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Full Length Research Paper

Incidence and distribution of seed-borne fungi associated with wheat in Markazi Province, Iran

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53 seed samples collected from harvested seed loads of irrigated wheat fields in Markazi province in the central of Iran was used for this study. Isolation and identification of seed-borne fungi were conducted according to standard tests described by the International Seed Testing Association (ISTA). A total of 15 fungal species including *Tilletia laevis*, *Tilletia tritici*, *Ustilago tritici*, *Fusarium graminearum*, *Fusarium culmorum*, *Microdochium nivale*, *Bipolaris sorokiniana*, *Alternaria alternata*, *Curvularia* sp., *Aspergillus niger*, *Aspergillus candidus*, *Aspergillus flavus*, *Penicillium* sp., *Mucor* sp. and *Rhizopus* sp. were identified in three wheat cultivars of Backcross Roshan, Alvand and C-78-14. The average of infection level in tested samples to both *T. laevis* and *T. tritici* was estimated as much as 7.1% in the province and the minimum and maximum infection levels were found in Lilian (Khomein) and Jirya regions (Arak), respectively. The average of infection rate by *U. tritici* in seed samples was 1.3% while it was as much as 17.4% for both *F. culmorum* and *B. sorokiniana* in the province. The frequency of *A. niger* and *Penicillium* sp. was predominant with an infection range of 37.8 and 29.1%, respectively. For the first time, the incidence and infection level of seed-borne fungi in wheat seeds have been determined in the central part of Iran.

Key words: Infection rate, seed-borne fungi, seed quality, wheat.

INTRODUCTION

One of the basic strategies to produce certificated and non-infected seeds is the identification of seed-borne pathogenic agents in wheat-growing fields. If infested grain is used as seed, not only would the seed-borne diseases reduce crop yield but also the seed will be a source of disease inoculum (Clear and Patrick, 1993). Seed-borne fungi are one of the most important biotic constrains in seed production worldwide. They are responsible for both pre and post-emergence death of grains, affect seedling vigor, and thus cause some reduction in germination and also variation in plant morphology (Van Du et al., 2001; Rajput et al., 2005; Niaz and Dawar, 2009). Furthermore, infection rate of seeds depending on some environmental conditions such as high relative humidity, suitable temperature and also high level of moisture content in seed is variable. Yield losses caused by seed-borne pathogens to wheat are reported between 15 to 90% of untreated seeds grown in fields (Wiese, 1984). The study of seed-borne pathogens is necessary to determine seed health and to improve germination potential of seed which finally leads to increase of the crop production.

Fungi are the principal organisms associated with crop seeds. A complex of seed-borne fungi including genera of *Tilletia*, *Ustilago*, *Bipolaris*, *Fusarium*, *Alternaria*, *Drechslera*, *Stemphylium*, *Curvularia*, *Cladosporium*, *Rhizopus*, *Aspergillus* and *Penicillium* has been convincingly reported as the most frequent seed-borne fungi of wheat throughout the world (Nirenberg et al., 1994; Glazek, 1997; Hashmi and Ghaffar, 2006; Rehman et al., 2011; Suproniene et al., 2011). Wheat (*Triticum aestivum* L.) is the most important agricultural crop in Iran that constitutes the main grain food and plays a crucial role in the agriculture market by reducing the imported volume of cereal grains. Some investigations have been reported regarding the mycoflora of seed which are responsible for reducing seed quality in wheat production systems in Iran (Kamran and Karbar, 1996; Babadost, 1997; Zare et al., 2006).

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Seed health testing to detect seed-borne pathogens is an important step in the management of crop diseases. Little is known about the status of this group of pathogens in wheat-growing regions in the central parts of Iran. This information is necessary for local growers to understand the seed-borne fungal pathogens status infecting wheat and their frequency to effectively apply control methods. The aim of this study therefore, was to survey and identify seed-borne mycoflora of wheat in Markazi province.

MATERIALS AND METHODS

Collection of seed samples

Wheat growing areas in central Iran have a semi-arid climate with cool to cold winters and hot dry summers. The average annual precipitation is in the range of 200 to 450 mm, which fluctuates from year to year. The precipitation is usually rain in November/ December and autumn and spring in April to June, respectively. Also, snow may fall from late December to March (Iranian Meteorological Organization, 2010, Markazi, Arak).

A total of 53 seed samples were collected from different locations of Markazi province in Iran, including Arak (Amrabad, Amanabad, Mobarakabad, Shahveh and Jirya regions), Khomein (Lilian, Ghorchibashi, Poshtkoh, Robatmorad and Khomein-Golpayegan junction regions) and Farahan (Moslehabad and Sarough regions). Samples of three wheat cultivars including Backcross Roshan, Alvand and C-78-14 were used for the isolation and identification of seed-borne fungi using the seed washing, deep freezing blotter and embryo count methods described by the International Seed Testing Association (ISTA).

