academicJournals

Vol. 13(5), pp. 258-271, 1 February, 2018
DOI: 10.5897/AJAR2015.9825
Article Number: 4A3EC8E55874
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Screening of wheat germplasm for seed associated fungi in geographical areas of Pakistan

Attiq-ur-Rahman[1], Riffat Tahira[2], Aqleem Abbas[3*], Shahid Iqbal[4], Israr Ahmad[1] and Waseem Ahmad[1]

[1]Department of Plant Breeding and Genetics, The University of Agriculture, Peshawar, Pakistan.
[2]Plant Genetic Resources Institute, National Agriculture Research Center, Islamabad, Pakistan.
[3]Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan.
[4]Department of Agronomy, Poonch Rawalkot University, Pakistan.

Received 18 April, 2015; Accepted 12 November, 2015

One hundred wheat (*Triticum aestivum* L.) accessions were selected on the basis of different geographical areas of Pakistan. Isolation and identification of seed born fungi were conducted according to standard blotter test and a total of five major seed borne fungi including *Alternaria alternata, Aspergillus niger*, *Fusarium* species, *Drechslera* species and *Phytophthora* species were isolated from the wheat seeds. The frequency of occurrence of these five seed born fungi was 49, 46, 42, 35, and 16%, respectively. Infection percentage varied from 0 to 90% in all 100 wheat accessions. Among the accessions, the highest infection (100%) of seed born fungi was recorded in 011185 and 011757 accessions while the lowest infection (10%) was recorded in 011415 accessions. Moreover, in accessions collected from Gilgit Baltistan and Azad Jammu Kashmir. *Alternaria niger* and *Alternaria fusarium* were dominant, while in Khyber Pakhtunkhawa province, *A. niger* was prevalent followed by *A. alternata.* In the case of Baluchistan province, the dominant seed born fungi was *A. alternata* followed by *Drechslera* spp. Similarly, in case of Punjab, the occurrence of *A. alternata, Drechslera* spp., *Fusarium* spp., and *A. niger* associated with seeds were similar. For accession collected from Sindh province, the dominant seed born fungi was *A. niger* and *Drechslera* spp. However, the *Phytophthora* spp. infection of wheat seeds accession of Baluchistan was the highest followed by wheat seeds accession collected from Gilgit Baltistan and AJK, Kpk and Punjab, whereas wheat seeds accessions collected from Sindh province were found to be free from *Phytophthora* spp.

Key words: Bread wheat, screening, wheat germplasm, seed born fungi.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple foods among agricultural crops since it constitutes the basis for human nutrition and is of enormous economic importance worldwide. Wheat is used mainly for human consumption and supports nearly 35% of the world population (Schuster et al., 2009). It supplies a

*Corresponding author. E-mail: aqlpath@gmail.com.

large fraction of the dietary protein, total food supply. It is also a principal source of carbohydrates and proteins both for human beings and animals (Ali et al., 2013).

In Pakistan, wheat (*T. aestivum* L.) crop is considered as the best cereal since it ranks the first among the cultivated cereals in the country and occupies about 66% of the annual food crop area (Ansari et al., 2006). Wheat contributes 10.1% to the value added in agriculture and 2.2% to gross domestic product (GDP). Area under wheat increased to 8693 thousand hectares in 2012 to 2013, from 8650 thousand hectares showing an increase of 0.5% over last year's area. In Pakistan, the production of wheat crop stood at 24.2 million tonnes during 2012 to 2013, against the target of 25.5 million tonnes which is 5.1% decrease while an increase of 3.2% over the last year production of 23.5 million tonnes has been witnessed. The yield per hectare in 2012 to 2013 stood at 2787 (kg/ha) posted a positive growth of 2.7% as compared to negative 4.2% growth last year (PBS, 2013).

Wheat is stored for a period of time before it can be marketed or used as feed or seed. The length of time cereal can be safely stored will depend on the condition it was harvested and the type of storage facility being utilized. Conditioning of grain has the single purpose of preserving the quality of grain. Low moisture content and temperature have been shown to be essential for successful storage of grain for a long period of time (Chaudhary et al., 2000).

The quality seed of improved wheat varieties is also considered as most important input for obtaining optimum production. Only the good seed can give an economic benefit to the wheat grower. It can maintain the quality of production, which fetches higher value in the market. Therefore, availability of healthy and pure seed should be confirmed, otherwise most of the seed-borne diseases of wheat could become responsible for heavy losses (Marwat et al., 2002).

Wheat crop is subjected to a number of diseases, which reduces its overall production to a great extent, because wheat plants in all stages of growth and in all natural environments are subject to various mechanical, physiologic and biological stresses that interfere with their normal growth and development. Biotic hazards, insects, viruses, fungi, nematodes, bacteria and weeds are primary hazards to wheat production (Ahmad et al., 2003).

The actual number of wheat diseases is unknown, but nearly 200 have been reported from the wheat-growing countries in the world. Over 100 infectious diseases caused by pathogens and by weeds are transmissible from plant to plant. Amongst these, about 50 are generally seed-borne. In Pakistan, 50 diseases are reported to occur which have great financial repercussions (Iftikhar et al., 1991). The rusts are considered the most destructive, but the problem of seed-borne diseases is also of great importance and could not be neglected. Smut, bunt, blight and root rot are some important seed-borne diseases, which are perpetuated through seeds and cause considerable losses to crops under favorable conditions (Dawson and Bateman, 2001). Seed-borne diseases have been found to affect the growth and productivity of crop plants and especially seed-borne fungi are important from the economic point of view as they render losses in a number of ways. Numerous examples exist in agriculture literature for the international spread of plant diseases as a result of importation of seeds that were infected or contaminated with pathogens (Clear and Patrick, 1993). 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 (Bishaw et al., 2013).

