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Incidence of Crown Rot Disease of Wheat Caused by *Fusarium pseudograminearum* as a New Soil Born Fungal Species in North West Iran

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Abstract: Root rot and crown rot is one of the main important fungal diseases on wheat in North West Iran. The disease was studied during 1999-2004 growing seasons in four provinces including Qazvin, Zanjan, East Azarbyjan and Ardabil. Different wheat fields in the areas studied were visited and samples of the plants showing symptoms like chlorosis, withering, whiting of spikes, growth reduction and white heads were collected and transferred to the laboratory. Samples were surface sterilized with sodium hypochlorite and then cultured on common media (PDA) and specific media (PPA and CLA). Totally 155 fungal isolates belonging to five genera were identified and the pathogen most frequently isolated was *Fusarium pseudograminearum* (formerly known as *F. graminearum* Group 1). This species normally causes crown rot resulting in severe damage in several locations under dry spring conditions. The disease caused losses from 18-45.5% in the fields where the season and crop rotation allowed the disease to build up. Prolonged moisture stresses coupled with relatively high soil temperature in the fall enhanced early disease development on the roots and sub crown internodes. Environmental conditions and genetic susceptibility of cultivars were the two main factors affecting diseases incidence.

Key words: Crown rot, *Fusarium pseuograminearum*, wheat, Iran

INTRODUCTION

Wheat (*Triticum aestivum* L.) occupies about 30% of the world's cultivated land area and is the most important agricultural commodity in international trade (Karimi, 1992). Wheat is also the most important agricultural crop of Iran and is cultivated approximately in 4.5 million hectares and its production was 8.7 million tones in 1999 growing season. Generally crown rot is a major disease of small grain cereals, caused mostly by *Fusarium* species which is the main disease limiting yields of wheat in North West Iran (Froutan *et al*., 1995).

Since the wheat plant is adapted to different climatic areas from cool to warm conditions, other soil born fungi can also cause seedling blight and common root rot in wheat, leading to yield losses (Bockus *et al*., 2007; Wise, 1998). However, crown rot of wheat in Iran is caused mostly by the new species named *Fusarium pseudograminearum* (Saremi and Farrokhi, 2004; Aoki and O'Donnell, 1999), formerly known as *F. graminarum* (Group 1). Generally the increased incidence and economic importance of the disease has been linked to environmental conditions such as dry weather and use of susceptible varieties.

Actually, crown rot is the most common diseases of wheat in northwest Iran and usually appears after flowering when white heads can be seen scattered in the crop. It usually occurs in large patches and is more common on the lower side of paddocks. The disease acquires economic proportions in the northwest of Iran, especially in East Azarbyjan, Ardabil and Zanjan provinces.

It has been reported that several soil-borne fungi are involved in causing crown rot of wheat in Iran. For example, Mansoury (1995) isolated different species of *Fusarium*, *Drechclera* and *Sclerotium* from the affected wheat fields in the Fars province. On the other hand, *F. culmorum*, *F. avenaceum* and *F. acuminatum* infections were correlated with yield losses (Ravanloo and Banihashemi, 1999). Other fungi, including *Rhizoctonia solani*, *R. cerealis*, *F. graminearum* and *Gaeumanomyces graminis* have also been associated with seedling blight and common root rot of wheat in north Iran and other countries (Bockus *et al*., 2007; Froutan *et al*., 1995).

It has bean reported that several *Fusarium* species especially *F. pseudograminearum* are associated with wheat root rot diseases in New Zealand (Bentley *et al*.,

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2006). Mostly four pathogenic species including *F. pseudograminearum*, *F. culmorum*, *F. crookwellense* and *F. graminearum* caused wheat root rot and crown rot. How ever it was frequently identified *F. pseudograminearum* based on morphological features. It is also a serious problem in Australia (Chakraborty *et al*., 2006; Akinsanmi *et al*., 2004; Burgess *et al*., 1996) and USA (Smiley and Patterson, 1996). In addition, the disease has been reported from the wheat tracts of South Africa (Klaasen *et al*., 1991) as well as Syria, Egypt and Italy (Balmas, 1994).

