tops, and progresses upward into the bases of the stems. Here, it disrupts the flow of water to the tops and causes premature death of the plant (Cook, 2003).

Common root and crown rot of wheat is widely documented in different Iranian wheat belt (Darvishnia et al., 2006), but no studies have been carried out to determine the species which cause these symptoms in the region. The objective of this study was to determine the most widespread fungi associated with the roots and crowns rot of wheat grown in Moghan area, Northwest of Iran.

MATERIALS AND METHODS

Experiment was carried out at Plant Pathology Laboratory of the Junior College of Agriculture, Mohaghegh Ardabili University, Ardabil, Iran during 2006 - 2008.

Sample collection

Injured plants of wheat with blighting, stunting, and death of mature plants, as well as necrotic lesions on seminal crown roots, subcrown internodes and crown, and basal stem tissue symptoms at the early milk to ripe maturity stages were collected from localities of different region of Moghan wheat growing area randomly (about 3-5 km apart). 5 to 10 symptomatic plants were collected within each locality. Plants including each sample were removed with intact roots and crown and were stored in coolers from the time of collection to isolation processing. All field observation and sampling were made between June and July before the harvest time of cereals during 2006 - 2008.

Isolation of wheat root and crown associated fungi

Fungi associated wheat root and crown were isolated using the following surface sterilization technique. The gathered samples were washed thoroughly under running water for 20 min after removing dead basal leaf-sheath. Infested root, subcrown internodes and crown pieces (5-10 mm) were surface-disinfected by submersing in 1.5% sodium hypochlorite solution for 1 - 2 min; they were washed 3 times in sterile distilled water and dried on sterile filter paper in a laminar air flow cabinet. Following surface sterilization the pieces were placed onto Potato Dextrose Agar (PDA, BDH Ltd, UK 39 g/l) supplemented by 100 mg/l Streptomycin Sulphate in 9 cm Petri dishes and incubated at 20 ± 1°C in the dark for 2 - 5 days or longer. Emerging fungi were subcultured onto PDA prior purified by single hyphae tip and single spore culture. For long term preservation, mycelium plugs were taken from the colony margin using a No3 sterile cork borer (8mm diameter) and stored at - 80°C in 15% glycerol. Original collections are deposited in the Department of Plant Production, University of Mohaghegh Ardabili.

Identification of common root and crown rot fungi

The cultures were identified based on general colony morphology, the morphology of mycelia and reproductive structure and taxonomic description. *B. sorokiniana* isolates were characterized by a profuse production of Bipolaris -type dark conidia identified with a binocular microscope and the species were identified according to their description (Sivanesan, 1987; Ellis, 1976; Ellis, 1971). Also, the germination of conidia produced on PDA was examined, using dispersed conidia in a drop of sterilized water on surface of PDA plates.

Fusarium cultures were transferred to Carnation Leaf Agar (CLA) (Nelson et al., 1983), Synthetischer Nahrstoffarmer Agar (SNA) (Gerlach and Nirenberg, 1982), Banana Leaf Agar (BLA) (Matsushima, 1971) and Potato Dextrose Agar (PAD) prepared from fresh potato (Nelson et al., 1983). The cultures were identified based on general colony morphology, morphology of microconidia, macroconidia, clamidospore, conidiophores and taxonomic description of the Fusarium diagnostic keys (Leslie and Summerell, 2006; Nirenberg, 1990; Nelson et al., 1983; Booth, 1971).

Single hyphae tip subcultured isolates, with lobed hyphopodia known as Gaeumannomyces/Phiallophora complex were subsequently identified through the following: distinction of conidia and growth rate at 25°C, ability to produce perithecia and having characteristic of asci and ascospore. Perithecia were induced to form on Wheat Leaf Agar (WLA: 100 g of green wheat leaves was boiled in distilled water for 10 min, then two layers of cheesecloth were poured, adjusted to 1 l with distilled water, and 20 g of agar was added, after which they were autoclaved) in 6 - 8 weeks (Mathre, 2000).

Pathogenicity test

Pathogenicity of 28 selected isolates from various identified species was determined on wheat (*T. aestivum* L. CV. Atila 4; a common grown cultivar of wheat in Moghan area) seedlings. For testing *B. sorokiniana* pathogenicity, the conidia of *B. sorokiniana* were scraped or washed from the surface of Potato Dextrose Agar (PDA, BDH Ltd, UK 39 g/l) with double distilled water and counted, using haemocytometer. They were adjusted to 10^6 conidia/ml. Four surface disinfected wheat seeds (surface sterilized by 1% NaClO for 5 min and rinsed in double distilled water) were planted in 15 cm diameter pots, containing sterilized wheat growing soil (autoclaving in 121°C for 1 h twice with 24 h distance) to a depth of 1 cm in each pot in a glass house (the temperature range were adjusted from 20 - 25°C). They were inoculated by 5 ml of *B. sorokiniana* inoculants.