Isolation and identification of fungi

Seed washing method

This test was used to study fungal inoculums located on the surface of wheat seed (especially for *Tilletia* spp.). 50 g of seed samples were taken in a 200 ml beaker containing 50 ml sterilized distilled water and 1 to 2 drops of Tween 20 shaken for 10 min over a mechanical shaker. The suspended spores were concentrated by centrifugation at 3000 rpm for 15 min. The supernatant was discarded and the deposit suspended in 500 µL distilled water (Khan, 1992; Mathur and Kongsdal, 2003). The fungal spores were identified on the basis of teliospores morphology under a microscope (Castlebury and Farr, 2011).

Blotter method

Three Whatman filter papers kept on each other as a layer were moistened and placed in 90 mm diameter sterilized Petri dish. 400 seeds of each sample were separated and placed at the rate of 25 seeds per plate. The plates were incubated at 25 ± 1°C for seven days and then the incubated seeds were studied under a stereomicroscope for identification of fungi (Khan, 1992; Mathur and Kongsdal, 2003; Habib et al., 2011). Infection levels were recorded as the percentage of infected seeds in each sample.

Deep freezing method

25 seeds per plate were placed on a 3-layered well soaked blotter as described above. Each sample had three replications. The Petri dishes were incubated for 24 h at 25°C and then transferred to -20°C in a freezer for 6 to 8 h followed by incubation at 25°C for 5 to 7 days (Mathur and Kongsdales, 2003). After the incubation period, each Petri dish was examined under a stereomicroscope in order to record the incidence of different seed-borne fungi. Primary identification of fungi grown on the wheat seeds was performed on the basis of their typical colony characteristics and conidial morphology. The percentage of seed infection in each sample and the percentage of infection in each region were determined by the following formulae;

$$\text{Mean rate of seed infection} = \frac{\text{Number of seed on which a fungal species identified}}{\text{Number of seed tested}} \times 100$$

$$\text{Mean of regional infection} = \frac{\text{Frequency of sample on which a fungus identified}}{\text{Number of sample collected}} \times 100$$

For complementary identification of the fungi up to species level, mycelium of fungi grown on the filter papers were isolated on potato dextrose agar (PDA). Cultures were maintained on PDA at 24 ± 1°C for 7 to 10 days and the identification was conducted using morphological characters such as spore size, shape, color and their arrangement on the conidiophores and morphology of the mycelium (Utobo et al., 2011) by referring to Nelson et al. (1983), Sivanesan (1987), Leslie and Summerell (2006) and Watanabe (2002).

Embryo count method

When the inoculum of a fungus as mycelium exist deep within the seed, separating of the embryo for microscope observations is necessary. In this test, 100 g seeds of each sample were soaked in 1 L of water and then mixed with 50 g NaOH and 0.1 g trypan blue at 20 to 24°C. After 24 h, the seeds were transferred to a sieve of 3.5 mm diameter which was placed over sieves of 2 and 1 mm diameter and then were washed using hot water tap (60 to 70°C) until the embryos passed through sieves and placed over the sieve of 1 mm diameter. The embryos and chaffs were transferred to a beaker containing 90 ml lactic acid, glycerol and water (30:30:30 v/v). The floated embryos were separated and transferred to grooving plates (Khan, 1992; Mathur and Kongsdal, 2003). The number of infected embryos was enumerated in each sample under a binocular microscope and infection rate of the seed was determined using the aforementioned formulas.

RESULTS AND DISCUSSION

It has long been noted that seed-borne fungal pathogens are responsible for reducing seed quality, protein and carbohydrate contents, reduction or elimination of germination capacity as well as seedling damage, which result in the reduction of crop yield (Mushtaq and Hashmi, 2005; Fakhrunnisa et al., 2006). Over the last decades, many studies have been made to test and detect seed-borne diseases of wheat throughout the world. The studying of seed microflora in Canada revealed that a total of 35 genera and 59 species of seed-borne fungi exist in seed samples of wheat. Khan (1992) reported that 17 genera and 45 species of seed-borne fungi were associated with wheat seeds in Pakistan.

In this study, the results show that a total of 15 fungal species including *Tilletia laevis*, *T. tritici*, *Ustilago tritici*,

Table 1. Incidence and frequency of bunt and loose smut fungi associated with wheat seed in Markazi province.