Fungi are the principal pathogen organisms associated with crop seeds. A complex of seed-borne fungi including genera of *Tiletia, Ustilago, Bipolaris, Fusarium, Alternaria, Drechslera, Stemphylium, Curvularia, Cladosporium, Rhizopus, Aspergillus* and *Penicillium* have been convincingly reported as the most frequent seed-borne fungi of wheat throughout the world (Kumar et al., 2008). The present study was conducted to isolate the wheat seed associated fungi from seeds collected from different geographical locations of Pakistan in order to know the importance of seed borne pathogens and their effects on wheat crop in these locations.

MATERIALS AND METHODS

Collection of seed samples

Wheat seeds (100 accessions) were obtained from the National Agriculture Research Center (NARC) gene bank, Islamabad. The accessions were selected on the basis of different geographical areas of Pakistan (Table 1a, b, c, d and e).

Isolation of seed associated fungi

For isolation of fungi associated with wheat seed, blotter test was used. Initially 90 mm size discs of blotting paper were moistened with autoclaved distilled water and placed at the bottom of 90 mm sterilized Petri plates. The seed were surface sterilized with 5% hypochlorite solution followed by a rinse with autoclaved water. Ten seeds of each wheat accession were placed at equal distance in separate Petri plates using a sterilized pair of forceps. The lids of the Petri plates ware held in place with parafilm. The plates were incubated at 27°C for a period of 5 to 7 days under 12 h alternating cycle of light and darkness. After the incubation period, fungi growing out from seeds were examined, identified and their percentage frequency (PF) and relative abundance of infected seeds were calculated by the following formula:

Frequency of occurrence (%) = No. of seeds on which a fungal species occurs/Total No. of seeds × 100

Purification of cultures

Pure cultures were obtained after repeated sub-culturing of fungi appearing on seeds on Potato Dextrose Agar (PDA) plates.

Table 1. Wheat accessions collected from Gilgit and Azad Kashmir.

S/N	Accession	District	Town
a. Gilgit and Azad Kashmir			
01	011451	Rawalakot	Ghel
02	011484	Gilgit	Jutial
03	011489	Gilgit	Hunza
04	011602	Gilgit	Gitch
05	011620	Gilgit	Ghizer
06	011768	Gilgit	Austor
07	011765	Skardu	Sarri
08	012104	Muzaffarabad	Kahala
09	012285	Diamer	Jal village
10	011443	Bagh	Bagh
11	011480	Skardu	Hussainabad
12	011813	Gilgit	Juglote
13	011797	Chilas	Governer farm
14	012271	Chilas	Chilas
15	011487	Rawalakot	Ghel
16	011485	Rawalakot	Palandari
17	011766	Skardu	Keris
18	011588	Baltistan	Sordas
19	025912	Gilgit	Sheesh kot
20	011619	Gilgit	Tero
b. Khyber Pakhtunkhwa			
01	012012	Abbottabad	Tarhanagala
02	012021	Mansehra	College Durai
03	012040	Sawat	Char bagh
04	012035	Sawat	Bihar
05	012065	Dir	Shingerdar
06	012066	Malakand	Batkhaila
07	012069	Mardan	Rashakai
08	012072	Nowsehra	Pubbai
09	012208	Chitral	Nohedes
10	012268	Kohistan	Dalowndassu
11	018926	Lakkimarwat	Basit sultan abad
12	018925	Tank	Patthan colony
13	018921	D.I.khan	Hathala
14	018934	Bannu	Domel
15	018936	Karak	Jahangiribanda
16	011513	Para chinar	Zaran
17	012231	Haripur	Hawalian
18	012244	Buner	Guswanda
19	018786	Mansehra	Battal
20	018898	Chitral	Ayun
c. Balochistan			
01	011542	Quetta	Baleli
02	011548	Pinhin	Gawal
03	011771	Chagai	Ahmad wal
04	011781	Kalat	Rodingo
05	011752	Khuzdar	Surgaz
06	011759	Kechb	Asiaabadtump

Table 1. Contd.

07	012151	Loralai	Loralai
08	012144	Ziarat	Zandra
09	012155	Bolan	Kalpor
10	013189	Qilasaifullah	Bakacheena
11	011200	Pishin	Barozai
12	011185	Kharan	Karan
13	011232	Sibi	Sabi
14	011266	Qilaabdullah	Kili haji babari
15	011285	Pinhin	Pinhin
16	011535	Quetta	Marghat
17	011521	Awaran	Jibri
18	011758	Kech	Churbak
19	011757	Panjgur	AandayDaz
20	012180	Chagai	Noshki
d. Sindh			
01	011452	Hydderabad	Sebhat
02	011524	Nawabshah	Near river bank
03	011525	Dadu	Sherm.dawtch
04	011526	Thatta	Haji qasim
05	018730	Shikarpur	Haji khan wastil
06	018728	Larkana	Shahdadkot
07	018731	Jacobabad	Goth ghulammuhammad
08	018712	Ghotki	Ubaro/chimni
09	018716	Sukkar	Jahan khan
10	011378	Hyderabad	Mori
11	011383	NaushahroFiro	Haji esaboughi
12	011386	Sukkar	Nasirabad
13	018726	Larkana	Channagoth
14	018715	Ghotki	Panuaqil
15	018717	Larkana	Geaja
16	018736	Jacobabad	Bakhshapur
17	018719	Larkana	Mauta
18	018713	Ghotki	Mir purmathel
19	018733	Jacobabad	Goth ghulam Muhammad
20	018714	Ghotki	Ghotki
e. Punjab			
01	010715	Islamabad	-
02	010734	Islamabad	-
03	010759	Islamabad	-
04	010947	Faisalabad	-
05	010982	Faisalabad	-
06	011491	Okara	RenalaKhurad
07	011784	Attock	FatehJhang
08	011786	Chakwal	Dhoklalkahn
09	012096	Rawalpindi	M. khan
10	018702	Mainwali	Shahazkhel
11	018711	Khushab	Sakesar
12	018900	Jhelum	Suhan
13	018904	Sheikhupura	Kotlakahlune
14	018908	Layyah	Islamabad walipul

Table 1. Contd.