For determination of *Fusarium* isolate pathogenicity, the seeds were inoculated by placing a colonized PDA 5 mm diameter plug in direct contacts with the planted seed and covering the inoculums and seed with the soil. For testing Gaeumannomyces/Phiallophora complex isolate pathogenicity, inoculums were prepared twice on autoclaved wheat grain and were distributed over the pot soil. The seeds were placed on to this surface and 1 cm of soil was placed over the seeds and inoculums (Smiley et al., 2005). There are four replicates which were used for each isolate and randomly distributed

Figure 1. Various symptom observed in wheat root and crown infected by common root and crown rotting fungi in Moghan area (A - H) in compared healthy plants (I).

in glasshouse. After four weeks, seedlings were evaluated.

RESULTS

A total of 135 fungal isolates from 76 samples that showed blighting, stunting, and death of mature plants, necrotic lesions on seminal crown roots, sub crown internodes and crown, and basal stem tissue symptoms (Figure 1) were obtained from wheat growing fields of four districts: Parsabad, Tazeh-kand, Billesouar and Germi (Table1). The predominant pathogens implicated in causing common root and crown rot in this study was *B. sorokiniana* that was isolated from crown and root tissue of wheat from districts in Moghan area. The colonies on PDA are olivaceaous brown to black, and in old cultures a profuse number of conidia and all the culture turn into shiny black. Under binocular observation, short, mostly single conidiophores, that bear laterally and terminally one to six of multicell (with five to nine cell) dark brown oval shape, with thick cross walls conidia, were observed in the old culture. The results, on the bases of isolated species from the different fields may suggest that in addition to *B. sorokiniana*, *F. culmorum*, *F. graminearum* and Gaeuma-

Table 1. Wheat root and crown rotting associated fungi isolated from Moghan wheat growing fields.

Species	Pathogenicity of tested isolates	Districts				Total
		Pars-abad	Tazeh-kand	Bilesouar	Germi	
Gaeumannomyces / Phiallophora	+	2	8	6	3	19
Bipolaris sorokiniana	+	6	13	7	5	31
Fusarium culmorum	+	4	7	9	7	27
F. graminearum	+	3	5	7	3	18
F. crookwellence	+	1	4	1	3	9
F. proliferatum	-	2	2	3	-	7
F. clamidosporum	-	-	1	4	2	7
F. babinda	-	1	-	1	-	2
F. udum	-	1	-	2	2	5
F. solani	-	2	1	1	2	6
F. poa	+	1	-	2	1	4
Total		23	41	43	28	135

nnomyces/Phiallophora complex are the most widely distributed species on wheat growing belt in Moghan area. *F. crookwellence*, *Fusarium clamido-sporum*, *F. proliferatum*, *Fusarium solani*, *F. udum*, *F. poa* and *Fusarium babinda* were abundant wheat root associated species of Fusarium respectively. This was ascertained during our investigation. However there were significantly differences in the occurrence of *F. culmorum* in comparison with the other ones. *B. sorokiniana*, *F. culmorum*, *Fusarium graminearum, F. solani*, *F. crookwellence*, *F. clamidosporum*, *F. proliferatum*, *F. poa*, *F. udum* had previously been reported in Iran. In the present study *F. babinda* are reported for the first time from wheat in Iran.

These *Fusarium* species can occur singly or in combination with *B. sorokiniana* and/or other identified species of Fusarium. In this study, *Rhizoctonia solani* AG8 was not isolated from wheat in all of the samples. The pathogenicity tests showed that all tested *B. sorokiniana* isolates were the most virulent on wheat and infected seedlings developed dark brown necrotic lesions on roots, crown and lower leaf sheaths. Also the pathogenicity tests of *F. culmorum*, *F. graminearum F. crookwellence* and *F. poa* as well as Gaeumannomyces/ Phiallophora were positive through seedling infection. It seems that these species are active pathogens without being the cause of injury.

DISCUSSION

Wherever wheat is grown as well as Moghan wheat belts, common root and crown rot is an important disease problem; it results in yield loss by damping off, reducing tiller number, head size and kernel yields (Backhouse et al., 2004). Several soilborne pathogen species occurring worldwide on cereals are caused by agents of wheat common root and crown rot (Sallans, 1965). In this study, the predominant pathogens implicated in causing common root and crown rot was *B. sorokiniana*. These findings are in general agreement with other several recent reports from different wheat growing areas of Iran where it was revealed that *B. sorokiniana* is the main cause of wheat common root rot in Tehran, Boshehr, Kermanshah, Lorestan and Fars provinces wheat growing fields. However, the name, common root rot is generally accepted when *B. sorokiniana* species are dominant members of the complex. *B. sorokiniana* was isolated more frequently from crown and sub crown internodes. It is revealed that the sources of *B. sorokiniana* inoculums in nature are infected seeds, infected residues after crop harvest, collateral hosts and free dormant conidia in the soil (Malaker et al., 2007). However, the survival of *B. sorokiniana* on the seed may be caused by a major source of primary inoculums (Chand et al., 2002; Malaker et al., 2007).