Since crown rot and root rot of wheat have become major diseases in the areas of continuous wheat cultivation in Iran, particularly in the North West, it is important to control them through management practices and use of tolerant cultivars. Crop rotation, stubble burnt in autumn and careful selection of nearly resistant varieties can be helpful to minimize the incidence of disease. By the way, there is no a general perfect method to be used in all instance of soil borne pathogens control. Thus any new method of control, even if restricted in its use, is of value because it adds to our rather limited arsenal of control methods.

Therefore soil solarization or solar heating which is relatively new approach can be good way for controlling soil borne pathogens. The aim of soil solarization is to harness solar energy to raise the temperature of moistened soil which can resulted the control of soil borne pathogens especially *Fusarium pseudograminearum*. Actually, we tried to use this method in one studied areas on summer season which is climatically marginal region for this method.

Crown rot usually causes yield losses under dry conditions in spring when infection of the crown or stem tissue occurs near the soil surface. Initial infections of plants are facilitated by wet conditions but the fungus grows rapidly through the plant tissues when the plants are under moisture stress (Wallwork, 1996). The present study has been carried out to assess the etiology of crown rot disease of wheat and the contribution of environmental conditions or susceptible varieties on the increase of the diseases and its management by soil solarization in northwest Iran.

MATERIALS AND METHODS

Sample collection: The 5 year study covered wheat fields in the Qazvin, Zanjan, East Azarbyjan and Ardabil provinces from 1999 to 2004. All these areas were visited and plants with symptoms of chlorosis, withering, growth reduction, crown necrosis, white heads and blighting of spike lets were collected and transferred to the laboratory of plant pathology in the Zanjan University. Six samples infected with crown rot were collected from each province each year and 72 complete samples were used to isolate and identify the causal agents. Various fractions of the infected samples and soils around the roots were cultured in different media.

Media: The samples were cultured in PDA (Potato, Dextrose and Agar) as common medium, PPA (Peptone, PCNB and Agar) as selective medium and CLA (Carnation, Leaf and Agar) as natural medium after surface sterilization with sodium hypochlorite. Cultures were kept in light room with fluctuating temperatures (25°C during day and 20°C at night) and UV lights (12 h light and 12 h dark) to allow the colonies of the pathogen to grow (Burgess *et al*., 1994). The soil dilution method was used to isolate pathogen inoculum from the soil around the roots of the wheat plant suspected to infected crown rot disease (Saremi, 1998). Pure culture was obtained from each isolate using the single spore culture method and all isolates were identified (Burgess *et al*., 1994).

Soil dilution: The technique involved the uniform dispersion of 1 mL of soil suspension of infected field across a selective medium such as PPA. Actually, one gram of infected soil was added to the water agar to produce soil suspension. Propagules in the soil sample suspension germinated within 2-3 days on PPA and produced small colonies by 5-7 days. The suspension was uniformly dispersed over the medium by carefully pipeting 1 mL of soil suspension onto the medium on one edge of he PPA. The plate was then held with a slight slope away from the suspension and gently shaken at right angles to the slope. The suspension slowly spread across the plate with a uniform wetting front (Burgess *et al*., 1994; Saremi, 1998).

Incubation: The cultures were incubated in a room lighted at near-ultraviolet wave lengths (black light tube, Philips TL 36 w/80 RS F40 BLB) and fluctuating temperature regimes, 25°C during day and 20°C in night under 12 h photoperiod. Sporulation and pigmentation of *Fusarium* species are favored by these conditions (Burgess *et al*., 1994). Soil dilution technique was also used to isolate inoculums from soil in the root zone suspected to be infected with crown rot disease.

Yield losses: Grain yield loss of wheat was assessed at one location of usual infected fields from each province. Grain yield from 100 m^2 area in each infected field with crown rot disease was compared with the same size plot in a no infected field of four studied provinces. Yield

(a) (b)

Fig. 1: Covering infested soil with *Fusarium pseudograminearum* by clear plastic (polyethylene) after cultivation and irrigation the bed in Zanjan (a). Increasing temperature on moistened soil in studied area (b)

production of all locations was evaluated to approximate the outcome of crown root disease. Annual infection rate with percentage of damage for each province was as well determined.

