IJBPAS, September, 2016, 5(9): 2203-2211

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

SEED MYCOFLORA OF WHEAT (*TRITICUM AESTIVUM* L.) IN NORTHEAST IRAN

MEHDI SADRAVI* AND MOHAMMAD ABDOLLAHI

Department of Plant Protection, Faculty of Agriculture, Yasouj University, Yasouj, Iran

***Corresponding Author: E.Mail: msadravi@yu.ac.ir; Phone: +989173877160**

Received 29th May 2016; Revised 29th June 2016; Accepted 16th July 2016; Available online 1st Sept. 2016

ABSTRACT

There are many important fungal seed borne diseases of wheat. The Golestan Province, in northeastern Iran, is a major wheat production area of the country. To identify the seed mycoflora of wheat, seventy five seed samples were collected from this area. The associated fungi were isolated using agar plate method. All samples were contaminated with (2-)3(-5) fungi, out of them fifteen species were found to belong to eight genera, *Alternaria*, *Aspergillus*, *Cladosporium*, *Bipolaris*, *Fusarium, Penicillium*, *Rhizopus* and *Rhizoctonia*. Frequency of contaminated seeds and associated fungi varied from 2-80% and 1.5-98%, respectively. The isolated fungi can cause some diseases in wheat, consist of black point, common root rot, head blight and seed rot. Effects of seed production area, rates of seeding and fertilizers, cropping system and storage time on contamination to these fungi and also suggested methods for reducing the seed contamination are discussed.

Keywords: *Alternaria*, *Fusarium*, *Bipolaris*, Seed, Wheat

INTRODUCTION

Some of the major diseases of wheat are seed-borne. Among them, *Fusarium* head blight, black point and common root rot, are of most importance [1]. Regarding to significance of seedborne diseases of wheat, study of seed mycoflora is recommended to ensure seed safety and disease prevention. Studies on seed mycoflora of wheat were carried out in Argentina [2], Australia [3,4], Bangladesh [5], Bolivia [6], Brazil [7,8], Canada [9,10], China [11], Crovasia [12], Egypt [13], Eslovenia [14], India [15], Italy [16], Pakistan [17] and USA [18,19]. In Iran, the seed mycoflora of wheat has been studied in Northwest Iran [20]. So far, some fungal

seedborne diseases of wheat, including head blight and black sooty head molds have been observed in wheat fields of the Golestan Province, in northeast Iran (Personal observations). In spring, during wheat grain development and seed ripening, in the west part of the province, on the southeastern coast of Iranian Caspian Sea, air humidity is high and the annual average rainfall is about 650 mm. On the other part of the province, in the east plains, relative humidity is low and anual precipitation is 550 mm. Considering the natural variability in province climate, this study aimed to investigate the diversity of fungi associated with wheat seeds in different parts of the region.

MATERIALS AND METHODS

Wheat fields of Golestan Province were surveyed and 75 seed samples were randomly collected from farmers. For each collected sample, required data of conditions of seed production including: cultivation method, seeding rate, fungicide seed treatment, levels of NPK fertilizers, period of seed storage, were recorded. Agar plate method with Potato Dextrose Agar and semi-selective Modified Nash and Snyder medium was used for fungal isolation [21]. All of isolated fungi were purified by single spore or hyphal tip methods [22]. Carnation leaf agar medium (CLA) was used for stimulation of macroconidia formation in *Fusarium* isolates [23]. *Rhizoctonia* isolates were identified by hyphae staining method [24]. Morphological characteristics of isolated fungi were studied and were measured with a calibrated Nikon bright-field microscope and the collected data were compared with the original descriptions [25, 1, 26, 27, 28, 23, 24]. Fungal diversity and the frequency of the contaminated seeds present in samples were recorded.

RESULTS AND DISCUSSION

Identified fungi and their frequency

Fungal contamination was detected in all of collected seed samples. Number of isolated species of fungi per seed varied from two to five with an average of three. Frequency of contaminated seeds per each sample was 1.5 to 98%. In this study, about 600 fungal isolates were obtained. After purification and study of the morphological characteristics, 15 fungal species belong to eight genera were identified (Table1.)

Two species, *Alternaria alternata* and *Cladosporium cladosporioides*, were isolated from seeds which were infected to black point disease (Fig.1A). *Alternaria* and *Cladosporium* species cause black (sooty) head molds and black point diseases of wheat [1]. *A. alternata*, has verrucose small conidia, measured 10-45 × 6-8 µm, which are produced in a long chain (Fig.1B). So far, *A. alternata* has been

reported from 72% of seed samples with symptoms of black point and low germination percentage, from Australia [3], Brazil [7], Canada [9], Croatia [12], Egypt [13] and India [15]. The fungus *C. cladosporioides* has 1-2 celled, verrucose, oblong-ellipsoid conidia, brown in color and borne in chains on long conidiophores. Terminal cell sometimes separated and formed ramoconidium (Figs.1C, D).