Based on the isolation results, out of *B. sorokiniana*, several species of Fusarium are associated in the common root rot disease in Moghan growing belt. *F. culmorum* and *F. graminearum* are the most predominant respectively. *F. culmorum* and *F. graminearum* that apparently are effective colonizers of sub crown internodes (Windels and Wiersma, 1992) may occur singly, but they often coexist in the same fields and even within individual wheat plants. Moreover, invasion of the same side or infections of different sites of wheat root and crown may occur by *B. sorokiniana* in combination with *F. culmorum* and/or other Fusarium species. It is determined that prepossession of the internodes by *B. sorokiniana* did not prevent later infection by Fusarium species (Windels and Wiersma, 1992). Also, predominance of specific species in a region is mostly influenced by cropping history and climatic conditions, especially temperature and moisture requirements.

F. culmorum is a one of the principal causal agent of common root rot in wheat especially in many wheat

growing regions as well as several wheat growing fields in Iran such as Fars, Mazandaran, West Azerbaijan, Khorasan, Yazd, Golestan, Ilam, Boushehr, Kermanshah provinces(Darvishnia et al., 2006). It is showed that *F. culmorum* is more pathogenic than *B. sorokiniana* to wheat seedling (Wiese, 1987; Windels and Wiersma, 1992).

Additionally, *F. graminearum* is the other important causal agents of wheat common crown and root rot, especially in warmer wheat-growing areas (Weise, 1987). In Iran, *F. graminearum* is reported from West Azerbaijan, Tehran, East Azerbaijan, Guilan, Mazandaran, Ilam, Ardabil, Hamadan, Golestan, Fars, Kerman, Markazi, Boushehr, Qom, Qazvin and Khorasan provinces (Darvishnia et al., 2006). In Moghan area, *F. graminearum* is reported as a major causal agent of Fusarium head blight in wheat (Davari et al., 2006). There are two subpopulations of *F. graminearum* referred to as groups 1 and 2. Accordingly, group 1 is associated with wheat. Other small grain cereals and grasses also cause crown and foot rot of wheat. Akoi and Donnel (1999) lately described *the* specie as *Fusarium pseudograminearum.* Prior to that, it was known as *F. graminearum* group 1. In this study, single spore that purified *F. graminearum* cultures on the CLA medium did not form perithecia even after 2 months, rather it was showing heterothallism in these isolates. In this regards, it seems that the isolate, named *F. graminearum* in this study may be identified as a *F. pseudograminearum* by Akoi and O'Donnell (1999). However, *F. graminearum* cultures can be confused easily with *F. pseudograminearum* cultures (Leslie and Summerell, 2006).

The results of pathogenicity test demonstrated that *F. culmorum*, *F. graminearum* and *F. crookwellence* attack wheat root and crown actively while *F. solani*, *F. clamidosporum*, *F. proliferatum*, *F. poa*, *F. udum* and *F. babinda* destroy root and crown tissue when the root and/or crown were weak. In this regards, some studies demonstrated that some Fusarium species form mycorrhizae in annual plants and when the host were weakened, the Fusarium making mycorrhizae pass from symbiotic relationship to parasitism (Sallans, 1965). It is an open aspect for further studies about these Fusarium roles in symbiotic relationship and plant nutrition.

The obtained results revealed that take all diseases are common in Moghan wheat growing area, especially on irrigated fields. It is mentioned that apart from *G. graminis,* other dematiaceous fungi such as *Phiallophora* sp. and nonpathogenic Gaeumannomyces may cause take-all disease symptoms such as blackened roots, stunted plant growth and whiteheads, either alone or in combination with *G. graminis* in wheat and other affected cereals (Smiely et al., 1986). In wheat plants presented take-all disease symptoms in Moghan fields, production of perithecia were not shown on affected wheat roots and crowns. Also single hyphae tip subcultures of Phiallophora like fungi on WLA two month later did not give perithecia on media. Because *G. graminis* varieties have Phiallophora like anamorphs and with other nonpathogenic Gaeumannomyces and Phiallophora species colonizing cereal roots (Bateman et al., 1992; Henson, 1992; Bryan et al., 1995 and Ulrich et al., 2000) therefore, in this study, these isolates constituted Gaeumannomyces-Phiallophora complex. For identification and characterization of these isolates further studies should be carried out.

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