15	018910	D.G Khan	Haibatkot
16	011415	Bhakkar	-
17	018911	D.G Khan	Dao Mor
18	018969	Chakwal	Mureed Air Base
19	018948	Attock	Chakki
20	018949	Attock	Thatti S.I wala

Figure 1. Appearance of seeds associated fungi on wheat seeds.

Identification of fungi

The pure cultures of fungi were identified on the basis of spore morphology and colony characteristics were examined using a stereo-binocular microscope (Barnett and Hunter, 1992).

RESULTS

Identification of seed born fungi associated with wheat seeds

After incubation, fungi infection appeared on the wheat seeds as shown in Figure 1. Pure culture of seed born fungi is as shown in Figures 3, 4, 5, 6 and 7.

Frequency of fungi in wheat seeds

The frequency of each fungus in wheat seeds is presented in Figure 2: *Alternaria alternata* (49%), *Aspergillus niger* (46%), *Fusarium* species (42%) *Drechslera* species (35%), and *Phytophthora* species (16%).

Isolation of fungi from wheat seeds

In total, five fungi including both saprophytic as well as pathogenic were isolated from wheat seeds (Table 2). Fungi isolated from wheat seeds were *A. alternata*, *A. niger, Fusarium* spp., *Drechslera* spp., and *Phytopthra* spp. Infection percentage varied from 0 to 90% in all the accessions tested with accessions no. 011185 and 011757 showing the highest 90% infection, three accessions numbers 011487, 011200 and 011232 showed 70% infection, 012285 showed 60% infection, two accessions numbers 011381 and 011565 showed 50% infection. Six accessions numbers 010947, 011443, 011758, 012069, 012144, and 011542 showed infection of 40%. Seventeen accessions 012065, 011619, 012104, 012180, 018714, 011521, 018731, 011378, 011561, 011588, 011781, 011771, 011752, 011602, 012271, 012268, and 012072 showed infection of 30%. Eighteen accessions 012040, 018715, 011620, 011489, 018925, 010982, 011386,012151, 011285, 018898, 010715, 011766, 012021, 011524, 018900, 012066, 011765, and 012036 showed infection of 20%. Eighteen accessions 018726, 011451, 011383, 018936, 018719, 018926, 012208, 018904, 018908, 011232, 018728, 018910, 018948, 012012, 011520, 011797, 011525, and 011415 showed 10% infection.

Occurrence of fungal species in relation to geographical areas of Pakistan

Table 3 reveals the occurrence of fungal species in wheat seed accessions in relation to different geographical

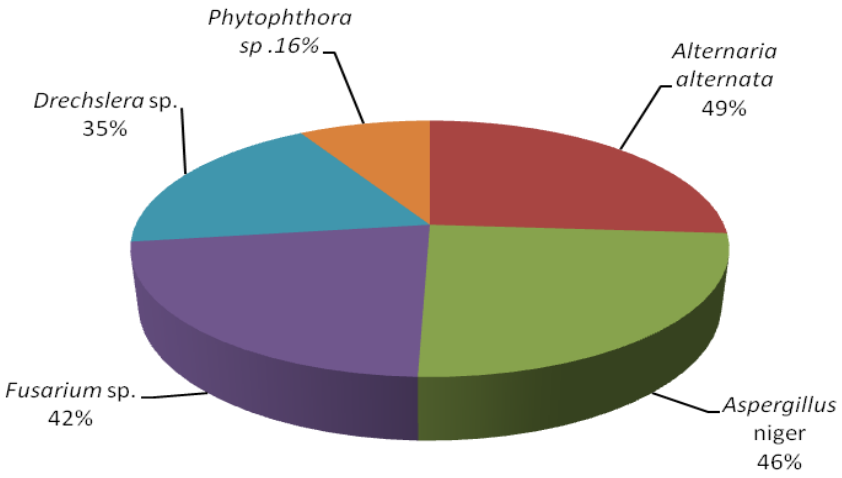

Figure 2. Frequency of occurrence of seed associated fungi isolated from wheat.

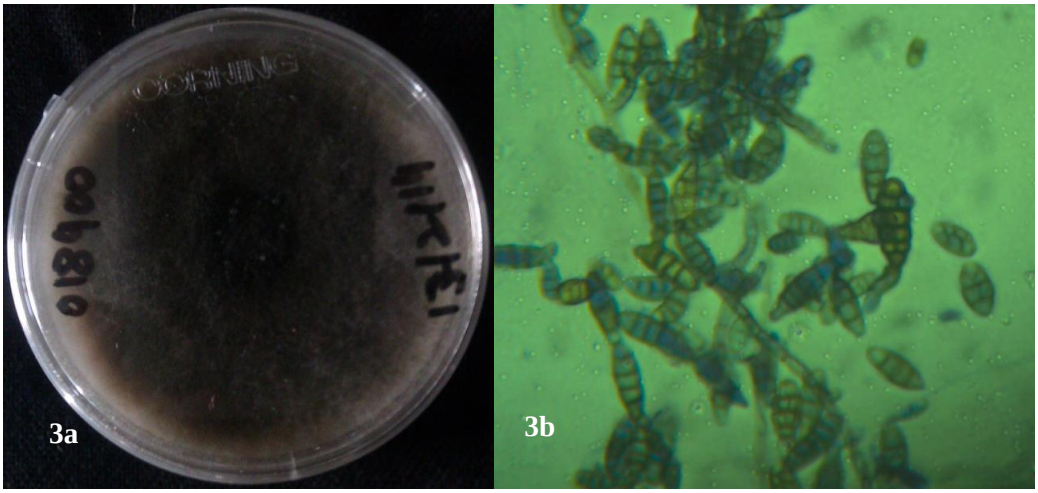

Figure 3. Morphological characteristics of *Alternaria alternate*: (a) Pure culture, (b) Microphotograph showing conidia at 40× magnification.