Bipolaris sorokiniana has dark brown mycelia, sympodial conidiophores and curved, fusoid to broadly ellipsoidal conidia in light brown color (Figs.1E,F). It has been found in Australia [3], Bangladesh [5], Brazil [8], Bolivia [6], Canada [9], China [11] and Pakistan [17]. *B. sorokiniana* causes common root rot (dry land root rot) disease in wheat which can be transmitted by seed [1].

Table 1: Wheat seed borne fungi in the northeast Iran

S. No	Fungi	Frequency of contaminated samples (%)	Frequency of contaminated seeds (%)
1-	*Alternaria alternata* (Fr.) Keissler	80	4-36
2-	*Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.	20	4
3-	*Cladosporium cladosporioides* (Fresen.) de Vries	21	4
4-	*Aspergillus flavus* Link ex Fries	49	0.5-16
5-	*Penicillium chrysogenum* Thom	15	0.5-8
6-	*Fusarium acuminatum* Ell. and Ev.	3	0.5
7-	*F. equiseti* (Corda) Sac.	3	0.5
8-	*F. graminearum* Schwabe	52	15-30
9-	*F. lateritum* Nees	2	4
10-	*F. moniliforme Sheldon*	17	4 -8
11-	*F. proliferatum* (Matsushima) Nirenberg	21	4 -15
12-	*F. semitectum* Berk. and Rav.	4	4 - 8
13-	*F. subglutinans* (Wollenw. & Reink.) Nel., Tous. & Mara.	10	4
14-	*Rhizopus arrhizus* Fischer	13	3-58
15-	*Rhizoctonia solani* Kühn	3	4

Fusarium graminearum (Fig.1G) has been isolated from wheat seed and is reported from northwest Iran [20], Italia [16], Canada and USA [29]. It has also isolated from barley grains and reported from northeast Iran [30]. *F. graminearum* infects the crop in the field, during wheat seed development and can greatly reduce the seed quality. Wheat seeds which are severely infected with *F. graminearum*, are mostly lighter and shriveled with creamy-white or pinkish color. Such infected seeds, will generally fail to pass the standard germination test [31]. *F. acuminatum* (Fig.1H) has been reported from wheat seeds in northwest Iran [20] and from wheat crop infected to ears blight disease in Canada [10, 32]. *F. semitectum* is reported from wheat seeds in Sao-Paolo, Brazil [7], Argentina [2] and Europe [33]. *F. lateritum*

has been reported from wheat seeds in Florida [18], *F. subglutinans* from Europe [33], *F. equiseti* (Fig.1I) from wheat seeds in northwest Iran [20] and from a cultivar of durum wheat in Argentina [2]. *F. graminearum*, *F. acuminatum*, *F. subglutinans*, *F. lateritum*, *F. semitectum* and *F. equiseti* cause severe to mild seed borne *Fusarium* head blight disease in most of wheat growing areas in the world [1].

F. moniliforme and *F. proliferatum* were isolated from wheat seeds in northwest Iran [20], Sao-Paolo, Brazil [7] and Argentina [2]. *F. moniliforme* and *F. proliferatum* are main cause of wheat, corn and sorghum seed rot and root rot of rice, and produce the mycotoxin Fumonisin, which can pose serious health risks to humans and animals [23]. These species are well known as main cause of cereal grains rot and to produce Fumonisin in cereals in central Europe [33].

The fungus *Rhizoctonia solani* has been reported from wheat seeds in USA, the states of Idaho, Oregon and Washington [19]. It causes root rot and sharp eyespot diseases in wheat. Change in the seed color and reduction of germination rate are mentioned as important symptoms of diseased plants. The fungus has potential of three years survival in infected seed [19].

Aspergillus flavus, has been reported from stored seeds of wheat in Crovesia [14] and from barley grains in northeast Iran [30]. The fungus has a worldwide distribution and contaminates seeds, grains, nuts and fruits of many plants, and produce carcinogen and mutagen aflatoxins [34]. *Penicillium chrysogenum* (Fig.1J) has been reported from wheat seeds in Turkey [35].