City	Region	Number of tested sample	Identified species	Mean rate of seed infection in each sample (%)
Arak	Amrabad	3	*Tilletia laevis*	4.2
			Tilletia tritici	5.5
			Ustilago tritici	1.4
	Amanabad	7	*T. laevis*	8.7
			T. tritici	9.2
	Jirya	6	*T. laevis*	10.5
			T. tritici	14.3
	Shahveh	2	*T. laevis*	5.2
			T. tritici	8.4
			U. tritici	1.7
	Mobarakabad	2	*T. laevis*	4.1
			T. tritici	7.8
			U. tritici	1.3
Khomein	Lilian	8	*T. laevis*	5.6
			T. tritici	2.7
	Ghorchbashi	8	*T. laevis*	6.5
			T. tritici	7.9
			U. tritici	0.6
	Khomein-Golpayegan junction	5	*T. laevis*	8.4
			T. tritici	7.1
			U. tritici	1.1
	Robatmorad	3	*T. laevis*	5.2
			T. tritici	8.7
			U. tritici	2.4
	Poshtkoh	8	*T. laevis*	2.4
			T. tritici	13.3
			U. tritici	1.2
Farahan	Moslehabad	1	*T. laevis*	12.6
			T. tritici	7.4
	Saroogh	1	*T. laevis*	5.3
			T. tritici	9.5
			U. tritici	0.8

Fusarium graminearum, *F. culmorum*, *Microdochium nivale*, *Bipolaris sorokiniana*, *Alternaria alternata*, *Curvularia* sp., *Aspergillus niger*, *A. candidus*, *A. flavus*, *Penicillium* sp., *Mucor* sp. and *Rhizopus* sp. were isolated and identified from the seeds of three wheat cultivars (Tables 1 and 2). In Iran, mycoflora of stored grains were investigated in Kerman province and the fungi of *A. alternata, Ulocladium alternariae, A. flavus, A. niger, Chaetomium globosum, F. proliferatum, Cladosporium cladosporioides, Rhizopus* spp. and *Penicillium* spp. were

Table 2. Seed-borne fungi Isolated from wheat seeds and their infection rate in Markazi province.

City	Region	Number of tested sample	Identified species	Mean rate of seed infection (%)	Mean of regional infection (%)
Arak	Amrabad	3	*Fusarium graminearum*	2.5	100
			Alternaria alternata	3.1	100
			Aspergillus niger	11.2	66.6
			A. flavus	9.4	66.6
	Amanabad	7	*Bipolaris sorokiniana*	1.2	85.7
			Fusarium culmorum	2.6	71.4
			A. niger	6.7	100
			Mucor sp.	10.4	71.4
	Jirya	6	*Microdochium nivale*	5.3	83.3
			A. alternata	4.8	66.6
			Rhizopus sp.	6.6	100
			A. candidus	4.3	83.3
	Shahveh	2	*B. sorokiniana*	0.7	100
			A. alternata	3.1	50
			Penicillium sp.	5.5	100
			A. candidus	1.3	100
	Mobarakabad	2	*F. culmorum*	1.7	100
			A. niger	3.8	100
			M. nivale	2.3	100
			Penicillium sp.	4.6	100
	Lilian	8	*A. alternata*	0.8	62.5
			F. culmorum	2.4	100
			M. nivale	4.3	37.5
			A. niger	6.8	75
	Ghorchbashi	8	*B. sorokiniana*	3.5	75
			F. graminearum	0.8	50
			A. alternata	0.4	37.5
			Penicillium sp.	2.4	62.5
Khomein	Khomein-Golpayegan junction	5	*M. nivale*	0.4	80
			F. culmorum	0.3	100
			Curvularia sp.	1.3	40
			Penicillium sp.	5.6	40
	Robatmorad	3	*A. alternata*	0.5	100
			B. sorokiniana	2.3	66.6
			A. candidus	2.1	66.6
			Penicillium sp.	2.3	100
	Poshtkoh	8	*B. sorokiniana*	1.8	87.5
			F. culmorum	1.1	87.5
			A. alternata	1.2	50
			Rhizopus sp.	1.9	75
			Penicillium sp.	0.7	87.5
			A. niger	5.6	87.5

Table 2. Contd.

			F. culmorum	0.9	100
	Moslehabad	1	*A. alternata*	2.1	100
			A. flavus	12.1	100
Farahan			*B. sorokiniana*	1.4	100
			F. culmorum	2.1	100
	Saroogh	1	*Penicillium* sp.	7.1	100
			A. niger	4.7	100
			Mucor sp.	1.4	100

reported. Zare et al. (2006) determined the fungi species and infection rate as 15.5% *F. culmorum*, 13.1% *F. graminearum*, 24.4% *B. sorokiniana*, 4.5% *Drecshslera tritici-repentis*, 8.5% *A. alternata*, 24.2% *Cladosporium sphaerospermum*, 4.7% *Penicillium* spp., 5% *A. niger* and 9% *A. flavus* in harvested wheat loads in Tehran, Fars, Ardebil, Golestan, Ilam, Semnan, and Kerman provinces.