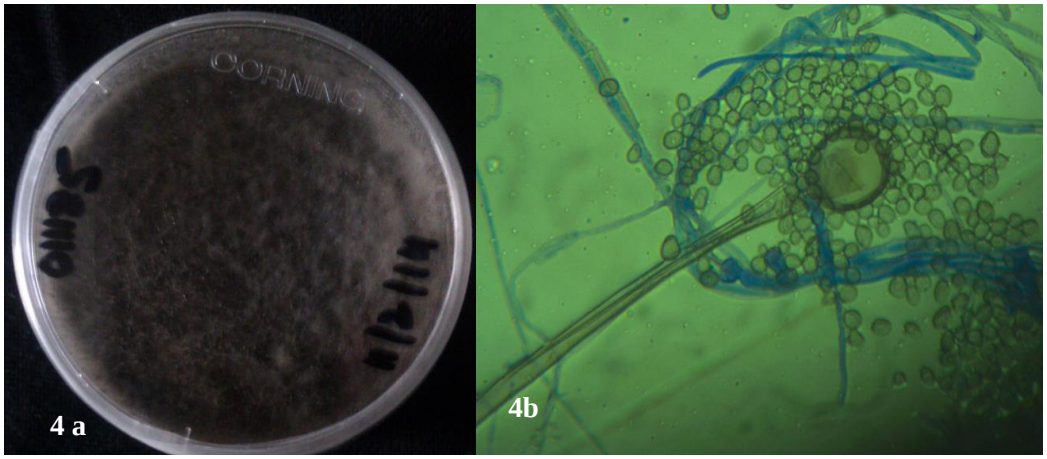

Figure 4. Morphological characteristics of *Aspergillus niger*: (A) Pure culture, (B) Microphotograph showing conidia at 40× magnification.

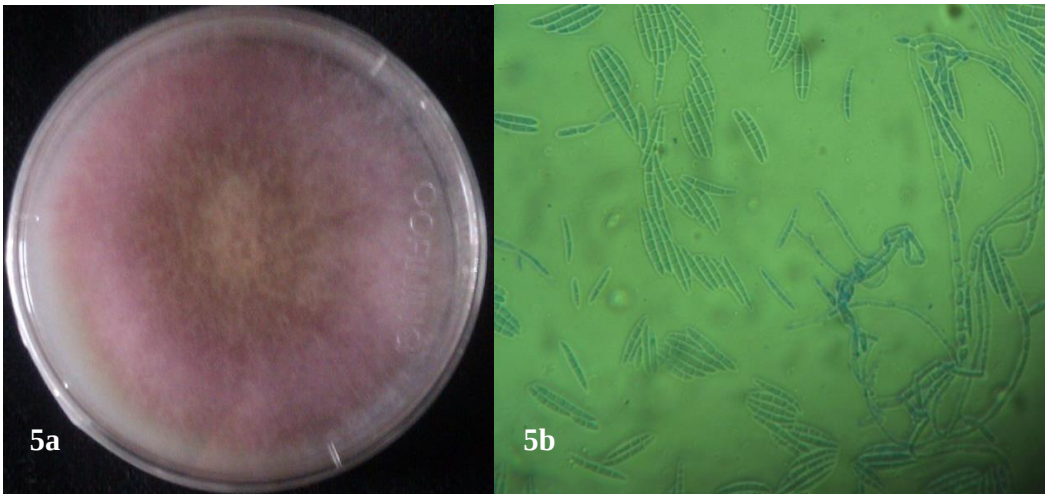

Figure 5. Morphological characteristics of *Fusarium* spp.: (a) Pure culture, (b) Microphotograph showing conidia at 40× magnification.

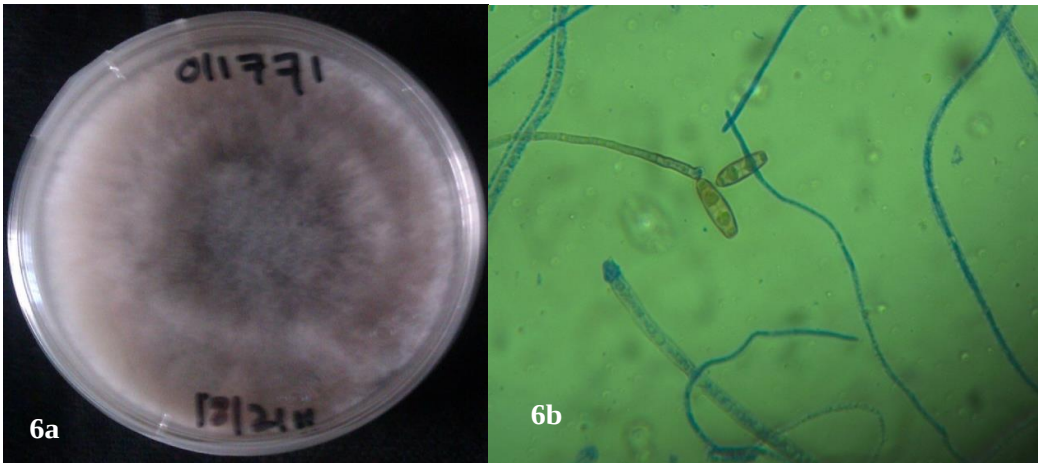

Figure 6. Morphological characteristics of *Drechlera* spp.: (a) Pure culture, (b) Microphotograph showing conidia at 40× magnification.