Factors affecting fungal contamination of seeds

The average frequency of contaminated seeds in conventional farming systems of Golestan Province is shown in Table 2. Based on the results, the farming system plays a critical role in fungal contamination of seed. Accordingly, the seeds that were produced in those fields with pre-plant fungicide application, in-row planting, lower seeding rate, low nitrogen and phosphorus fertilizers and using potassium fertilizer, were less contaminated to fungi, compare to other fields. More fungal contamination have been detected in seed samples from the west of province. The influence of percentage of fungal contamination on storage life in the west of the province was more than the east of province. This is the first report on effect of climate condition and storage duration on wheat seed contamination to fungi.

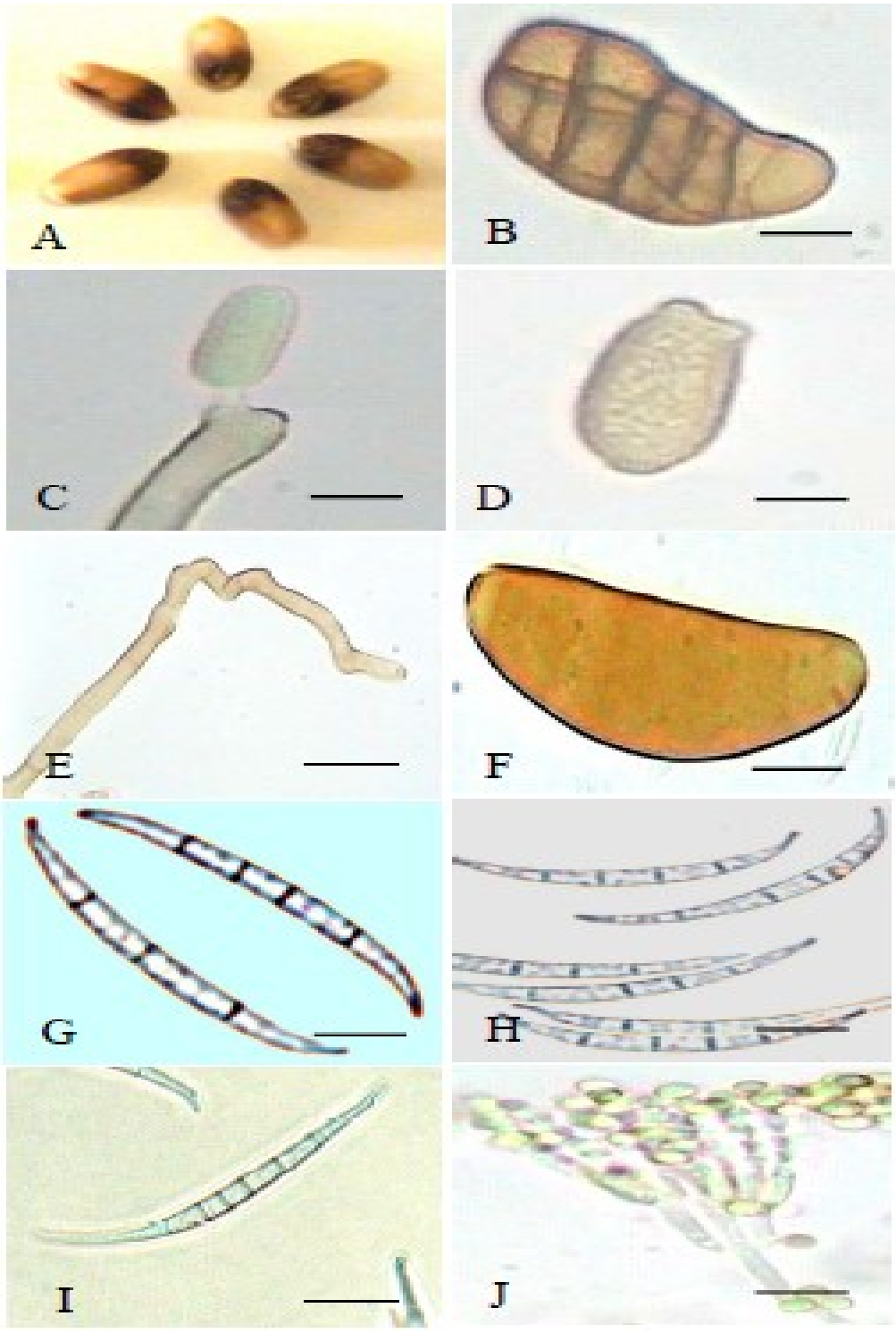

Fig. 1. Wheat seedborne fungi, A. Black pointed wheat seeds, B. Conidium of *Alternaria alternata* with verucosate surface, C,D. *Cladosporium cladosporioides*, *C. Conidiophore* with one new borned conidium, D. Ramoconidium, E, F. *Bipolaris sorokiniana*, E. *Sympodial conidiophore, F. Conidium, G. Macroconidia* of *Fusarium graminearum*, H. Macroconidia of *F. acuminatum*, I. Macroconidium of *F. equiseti*, J. Conidiophore conidia of *Penicillium chrysogenum* (Bars= 5 µm).