Control managements of pathogen: Soil disinfestation by soil solarization method was carried for the relatively control the pathogen at summer season after yield production in zanjan province. This method facilitates solar energy to raise the temperature of moistened soil which can be resulted the control of soil borne pathogen (Fig. 1a and b). By this way the infested soil was covered with transparent polyethylene to raise soil temperatures high enough for controlling the pathogen. The plastic edges were buried in the trench to ensure that the plastic is held in place to prevent heat.

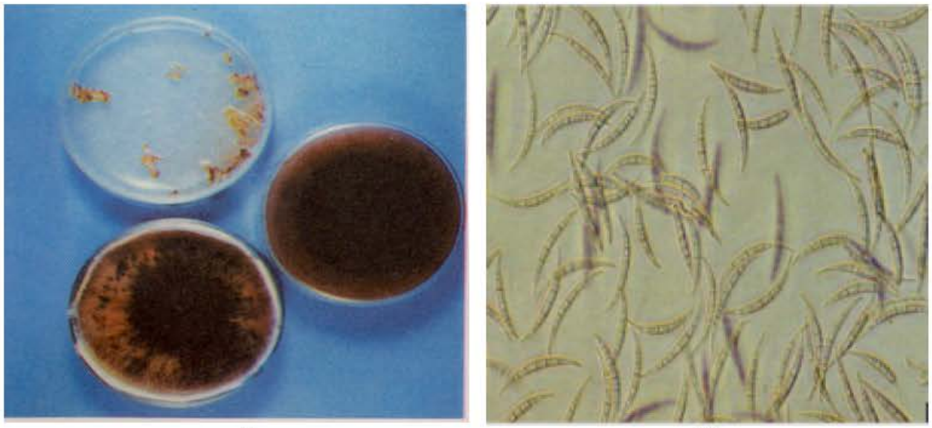
Furthermore, it was obvious that using susceptible cultivars can be resulted in high epidemic crown rot disease in cultivated locations. By the way, it is important to extend resistant cultivars through breeding effort by applying recurrent selection. Consequently, research is under way to find resistant cultivars through attempt by applying frequent selection as it has been prepared by a number of researchers in other countries (Akinsanmi *et al*., 2006a; Liu *et al*., 2004; Mitter *et al*., 2006; Xie *et al*., 2006).

RESULTS AND DISCUSSION

Symptom of crown rot disease in wheat: Generally, various fungal species produced different symptoms on the infected wheat plants. However, the main symptoms of common root rot and crown rot in the areas studied were yellowing, growth reduction, pink coloration around the crown and white heads (spikes) caused by *F. pseudograminearum* (Fig. 2). The diseased plants were mostly stunted and the symptoms were most striking near or below the surface. They include brown spots, blotches and rotting on the crown, roots and subcrown internodes (Fig. 3). Some of the infected plants had stunted growth. In such cases, the rootlets were necrotic or rotten and engulfed in a mass of white fungal growth.

Other soil-borne fungi, such as *Rhizoctonia solani* and *Drechslera* sp., were also found in rare situations. However, crown rot disease caused mostly by *Fusarium graminearum* (group 1), which has been named *F. pseudograminearum* which is the causal agent of the disease in many other countries (Aoki and Donnell, 1999). The fungus leads to formation of white heads with little or no grain. The disease appears when the fungus was able to build up sufficient inoculum in the soil over two or more years on susceptible varieties.