areas of Pakistan. In the case of Gilgit Baltistan and AJK, from a total amount of 200 tested seeds, 9 were found to be infected with *A. alternata,* 10 with *A. niger* and *Fusarium* spp., 9 with *Drechslera* spp., 5 with *Phytopthora* spp. whereas the remaining seeds were free from seed born fungi. In case of wheat accessions for kpk, out of 200 tested seeds, 7 seeds were found to be infected with *A. alternata,* 8 seeds were found to be infected with *A. niger*, 5 were found to be infected with *Fusarium* spp., 3 were found to be infected with *Drechslera* spp. and 2 were found to infected with *Phytopthora* spp. whereas the rest of the seeds were found to free from seed born fungi. In case wheat accession for Baluchistan province, out of total 200 tested seeds, 15 were found to be severely infected with *A. alternata*, 13 seeds were recorded to be infected with *A. niger,* 12 seeds were found to be infected with *Fusarium* spp., 14 seeds were found to be infected with *Drechslera* spp. and 6 seeds were infected with *Phytopthora* spp. In case of wheat accessions from seeds, out of total 200 tested seeds, 2 seeds were infected with *A. alternata*, 6 seeds were found to be infected with *A. niger,* 5 seeds were infected with *Fusarium* spp., 6 seeds were found to be infected with *Drechslera* spp. and no seeds were found to be infected with *Phytopthora* spp. whereas the rest of the seeds were free from seed born fungal infections. In case of wheat accession collected from Punjab province, out of 200 tested seeds, 3 seeds exhibited infection of *A. alternata,* 3 seeds were found to be infected with *A. niger,* 3 seeds

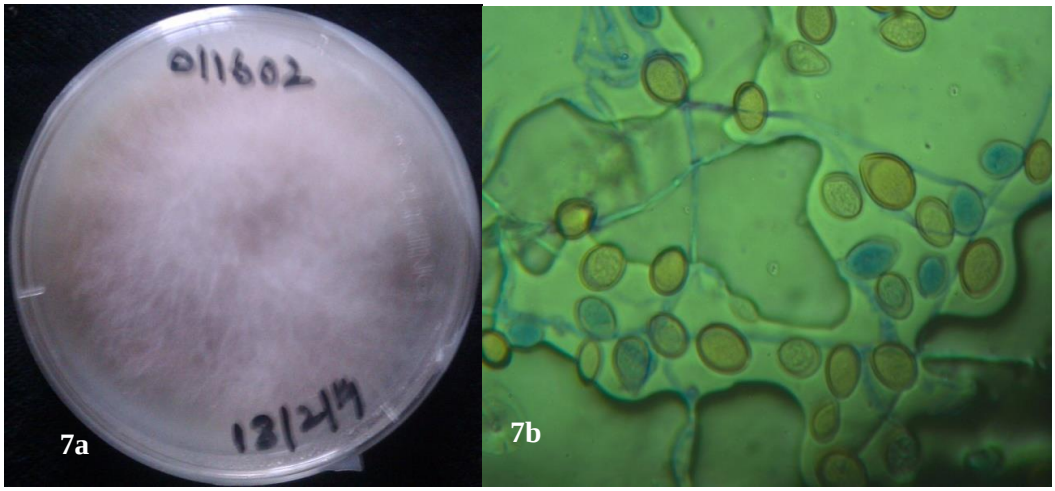

Figure 7. Morphological characteristics of *Phytopthora* spp.: (a) Pure culture, (b) Microphotograph showing conidia at 40X magnification.

Table 2. Frequency of occurrence of seed associated fungi from wheat.

S/N	Accession No.	*A. alternata*	*A. niger*	*Fusarium* spp.	*Drechslera* spp.	*Phypthera* spp.	Total	Frequency (%)
1	012096	-	-	-	-	-	-	-
2	010947	2	1	1	-	-	4	40
3	012040	1	1	-	-	-	2	20
4	011480	-	-	-	-	-	-	-
5	011487	-	2	3	1	1	7	70
6	012065	2	-	-	1	-	3	30
7	011443	1	1	2	-	-	4	40
8	018715	-	-	1	1	-	2	20
9	011185	2	2	3	1	1	9	90
10	011619	-	1	-	1	1	3	30
11	018786	-	-	-	-	-	-	-
12	011620	1	-	1	-	-	2	20
13	011489	-	1	-	-	1	2	20
14	018726	-	-	-	1	-	1	10
15	018713	-	-	-	-	-	-	-
16	018717	-	-	-	-	-	-	-
17	011451	-	1	-	-	-	1	10
18	010759	-	-	-	-	-	-	-
19	010734	1	-	-	-	-	-	-
20	011786	-	-	-	-	-	-	-
21	011758	1	-	2	1	-	4	40
22	018768	-	-	-	-	-	-	
23	012104	1	-	-	1	1	3	30
24	011383	-	1	-	-	-	1	10
25	011757	2	3	1	2	1	9	90
26	018730	-	-	-	-	-	-	-
27	012180	1	-	1	1	-	3	30
28	011200	2	1	1	2	1	7	70
29	018925	-	1	-	1	-	2	20
30	018736	-	-	-	-	-	-	-

Table 2. Contd.