Table 2: The average frequency of wheat seeds contaminated to fungi in northeast Iran produced with conventional farming systems.

	Cultivation method		Seed treatment with a fungicide before planting		Seeding rate (Kg/ha)		Fertilizers (Kg/ha)					
			Yes	No			N		P		K	
	Row	Non row			180-200	201-220	50-100	150-250	50-100	150-250	0	50-100
Average frequency of contaminated seeds to fungi (%)	9	60	20	73	2	36	9.5	63	9	62	45	26

CONCLUSION

Based on the sources of seed contamination, the isolated fungi can be divided into two groups. Species of the genera *Alternaria*, *Cladosporium*, *Fusarium*, *Bipolaris* and *Rhizoctonia* fell in a group that invade the seeds either during the seed development or after its maturity. Because their damage generally occur in the field and they have a little or no damage at storage, they are considered as field fungi. Species of *Alternaria* and *Cladosporium* occur any time from grain filling to near harvest. High air humidity or any type of water application during the milk stage to soft dough stage and also crop lodging, often provide suitable conditions for seed infection to these fungi. Considering the harms of high humidity and rainfall in the period of seed development to seed maturity in the west part of the province, the seed production should be done in east part of the Golestan Province. Moreover, it will recommend that in the seed production field, seed must be treated with a fungicide before sowing. In-row cultivation method, optimum rates of seeding and NPK fertilizing will also recommended.

The second group of the isolated fungi, which are considerd as storage molds, consists of the species of the genera *Aspergillus*, *Penicillium* and *Rhizopus*. Seed contamination to these fungi occurs during harvest or at storage period. In high moisture seed content or warm storage with high relative humidity, they can cause complete seed decay. More fungal contamination was found associated with seeds with long time storage as well as in seeds that were produced in the previous growing season. Therefor, for mangement of the storage molds, it will recommended that store the seeds with low moisture in a dry and cool store, for a few weeks or months.

ACKNOWLEDGEMENT

The authors are grateful of Golestan Province Management and Planning Organization and Gorgan University of

Agricultural Sciences and Natural Resources for their financial support of this research.

REFERENCES

[1] Wiese MV .1987. Compendium of Wheat Diseases. APS Press, St. Paul, Minnesota, USA.

[2] Lori GA and Sisterna MN .2001. Occurrence and distribution of Fusarium species associated with durum wheat seed from Argentina. Journal of Plant Pathology 83:63-67.

[3] Rees RG, Martin DJ and Law DP .2006. Blackpoint in bread wheat: effects on quality and germination, and fungal association. Australian Journal of Experimental Agriculture 127:601-605.

[4] Wildermuth GB, Tinline RD and McNamara RB .1992. Assessment of yield loss caused by common root rot in wheat cultivars in Queensland. Australian Journal of Agricultural Research 43:43-58.

[5] Banu SP, Duxbury JM, Lauren JG, Meisner C and Rafiqul I .2004. Wheat seed quality-A study on farmers' seed. Proceedings of the 4th International Crop Science Congress, Brisbane, Australia, pp: 10-12.

[6] Toledo BJ and Guzman AE .1998. Importance of spot blotch caused by *Bipolaris sorokiniana* in Bolivia. *Helminthosporium* blights of wheat: Spot blotch and Tan spot. Pp: 146-149. E. Duveiller, H.J. Dubin, J. Reeves and A. McNab (Eds.). CIMMYT, Mexico.

[7] Badoniali-Furlong E, Valente Soares LM, Campos Lasca C and Yokokohara E .1995. Mycotoxins and fungi in wheat harvested during 1990 in test plots in the state of Sao Paolo, Brazil. European Journal of Plant Pathology 3:185-190

[8] Diehl JA, Tinline RD and Kochhann RA .1983. Losses in wheat caused by common root rot in Rio Grande do Sul in 1979-81. Fitopatologia Brasileira 8:507-511.

[9] Clear RM and Patrick SK.1993. Prevalence of some seed borne fungi on soft white winter wheat seed from Ontario, Canada. Canadian Plant Disease Survey 73:143-149.