Bunt fungi have been very important diseases of wheat since contaminated commodities (seeds, foods and feeds) affect the marketability of the crop on both domestic and export markets. In our study, the lowest infection rate of seed to both *T. laevis* and *T. tritici* was 8.3% in Lilian region and the highest infection rate was up to 24.8 in Jirya region (Table 1). Our results show that the frequencies of *T. laevis* and *T. tritici* teliospores were between 1 to 48 spores in each wheat seed. Results of a field survey in Syria revealed that the mean frequencies of *T. caries* and *T. foetida* teliospores in durum wheat were 87.7 and 12.3%, while in bread wheat, it was 19.1 and 80.9%, respectively (Kyali et al., 2010). In Iran, Hamidi et al. (2010) reported that the average of infection rate in commercial wheat seeds to *T. laevis* was 1.2 spores on each seed with a minimum and maximum level of 0.4 and 2.8 in Golestan and South Khorasan provinces, respectively.

Our results reveal that the average of infection level of *U. tritici* in wheat seeds was 1.8% in the province with a minimum and maximum level in Khomein (0.6%) and Arak (1.7%) respectively (Table 1). The results show that both *B. sorokiniana* and *F. culmorum* were widespread in different parts of the province, while *F. graminearum* was limited to two regions. It was noticed that *Fusarium* head blight may cause severe yield reduction, as high as 70%, grain quality losses and germination reduction in years with high epidemics in Iran (Zamanizadeh and Khorsandi, 1995). Our results show that the average infection level of *B. sorokiniana* and *F. culmorum* was 9.7 and 12.4% in the province, respectively. Babadost (1997) isolated some species of *Fusarium* fungus in wheat seeds collected from cereal fields in the North West of Iran. Zare et al. (2006) also reported an average of infection level up to 15.5 and 24.4% in wheat seeds to *F. culmorum* and *B. sorokiniana*, respectively, in seven provinces of Iran.

In this study, the average contamination level of the seeds was determined for *A. niger* (10.6%), *A. candidus* (4.7%), *A. flavus* (3.1%), *Penicillium* sp. (11.1%), *Mucor* sp. (3.2%) and *Rhizopus* sp. (3.3%) (Table 2). The species of *A. niger* and *Penicillium* sp. were the most frequent isolated fungi in the seed samples. These species have been reported to reduce the germination of seed and seed loss in storage (Christensen, 1973). The presence of saprophytic or weakly pathogenic fungi of *Alternaria, Helminthosporium, Curvularia, Stemphylium, Rhizopus, Cladosporium, Aspergillus, Penicillium, Gonatobotrys* and *Nigrospora* is demonstrated in wheat seeds (Bhutta and Hussain, 1999; Suproniene et al., 2011; Habib et al., 2011). Saberi et al. (2004) reported the isolation of the seed-borne fungi (*Aspergillus* spp., *Alternaria* spp., *Cladosporium* spp., *Penicillium* spp. and *Ulocladium* spp.) associated with wheat grains in Markazi province. Gohari et al. (2007) also isolated the fungi of *Aspergillus* spp., *Alternaria* spp., *Cladosporium* spp., *Penicillium* spp. and *Ulocladium* spp. in wheat seeds in Kerman province.

It is well accepted that wheat production must be increased considerably in the foreseeable future to meet the nutritional requirements of a rising human population (Oerke and Dehne, 2004). Certificated and healthy seed is an important input for crop production and hence reduction of yield loss caused by seed-borne fungi is one way to contribute to the food security in the world. Our results indicate the incidence and geographical distribution of the seed-borne fungi in the irrigated wheat fields of Markazi province. More research could be done in order to determine economical importance of seed-borne diseases of wheat in infested regions since yield losses caused by these fungal pathogens are not yet known in Iran.

Desirable crop traits considered essential by farmers are higher grain yield, drought tolerance, disease resistance, and grain quality. Hence, there is need for reducing the pathogenic fungi in produced seeds by applying different control options. Seed-borne fungi are the easiest pathogens to be controlled through treatment of seed using suitable chemicals and biological

compounds. In addition, using standard stores for preserving seed and planting resistant cultivars for protecting the infection level below damage threshold have been recommended (Rennie, 1993; Maude, 1996; Clark et al., 2004).

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