31	012069	2	1	-	-	1	4	40
32	018714	-	1	1	1	-	3	30
33	018712	-	-	-	-	-	-	-
34	018936	-	-	1	-	-	1	10
35	011484	-	-	-	-	-	-	-
36	011521	-	1	1	-	1	3	30
37	010982	1	-	1	-	-	2	20
38	011381	1	2	1	-	1	5	50
39	018731	-	1	1	1	-	3	30
40	011759	-	-	-	-	-	-	-
41	011386	1	-	-	1	-	2	20
42	011378	-	2	-	1	-	3	30
43	011284	-	-	-	-	-	-	-
44	018719	-	-	1	-	-	1	10
45	018934	-	-	-	-	-	-	-
46	018926	1	-	-	-	-	1	10
47	018700	-	-	-	-	-	-	-
48	012151	-	1	-	1	-	2	20
49	011565	2	1	-	1	1	5	50
50	011561	1	-	2	1	-	3	30
51	018769	-	-	-	-	-	-	-
52	011588	-	1	1	1	-	3	30
53	011485	-	-	-	-	-	-	-
54	018921	-	-	-	-	-	-	-
55	011781	1	-	1	-	1	3	30
56	018733	-	-	-	-	-	-	-
57	011285	-	1	-	1	-	2	20
58	012144	2	-	1	-	1	4	40
59	018716	-	-	-	-	-	-	-
60	012155	-	-	-	-	-	-	-
61	011542	1	2	-	1	-	4	40
62	012208	-	-	1	-	-	1	10
63	011771	2	-	1	-	-	3	30
64	018728	-	-	-	-	-	-	-
65	011752	-	2	-	1	-	3	30
66	018969	-	-	-	-	-	-	-
67	018898	1	-	-	-	1	2	20
68	018904	-	1	-	-	-	1	10
69	011602	1	-	-	2	-	3	30
70	010715	-	1	-	1	-	2	20
71	018911	-	-	-	-	-	-	-
72	011491	-	-	-	-	-	-	-
73	018908	-	-	1	-	-	1	10
74	011784	-	-	-	-	-	-	-
75	011232	2	2	1	1	1	7	70
76	012244	-	1	-	-	-	1	10
77	018949	-	-	-	-	-	-	-
78	011766	1	-	1	-	-	2	20
79	012021	-	1	1	-	-	2	20
80	018728	-	-	-	1	-	1	10
81	011524	1	-	1	-	-	2	20
82	018910	-	-	-	1	-	1	10

Table 2. Contd.

83	012285	2	2	1	-	1	6	60
84	012271	1	1	-	1	-	3	30
85	018948	-	1	-	-	-	1	10
86	012268	2	-	1	-	-	3	30
87	018711	-	-	-	-	-	-	-
88	018900	1	-	1	-	-	2	20
89	012012	-	1	-	-	-	1	10
90	018702	-	-	-	-	-	-	-
91	011513	-	-	-	-	-	-	-
92	012072	1	1	-	1	-	3	30
93	012066	-	1	1	-	-	2	20
94	011520	-	-	-	1	-	1	10
95	011813	-	-	-	-	-	-	-
96	011797	-	-	1	-	-	1	10
97	011525	1	-	-	-	-	1	10
98	011415	-	1	-	-	-	1	10
99	011765	1	-	-	1	-	2	20
100	011768	-	-	-	-	-	-	-
101	012036	1	-	1	-	-	2	20
Total		49	46	42	35	16	-	-

Table 3. Occurrence of fungal species in relation to geographical areas of Pakistan.

S/N	Accession	District	*A. alternata*	*A. niger*	*Fusarium* spp.	*Drechslera* spp.	*Phytopthora* spp.
Gilgit Baltistan and AJK							
01	011451	Rawalakot	-	1	-	-	-
02	011484	Gilgit	-	-	-	-	-
03	011489	Gilgit	-	1	-	-	-
04	011602	Gilgit	1	-	-	2	-
05	011620	Gilgit	1	-	1	-	-
06	011768	Gilgit	-	-	-	-	-
07	011765	Skardu	1	-	-	1	-
08	012104	Muzaffarabad	1	-	-	1	1
09	012285	Diamer	2	2	1	-	1
10	011443	Bagh	1	1	2	1	1
11	011480	Skardu	-	-	-	-	-
12	011813	Gilgit	-	-	-	-	-
13	011797	Chilas	-	-	1	-	-
14	012271	Chilas	1	1	-	1	-
15	011487	Rawalakot	-	2	3	1	1
16	011485	Rawalakot	-	-	-	-	-
17	011766	Skardu	1	-	1	-	-
18	011588	Baltistan	-	1	1	1	-
19	025912	Gilgit					
20	011619	Gilgit	-	1	-	1	1
Total tested seeds = 200			9	10	10	9	5
Khyber Pakhtunkhwa							
01	012012	Abbottabad	-	1	-	-	-

Table 3. Contd.

02	012021	Mansehra	-	1	1	-	-
03	012040	Sawat	1	1	-	-	-
04	012035	Sawat	-	-	-	-	-
05	012065	Dir	1	-	-	1	-
06	012066	Malakand	-	1	1	-	-
07	012069	Mardan	1	1	-	-	1
08	012072	Nowsehra	1	1	-	1	-
09	012208	Chitral	-	-	1	-	-
10	012268	Kohistan	1	-	1	-	-
11	018926	Lakkimarwat	1	-	-	-	-
12	018925	Tank	-	1	-	1	-
13	018921	D.I.khan	-	-	-	-	-
14	018934	Bannu	-	-	-	-	-
15	018936	Karak	-	-	1	-	-
16	011513	Para chinar	-	-	-	-	-
17	012231	Haripur	-	-	-	-	-
18	012244	Buner	-	1	-	-	-
19	018786	Mansehra	-	-	-	-	-
20	018898	Chitral	1	-	-	-	1
	Total tested seeds=200		7	8	5	3	2
Balochistan							
01	011542	Quetta	1	1	-	1	1
02	011548	Pinhin	-	-	-	-	-
03	011771	Chagai	2	-	1	-	-
04	011781	Kalat	1	-	1	1	-
05	011752	Khuzdar	-	1	-	1	-
06	011759	Kechb	-	-	-	-	-
07	012151	Loralai	-	1	-	1	-
08	012144	Ziarat	1	-	1	1	-
09	012155	Bolan	-	-	-	-	-
10	013189	Qilasaifullah	-	-	-	-	-
11	011200	Pishin	2	1	1	2	1
12	011185	Kharan	2	2	3	1	1
13	011232	Sibi	2	2	1	1	1
14	011266	Qilaabdullah	-	-	-	-	-
15	011285	Pinhin	-	1	-	1	-
16	011535	Quetta	-	-	-	-	-
17	011521	Awaran	-	1	1	-	1
18	011758	Kech	1	-	1	1	-
19	011757	Panjgur	2	3	1	2	1
20	012180	Chagai	1	-	1	1	-
	Total tested seeds= 200		15	13	12	14	6
Sindh							
01	011452	Hydderabad	-	-	-	-	-
02	011524	Nawabshah	-	1	1	-	-
03	011525	Dadu	1	-	-	-	-
04	011526	Thatta	-	-	-	-	-
05	018730	Shikarpur	-	-	-	-	-
06	018728	Larkana	-	-	-	-	-
07	018731	Jacobabad	-	1	1	1	-

Table 3. Contd.

