Mycopath (2019) 17(2): 59-62 **Research Article**

Isolation and identification of seed associated fungi of wheat

[*]Nadeem Shad[1], Rehman Ejaz[1], Atif Jamal[2], Iqra Haider Khan[1] and Arshad Javaid[1]

[1]Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan
[2]National Agricultural Research Council, Islamabad, Pakistan
[*]Corresponding author's email: nadeem.iags@pu.edu.pk

Abstract

This study reports the occurrence of fungi associated with 11 accessions of wheat procured from NARC, Islamabad, Pakistan. Eight to twenty eight percent seeds were found affected by these fungi. The highest fungal association was recorded in accessions 11238 and 11241 (28% each) followed by 11242 (24%), 11239 and 11246 (16% each), 11240, 11243 and 11247 (12% each), and 11245 (8%). Fungi associated with these seed(s) included *Aspergillus flavus, A. niger, Penicillium* sp., *Bipolaris* sp., *Curvularia* sp. and *Fusarium oxysporum. Penicillium* sp. was found the most abundantly occurring species with 24% occurrence followed by *Bipolaris* sp. (20%), *Curvularia* sp. (18%), *A. niger* (16%) and *F. oxysporum* (9%).

Keywords: Mycoflora, Seeds, Storage fungi, Wheat accessions.

Introduction

Wheat (*Triticum aestivum* L.) is consumed as a staple food for more than one third of the world's population. Globally, its cultivation is concentrated in China, Canada, European Union, Australia, Russia, Pakistan, India, United States, Turkey and Ukraine accounting for over 80% of the total production (Senapati and Semenov, 2019). In Pakistan, numerous wheat varieties are sown based on diverse agro-climatic conditions in the country. Pakistan is the 8th largest wheat producing country in the world, having a share of 3.27% in the world's wheat production from an area spanning over 8958.4 thousand hectares in 2018-2019 (Agricultural Statistics of Pakistan, 2019). It is considered as an excellent health building food, rich in proteins 9.2–11.3%, dietary fibers 1.8–1.9%, B-group vitamins, moisture content 8.4–9.8%, and a blend of minerals like Zn, Cu, Fe, P and Mg (Johansson *et al*., 2020). It is consumed in the form of confectionary products, bread, biscuits, noodles, ethanol, cosmetics, and seitan or vital wheat gluten (Cappelli *et al.*, 2020).

Various seed characteristics such as germination, nutritional value, moisture content and discoloration are influenced by a number of biotic and abiotic factors during seed storage. The stored seeds are more prone to be attacked by the fungi making them unacceptable as food and feed (Chattha *et al*., 2016). Approximately 15 to 18 species of *Penicillium, Aspergillus, Alternaria, Bipolaris, Curvularia* and *Fusarium* have been reported as important contaminants of wheat grains (Sadhasivam *et al*., 2017). These fungi invade the seeds after their harvest or under field conditions and remain alive for years (Ulziijargal *et al*., 2019). Hot and humid climatic conditions favor the rapid spread of fungal growth that adversely affects the grain quality and also cause reduction in germination (Fleurat-Lessard, 2017). In the present study, 11 accessions of wheat were studied to identify the associated seed-borne mycoflora.

Materials and Methods

Seeds of 10 wheat accessions were obtained from gene bank (PGRI) at NARC (National Agricultural Research Centre), Islamabad. For isolation of seed-associated fungi, filter papers were placed at the base of sterilized Petri plates (90 mm) and moistened with 25 mL of distilled water. In each Petri plate, 25 wheat seeds were placed and incubated at room temperature. Each treatment was replicated thrice. The seeds were subjected to regular visual observations daily and examined under stereoscopic microscope to find any appearance of fungi. Seeds showing any distinct signs of fungal growth were transferred to the PDA (potato dextrose agar) plates after 3 days of incubation. Para film was raped around the plates, and placed them in the incubator for three days at 27 ºC. After 24–72 hours of incubation, these seeds had some fungal growth around them. The fungi along with media plug were transferred to the newly prepared PDA media plates for purification purpose. After 7 days, pure fungal colonies were grown in the Petri plates. From these purified fungal cultures, slides were prepared and characteristics of fungi were observed under the microscope. Fungi were identified with the help of relevant literature (Domesch *et al*., 1980). Percentage of fungal infection was calculated by counting the infected seeds in comparison to the total number of incubated seeds by using the following formula:

$$\text{Fungal infection} \quad (\%) = \frac{\text{No. of infected seeds}}{\text{Total no. of seeds}} \times 100$$

Results and Discussion

There was a variation in fungal infection in different accessions of wheat used in the present

study that ranged from 8–29%. The highest fungal association was recorded in accessions 11238 and 11241 that was 28% in each case. Likewise, accession 11242 also showed a comparatively high percentage of infection *i.e.* 24%. In other accessions *viz.* 11239 and 11246, infection was 16%, in 11240, 11243 and 11247, it was 12%; while it was only 8% in accession 11245 (Fig. 1). Variation in fungal infection among the wheat varieties has also been reported in many earlier studies. Rajput *et al*. (2005) collected 120 seed samples of 12 wheat varieties from Sindh, Pakistan and checked their seed-associated mycoflora. The highest fungal infection was observed on seeds of var. Pak-70 followed by Mehran-89 and Soghat. Conversely, wheat var. Anmol showed the lowest fungal infection followed by varieties Sarsabz, H-68 and Sindh-81, respectively.