Isolation of causal agents and other fungi: During the 5 year study in different wheat fields, 155 fungal isolates were identified. All the soil-borne fungi that cause common root rot and crown rot were isolated from the fields that were nearly dry. However, only seven genera were identified to be associated with the root rot and crow rot diseases. There was significant difference in the occurrence of *F. pseudgraminearum* and other species isolated in considered area. Commonly, the incidence of crown rot was more in the relatively drier provinces of Ardabil, East Azarbyjan, Zanjan and Qazvin, which had lower rainfall than other northern provinces, such as,



(a) (b)

Fig. 2: Colony morphology (a) and macroconidia of *Fusarium pseudograminearum* (b)

(a) (b) (c)

Fig. 3: Symptom of crown rot disease on wheat: White head (a), root rot (b) and crown rot (c)

Golestan and Mazenderan. The last two humid provinces were close to the Caspian Sea that receive nearly heavy rainfall in the crop season (April and May). In this situation, usually *Fusarium graminearum* (Group 2) was isolated from the head blight affected wheat samples.

Frequency of isolated fungi: Totally, eight species were isolated from all the regions however *F. graminearum* G2 was isolated in low from drier area than in the humid regions The *F. pseudograminearum* population was mostly recovered from the sub-crown internodes and roots of the infected plants. Its frequency was approximately 50% among the soil-borne fungi isolated in the areas under study. The frequency of the other fungi was 20% for *Rhizolania solani*, 3% for *R. cerealis*, 7% for *F. culmorum*, 10% for *Drechsera* sp., 3% for *Sclerotium rolfsil* and 5% for *Bipolaris* sp.

Yield losses: Crown rot disease caused white heads and resulted in poor seed filling, leading to significant yield losses. Yield productions of infected plants from four locations were compared with no infected plants yield. Investigation showed that there were differences in the extent of yield loss in different areas (Table 1). Yield loss in Ardabil province was 44.5% which showed more loss than other locations. Yield loss was 38% for East

Table 1: Yield losses of wheat productions due to crown rot disease caused by *Fusarium pseudograminearum* in four provinces, northwest Iran

	Yield, (kg 100 m^{-2})		
Province	Healthy crop	Diseased corp	Yield loss (%)
Ardabil	22.7	12.4	44.5
East Azarbyjan	18.6	11.6	38.0
Zanjan	16.8	11.5	32.0
Qazvin	14.5	11.9	18.0

Azarbyjan and 32% for zanjan and 18% for Qazvin provinces. The result showed the occurrence of crown rot disease in deferent wheat cultivated areas, while the incidence of disease was in nearly drier areas than humid area. Off course, distribution of the disease in country can cause economical problems for wheat growers due to loss production.

Soil solarizarion effect: Population density of colony forming unit (cfu g^{-1} soil) of *Fusarium pseudograminearum* was decreased quickly after application two weeks soil solarization in studied areas. Results showed that the population density reduced from 1800 soil to 1000 cfu g^{-1} soil after two weeks and then reduced to 6000 cfu g^{-1} soil after five weeks (Fig. 4). Normally, solarization heats the soil through repeated daily cycle. Commonly, the temperatures in solarized soil were 5 to 10°C higher than those in comparable non-solarized ones. Generally, soil solarization, as a hydrothermal process in moist soil is now very common in controlling soil borne plant pathogens all over the world. Solarization had been carried out at least 38 countries such as Italy, Australia UK, Canada, Germany and USA (Lopez *et al*., 1997; Hall, 1996; Annesi and Motta, 1994; Duff and Connelly, 1993). This method as natural solar heating, would be beneficial for the control of soil borne fungi, bacteria, nematodes and weeds in the fields. Solarization method can become more effective in controlling the soil borne causal agents since high temperatures in soil can be generated and kill pathogens.

Environmental effect: Commonly, the effect of climate, especially rainfall and temperature, on the abundance of *Fusarium* species has been reported by Burgess *et al.* (1988), Marasas *et al.* (1988) and Sangalang *et al.* (1995). The incidence of *Fusarium graminearum* (Group 2) in humid areas, isolation *F. compactum* only from warmer sites and recovering *F. sambucium* only from temperate to cold areas supported the contention of environmental effect on Fusareium species (Saremi *et al*., 1999). The incidence of crown rot and head blight of wheat were also significantly affected by environmental factors, mainly humidity and temperature.