08	018712	Ghotki	-	-	-	-	-
09	018716	Sukkar	-	-	-	-	-
10	011378	Hyderabad	-	2	-	1	-
11	011383	NaushahroFiro	-	1	-	-	-
12	011386	Sukkar	1	-	-	1	-
13	018726	Larkana	-	-	-	1	-
14	018715	Ghotki	-	-	1	1	-
15	018717	Larkana	-	-	-	-	-
16	018736	Jacobabad	-	-	-	-	-
17	018719	Larkana	-	-	1	-	-
18	018713	Ghotki	-	-	-	-	-
19	018733	Jacobabad	-	-	-	-	-
20	018714	Ghotki	-	1	1	1	-
	Total tested seeds= 200		2	6	5	6	0
Punjab							
01	010715	Islamabad	-	1	-	1	-
02	010734	Islamabad	-	-	-	-	-
03	010759	Islamabad	-	-	-	-	-
04	010947	Faisalabad	-	-	-	-	-
05	010982	Faisalabad	1	-	1	-	-
06	011491	Okara	-	-	-	-	-
07	011784	Attock	-	-	-	-	-
08	011786	Chakwal	-	-	-	-	-
09	012096	Rawalpindi	-	-	-	-	-
10	018702	Mainwali	-	-	-	-	-
11	018711	Khushab	-	-	-	-	-
12	018900	Jhelum	1	-	1	-	-
13	018904	Sheikhupura	1	-	-	1	1
14	018908	Layyah	-	-	1	-	-
15	018910	D.G Khan	-	-	-	1	-
16	011415	Bhakkar	-	1	-	-	-
17	018911	D.G Khan	-	-	-	-	-
18	018969	Chakwal	-	-	-	-	-
19	018948	Attock	-	1	-	-	-
20	018949	Attock	-	-	-	-	-
Total tested seeds = 200			**3**	**3**	**3**	**3**	**1**

were found to be infected with *Fusarium* spp., 3 were infected with *Drechslera* spp. and 1 was found to be infected with *Phytophthora.*

Identification of fungi

The isolated fungi were identified on the basis of spore morphology and colony characteristics. Some features on the basis of which fungi were identified are as follows:

A. alternata

The fungus *A. alternata* was identified as it produced woolly or powdery chains of dark brown conidia of uneven shapes and lengths. The colony colour was dark brown (Figure 3a). The mycelium was abundant and variable in colour, usually light olive green to dark brown. Hyphae were thick, septate, dark brown and branched, conodiophores were erect and simple with septate conidia (Figure 3b).

A. niger (van Tieghem)

Colony of *A. niger* on seed grew slowly, consisting of a compact to fairly loose white to faintly yellow basal mycelium, which bears abundant erect and usually

crowded conidial structures (Figure 4a). Conidiophores arise directly from the seeds coat and are smooth, hyaline or faintly brownish near the apex (Figure 4b).

Fusarium spp. *(Sacc)*

Fusarium spp. had a rapid growth on Potato dextrose agar PDA. The texture of colony was flat to wooly and pink in colour (Figure 5a). Conidia were 2 or more celled, curved, thick-walled, smooth, and canoe-shaped (Figure 5b)

Drechslera halodes (Ito) and Phytopthora spp.

Colony on PDA was dark brown (Figure 6a). Conidiophores were thick, septate, cylindrical, and paler toward the apex and were simple. Conidia were straight and slightly curved and thick walled (Figure 6b) and *Phytopthora* (Figure 7a and b).

DISCUSSION

Seed borne fungal pathogens transmit most of the major disease of wheat crop and reduce seed quality, nutrient contents, germination capacity as well as seedling collapse, which consequently reduce crop yield (Mushtaq and Hashmi, 2005). Over the last two decades, various studies have been carried out to identify seed born fungal pathogen of wheat crop throughout the world. For example in Canada, 35 fungal genera and 59 seed born fungi exist in association with wheat seeds. From Pakistan, Khan (1992) reported 17 genera and 45 species of seed born fungal pathogens associated with wheat seeds. In this study, the results show that a total of 5 major fungal pathogen including *A. alternate*, *A. niger*, *Fusarium* spp., *Drechslera* spp. and *Phytophthora* spp. were identified and isolated from the seeds of wheat crop. Zare et al. (2006) who determined the fungi species and infection rates as 15% *Fusarium culmorum,* 13.1% *Fusarium graminearum,* 4.5% *Drechslera* spp*.,* 24.2% *A. alternate* and 5% *A. niger* in harvested wheat loads in different provinces of Iran. In the present study, the lowest infection rate of seed to *Pytophthora* was determined in seed accession collected from Sindh province and the highest infection rate was reported from Baluchistan province. The results also show that infection percentage of five major seed borne pathogens varied from 0 to 90% in all accessions collected from different geographical areas of Pakistan. Moreover, the frequencies of five mentioned major fungus were higher in wheat seed accessions collected from Baluchistan and Gilgit Baltistan and AJK as compared to other geographical regions of Pakistan. It was found that among the seed born fungi frequency, *A. alternata* in all accessions was the highest as compared to other fungi associated with wheat seeds. Rajput et al. (2005) tested on hundred and twenty sample of wheat seeds for the presence of fungal seed borne pathogens collected from wheat growing of Sindh. From twelve wheat varieties five seed borne fungi that are *A. niger, Alternaria tenuis, Fusarium moniliformie, Stemphylium herbarum* and *Curvuluria lunata* were isolated. Same experiment is performed by Babadost (1997) who detected some species of *Fusarium* fungus in wheat seeds collected from cereal fields in the North West of Iran. In the results, *A. alternata* was the most frequent fungi associated with the wheat seeds. The presence of weakly pathogenic or saprophytic fungi such as *Helminthosporium, Curvularia, Stemphylium, Rhizopus, Cladosporium, Aspergillus, Penicillium, Alternaria, Gonatobotrys* and *Nigrospora* has been reported from wheat seeds (Habib et al., 2011). Saberi et al. (2004) also isolated seed-borne fungi (*Aspergillus* species, *Alternaria* spp., *Cladosporium* species, *Penicillium* species and *Ulocladium* spp.) associated with wheat grains in Markazi province. Hussain et al. (2013) reported *Bipolaris sorokiniana* (11.125%), *Aspergillus flavus* (9.825%), *A. alternata* (7.15%) and *A. niger* (6.225%) associated with wheat seeds. It is apparent from the present research that all the accessions of wheat crop tested were contaminated by fungi. Knowing the major contribution of wheat crop in world food, its production must be enhanced to meet the nutritional requirements of a rising human populations (Oerke and Dehne, 2004). Certified and healthy seeds of wheat crop are significant input for crop production and consequently reduction of yield loss caused by these seed born fungi and is a main way to contribute to the food security in the world. Moreover, seed born fungi can be easily controlled through treatment of seeds using fungicides and biological compounds. Further, using standard storage facilities for preserving wheat seeds and developing resistant germplasm to reduce the infection level caused by these seed-borne fungi below damage threshold have been recommended (Clark et al., 2004).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahmad J, Marwat MI, Ahmad HK (2003). Effect of herbicides and row spacing on different traits of wheat (*Triticum aestivum* L.). Pakistan J. Weed Sci. Res. 9(1-2):33-40.