Six fungal species were found associated with different accessions of wheat. Percentage occurrence of these fungi was highly variable. *Penicillium* sp. was the most abundant with 24% frequency of occurrence. *Bipolaris* sp. was the second most frequent fungal species with 20% frequency of occurrence. Other species namely *Curvularia* sp., *A. niger, A. flavus* and *F. oxysporum* were found with a frequency of 18%, 16%, 13% and 9%, respectively. *Bipolaris* sp. was isolated from 7 accessions. *Curvularia, Penicillium, A. flavus, A. niger* and *F. oxysporum* were found associated with 6, 6, 5, 5, and 4 wheat accessions (Table 1, Fig. 2). Islam *et al*. (2015) collected seed samples of wheat from different villages of Bangladesh and identified various fungal species which were very similar to those identified in the present study. They identified *Bipolaris sorokiniana, Curvularia lunata, Ahernaria tenuis, Fusurium* spp., *A. flavus* and *A. niger* as the frequently occurring species in association with seeds of wheat. Likewise, Hajihasani *et al*. (2012) collected 53 wheat seed samples of three wheat varieties namely Backcross C-78-14, Alvand and Roshan from different areas of Markazi province, Iran and reported various seed-borne fungi including *Alternaria alternata, A. niger, A. candidus, A. flavus, Bipolaris sorokiniana, Curvularia* sp., *Tilletia laevis, T. tritici, Fusarium graminearum, F. culmorum, Microdochium nivale*, *Penicillium* sp., *Mucor* sp., *Rhizopus* sp. and *Ustilago tritici*. Previously, Rajput *et al*. (2005) identified 5 fungal species from seeds of different wheat varieties collected from Sindh. These included *A. niger, Alternaria tenuis, Curvuluria lunata, Stemphylium herhurum* and *Fusarium moniliforme* ranging in their frequency from 1–48%. *A. tenuis* was the most abundant (22.5-47.5%) fungal species followed by *A. niger* (3.5-15 %). Other researchers have reported presence of species of *Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Helminthosporium, Penicillium, Rhizopus* and *Stemphylium* in wheat seeds (Khan *et al*., 1974; Bhutta and Hussain, 1999). Most of the workers have reported association of *Aspergillus* and *Penicillium* with the stored seeds of wheat (Ghosh and Nandi, 1986; Kunwar, 1989).

Conclusion

Penicillium sp. was found the most abundantly occurring species followed by *Bipolaris* sp., *Curvularia* sp., *A. niger* and *F. oxysporum.*

Table 1: Frequency of occurrence of seed-associated fungi isolated from wheat.

Sr. No.	Wheat Accession No.	No. of infected seeds						
		AF	**AN**	**P**	**B**	**C**	**FO**	**Total**
1	11238	2	1	2	1	1	-	7
2	11239	1	-	3	-	-	-	4
3	11240	-	-	1	-	1	1	3
4	11241	1	2	-	2	1	1	7
5	11242	1	2	-	2	-	1	6
6	11243	-	1	-	1	-	1	3
7	11244	-	1	2	1	2	-	6
8	11245	-	-	1	1	-	-	2
9	11246	1	-	2	-	1	-	3
10	11247	-	-	-	1	2	-	3
	Total	6	7	11	9	8	4	

AF: *Aspergillus flavus,* **AN:** *A. niger,* **P:** *Penicillium* sp., **B:** *Bipolaris* sp., **C;** *Curvularia* sp., **FO:** *Fusarium oxysporum.*

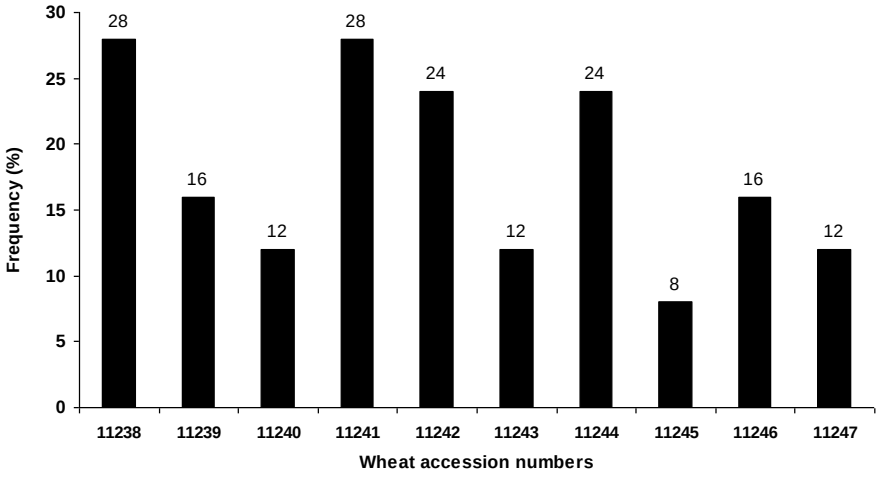

Fig. 1: Frequency of occurrence of fungi associated with seeds of different accessions of wheat.