[10] Turkington TK, Clear RM, Burnett PA, Patrick SK, Orr DD and Xi K. 2002. Fungal plant pathogens infecting barley and wheat seed from Alberta, 1995–1997. Canadian Journal of Plant Pathology 24:302–308

[11] Zhang TY, Wang HL and Xu FL .1990. Effects of grain black point

disease of wheat and the pathogenic fungi. Acta Phytophylacica Sinica 17:313-316.

[12] Jurkovic D, Cosic J, Popovic R and Vrandsic K .2002. Presence of some important fungi on wheat seed. Petria 12:229-233.

[13] Mazen MB, Abdel–Hafez SII and Shaban GMM .1984. Survey on the mycoflora of Egyptian wheat grain and their lemma and paleae. Mycopathologia 3:155-159.

[14] Tancinová D, Kacániová M and Javoreková S.2001. Natural occurrence of fungi in feeding wheat after harvest and during storage in the agricultural farm facilities. Biologia 56:247-250.

[15] Agarwal PC, Anitha K, Usha D, Baleshwar S and Ram N.1993. *Alternaria alternata*, real cause of black point and differentiating symptoms of two other pathogens associated with wheat (*Triticum aestivum*) seeds. Indian Journal of Agricultural Sciences 63:451-453.

[16] Shah DA, Pucci N and Infantino A .2005. Regional and varietals differences in the risk of wheat seed infection by fungal species associated with *Fusarium* head blight in Italy. European Journal of Plant Pathology 112:13-21.

[17] Hossain I and Hossain MM .2001. Effect of black pointed grains in wheat seed samples on germination, seedling vigor and plant stand. Pakistan Journal of Phytopathology 13:1-7.

[18] Weber Z, Berger DA, Shokes FM, Barnett RD and Wright DL .1992. Mycoflora of seed from advanced wheat breeding in north Florida. North Florida Research and Education Center Newsletter 51:96-99.

[19] Ogoshi A, Cook RJ and Bassett EN .1990. *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in Pacific Northwest. Phytophathology 80:784-788.

[20] Babadoust M .1995. *Fusarium* species on wheat seeds and plants in south Azarbayejan and Ardebil provinces of Iran. Iranian Journal of Plant Pathology 31:88-100.

[21] Jha DK .1995. Laboratory Manual on Seed Pathology. Vikas Publishing House PVT LTD, New Delhi, India.

[22] Dhingra OD and Sinclair JB .1987. Basic Plant Pathology Methods. CRC Press, Boca Raton, Florida, USA.

[23] Leslie JF and Summerell BA .2006. The Fusarium Laboratory Manual. Blackwell Publishing, USA.

[24] Sneh B, Burpee L and Ogoshi A .1991. Identification of *Rhizoctonia* Species. APS Press, St. Paul, MN., USA.

[25] Ellis MB .1971. Dematiaceous Hyphomycetes. CAB International, Kew, Surrey, UK.

[26] Onions AHS, Allsopp D and Eggins HOW .1981. Smith's Introduction to Industurial Mycology. Edward Arnold, London, UK.

[27] Rotem J .1994. The Genus *Alternaria*, Biology, Epidemiology and Pathogenicity. APS Press, St. Paul, MN., USA.

[28] Barnett HL and Hunter BB .1998. Illustrated Genera of Imperfect Fungi. 4th Edition. APS Press, St. Paul, MN, USA.

[29] McMullen M, Jones R, Gallenberg D .1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Disease 81:1340-1348.

[30] Ahmadi M and Sadravi M .2008. Six new fungi for Iran from barley grains in Golestan Province (N.E. Iran). Rostaniha 9:113-124.

[31] Argyris J, Van Sanford D and Tekrony D .2003. *Fusarium graminearum* infection during wheat seed development and its effect on seed quality. Crop Science 43:1782-1788.

[32] Xue AG, Armstrong KC, Voldeng HD, Fedak G and Babcock C .2004. Comparative aggressiveness of isolates of *Fusarium* spp. causing head blight on wheat in Canada. Canadian Journal of Plant Pathology 26:81-88.

[33] Bottalico A and Perrone G .2004. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. European Journal of Plant Pathology 108:611-624.

[34] McGee DC, Olanya OM, Hoyos GM and Tiffany L H .1996. Populations of *Aspergillus flavus* in the Iowa corn field ecosystem in years not favorable for aflatoxin contamination of corn grain. Plant Disease 80:742-746.

[35] Asan A .2004. *Aspergillus*, *Penicillium* and related species reported from Turkey. Mycotaxon 89:155-157.