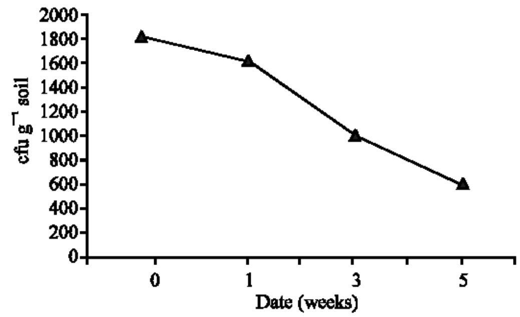


Fig. 4: Population density of *Fusarium pseudograminearum* after 14 and 35 days soil Solarization method in Zanjan province, Iran

Resistant cultivars: It is important to develop resistant cultivars through breeding effort by applying recurrent selection. It was obvious that using mostly susceptible cultivars such as Flat, Golestan and other national races can be resulted high epidemic of wheat crown rot and white head diseases in north Iran. Actually some cultivars were also less infected due to physical barriers and other relatively resistances. There was difference in the incidence of head blight among cultivars growing in adjacent fields in northern Iran (Etebarian and Torabi, 1996). Researchers reviewed also different methods to find sources of resistance to head blight in Iran (Allizadeh *et al*., 2001). Some physiological and morphological characters in wheat were associated with disease resistance. Noticeably using susceptible cultivars as the most key factor for increasing the disease should be changed. Furthermore, we have to expand resistant cultivars by applying repeated selection in different institute. That is why we follow our research to find resistant cultivars as it has been done by other researchers (Akinsanmi *et al*., 2006b; Liu *et al*., 2004). They introduced some resistant cultivars to wheat crown rot and head blight diseases in different counties (Mitter *et al*., 2006; Xie *et al*., 2006).

On the whole, controlling the soil borne pathogens, especially *Fusarium* species has changed over the last few decades. For example, soil solarization approach is to harness solar energy to raise the temperature of moistened soil. Application of soil solarization to control *Fusarium* and *Verticillium* wilt on some trees is carried out in at several countries such as Greece, Italy, Iraq and others (Annesi and Motta, 1994). Control of *Fusarium* wilt by soil solarization in many crops and orchards in different countries has been reported (Hall, 1996). Solarization method can become more effective in controlling the soil borne causal agents since high temperatures in soil can be generated and kill pathogens. Solarization method can become more effective in controlling the soil borne causal agents since high temperatures in soil can be generated and kill pathogens.

Solarization heats the soil through repeated daily cycle. Commonly, the temperatures in solarized soil are 5 to 10°C higher than those in comparable non-solarized ones.

Solarization is already being used for controlling different plant pathogens in several countries such as Greece, Italy, Iraq and others (Hall, 1996; Al-Ahmad, 1993). The practice of deep plowing to control soil borne pathogens has long been known in many countries, but has unfortunately been neglected. Thus, the present version of soil solarization based on mulching the soil with transport polyethylene is a modern modification of known principles, using technologies that were not previously available.

However, the incidence of crown rot disease was mostly correlated with stubble management as the fungus survives in the infected residues (Wearning and Burgess, 1977). Burgess *et al.* (1993) determined the consistency of the effects of stubble management on crown rot disease over longer periods of continuous wheat cultivation and on the disease incidence. The experience showed that stubble retained in some plots enhanced infection of crown rot disease. It was observed that in the Zanjan province the development of crown rot disease was more in poor soils than in soils rich in organic matter. It has been reported that the soil-borne pathogens are generally less than fungal saprophytes in the soil with high organic matter content (Van Bruggen, 1995). However, crown rot was more on deep heavy clay soils under continuous wheat cultivation. Since crown rot and head blight have become major diseases in the areas of continuous wheat cultivation in Iran, particularly in the northern wheat belt, it is important to control them through management practices and use of tolerant cultivars. Crop rotation, burning of stubbles in autumn and careful selection of nitrogen dose can help to minimize the incidence of disease. We have to stop the practice of stubble retention, which leads to significant increase in the incidence of crown rot caused by *F. pseudograminearum*.

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