Ali S, Shumaila U, Zahida N, Naseem Z, Saima N, Ammara Y, Tehseen Y (2013). Nutritional evaluation and stabilization studies of wheat germaplasm. Pak. J. Food sci. 23:148-152.

Ansari MA, Meman HR, Tunio SD, Keerio SA (2006). Effect of planting pattern on growth and yield of wheat. Pak. J. Agric. Agril. Eng. Vet. Sci. 22(2):22-24.

Babadost M (1997). Species of Fusarium in seeds and wheat plants in West Azarbayjan and Ardabil. Iran. J. Plant Pathol. 31:88-100.

Bishaw Z, Struik PC, Van Gastel AJG (2013). Farmer's seed sources

and seed quality: 2. Seed health. Int. J. Plant Prod. 7:637-657.
Chaudhary MA, Ali A, Siddique MA, Sohail R (2000). Growth and yield response of wheat to different seed rates and wild oat (*Avena fatua*) competition durations. Pak. J. Agric. Sci. 37:152-154.
Clark B, Cockerell V, Thomas J (2004). Wheat seed health and seedborne diseases-A guide. HGCA, Caledonia House, London.
Clear RM, Patrick SK (1993). Prevalence of some seedborne fungi on soft white winter wheat seed from Ontario, Canada. Can. Plant Dis. Surv. 73:143-149.
Dawson, WAJM, Bateman GI (2001). Fungal communities on roots of wheat and barley and effect on seed treatment containing fluquiconazol applied to control take-all. Plant Pathol. 50:5-82.
Habib A, Sahi ST, Javed N, Ahmad S (2011). Prevalence of seed-borne fungi on wheat during storage and its impact on seed germination. Pak. J. Phytopathol. 23(1):42-47.
Hussain M, Muhammad UG, Muhammad IH, Raza M (2013). Seed borne mycoflora of some commercial wheat cultivars in Punjab, Pakistan. Int. J. Pathol. 2:2.
Iftikhar, S, Ahmad I, Aslam M (1991). Seed born diseases of wheat in Pakistan and their control. Pak. Agric. Res. Council Islamabad 11(6):14-18.
Khan SAJ (1992). Studies on fungi causing seed-borne diseases of wheat and rice and their control. PhD dissertation, University of Karachi, Karachi, Pakistan. Ph.D. Thesis, Department of Botany/ University of Karachi, Pakistan 261pp.
Kumar A, Singh US, Kumar J, Garg GK (2008). Application of molecular and immune diagnostic tools for detection, surveillance and quarantine regulation of Karnal bunt (*Tilletia indica*) of wheat. Food Agric. Immunol. 19:293-311.
Marwat MI, Ahmad HK, Khan HH, Khan A (2002). Integrated weed management in wheat. 1. Weed density, dry weed biomass, absolute growth rate and grain yield. J. Weed Sci. Res. 8(1-2):81-93.
Mushtaq SNM, Hashmi MH (2005). Seed-borne mycoflora of Sunflower (*Helianthus annuus* L.). Pak. J. Bot. 37(2):451-457.
Oerke EC, Dehne HW (2004). Safeguarding production-losses in major crops and the role of crop protection. Crop Prot. 23:275-285.
PBS (2013). Pakistan Bureau of statistics, Ministry of National Food Security and Research (Economic survey of Pakistan, report).
Rajput MA, Pathan MA, Lodhi AM, Shah GS, Khanzada KA (2005). Studies on seed-borne fungi of wheat in sindh province and their effect on seed germination. Pak. J. Bot. 37(1):181-185.
Saberi R, Javan-Nikkhah M, Heidarian R, Hosseni S, Soleimani P (2004). Detection of fungal infectious agent of wheat grains in storepits of Markazi province. Iran. Agric. Appl. Biol. Sci. 67:418-423.
Schuster I, Vieira ESN, da Silva GC, de Assis Franco F, Marchioro VS (2009). Genetic variability in Brazilian wheat cultivars assessed by microsatellite markers. Genet. Mol. Biol. 32(3):557-563.
Zare L, Harsini M, Hosseini-bai S, Motieshare B (2006). Contamination of seed harvested wheat loads to a number of seed-borne fungal pathogen. In Proceeding 17th Iranian Plant Protection Congress. P 24.