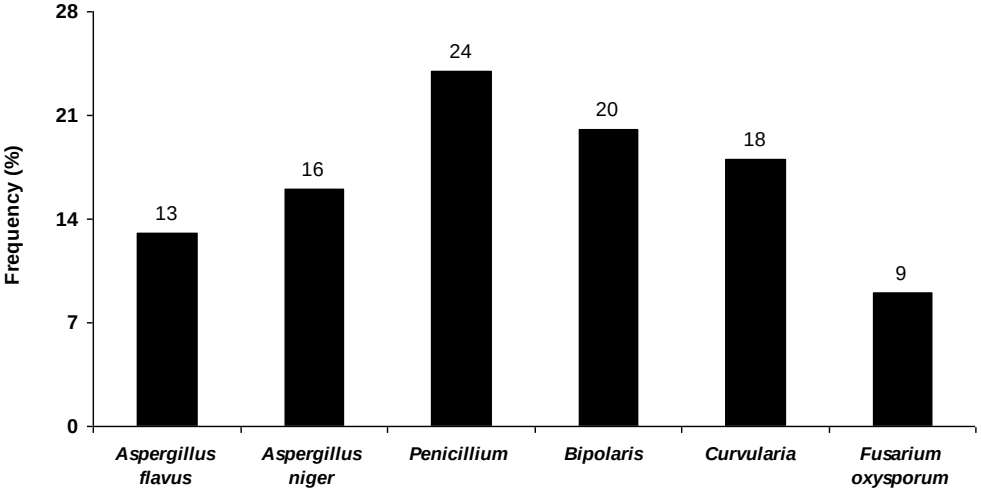

Fig. 2: Comparative occurrence of different fungal species associated with wheat seeds.

References

Agricultural Statistics of Pakistan (2019). Government of Pakistan, Ministry of Food and Agriculture (Economic Wing) Islamabad.

Bhutta AR, Hussain SA, 1999. Seed-borne pathogens of wheat in Pakistan. *Rachis*, **18**: 66-68.

Cappelli A, Oliva N, Cini E, 2020. Stone milling versus roller milling: a systematic review of the effects on wheat flour quality, dough rheology, and bread characteristics. *Trends Food Sci. Tech*., **97**: 147-155.

Chattha SH, Hasfalina CM, Mirani BN, Mahadi MR, Lee TS, 2016. Food grain losses associated with indigenous storage methods and development of storage facilities for food security. *Int. Food Res. J*., **23**: 57-63.

Domesch KU, Gams W, Traute-Heidi A, 1980. *Compendium of Soil Fungi*. Volume 1, Academic Press London.

Fleurat-Lessard F, 2017. Integrated management of the risks of stored grain spoilage by seedborne fungi and contamination by storage mould mycotoxins–an update. *J. Stored Prod. Res*., **71**: 22-40.

Ghosh J, Nandi B, 1986. Deteriorative abilities of some common storage fungi of wheat. *Seed Sci. Technol*., **14**: 141-149.

Hajihasani M, Hajihassani A, Khaghani S, 2012. Incidence and distribution of seed-borne fungi associated with wheat in Markazi Province, Iran. *Afr. J. Biotechnol*., **11**: 6290-6295.

Islam MS, Sarker MNI, Ali MA, 2015. Effect of seed borne fungi on germinating wheat seed and their treatment with chemicals. *Int. J. Nat. Social Sci*., **2**: 28-32.

Johansson E, Branlard G, Cuniberti M, Flagella Z, Husken A, Nurit E, Peña RJ, Sissons M, Vazquez D, 2020. Genotypic and

environmental effects on wheat technological and nutritional quality. In: *Wheat Quality For Improving Processing and Human Health*. Springer, Cham. pp. 171-204

Khan SA, Mathur SB, Neergaard P, 1974. Survey on new seed organisms of Pakistan. *Seed Sci. Technol*., **2**: 477-479.

Kunwar IK, 1989. Mycoflora associated with stored wheat and its milling fractions in India. *Plant Sci*., **99**: 437-443.

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**: 181-185.

Sadhasivam S, Britzi M, Zakin V, Kostyukovsky M, Trostanetsky A, Quinn E, Sionov E, 2017. Rapid detection and identification of mycotoxigenic fungi and mycotoxins in stored wheat grain. *Toxins*, **9**: 302-319.

Senapati N, Semenov MA, 2019. Assessing yield gap in high productive countries by designing wheat ideotypes. *Sci. Rep*., **9**: 1-12.

Ulziijargal E, Gorgo YP, Skorochod IO, 2019. Detection of seedborne mycoflora in wheat. *Int. J. Innov. Sci. Res. Technol*., **4**: 532-536.