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Fungi associated with wheat seed discolouration and abnormalities in *in-vitro* study

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ABSTRACT

The main aim of present study is to ascertain the fungal species and their effect on germination associated with wheat seeds. Seeds of three varieties WH896, PBW-373 and HD264 of wheat (*Triticum aestivum*) were collected from Quarsi Agriculture Farm Aligarh. These three seed samples of wheat showing different forms of discouloration and abnormalities were screened for associated fungi. Microscopic examination of wheat seeds reveals that seeds of all the varieties of wheat possess injuries to varying extent. Detailed examination of the seeds has shown that the seeds can be classified on the basis of extent of injury in the three categories viz., seeds having minor cracks, cracks without exposed embryo and cracks with exposed embryo. Seed soaking and washing techniques were also employed. *Fusarium moniliforme* and *Alternaria alternata* were isolated from all the categories of seed tested. Floating mycelial bits and conidia of *Alternaria*, *Fusarium*, *Drechslera*, *Curvularia lunata*, *Mucor* were found in all the three varieties of wheat. The seeds were subjected to visual observation and examination under stereoscopic microscope. These findings are to study and detect the phytopathogenic mycoflora which causes damage and loss to our seeds and crops.

Keywords: Wheat; Seed-Borne Fungi; Incidence; Discolouration; Wrinkled Seeds

1. INTRODUCTION

Wheat (*Triticum aestivum* L) is an important cereal crop belonging to Gramineae family .The production rates of these crops have failed to keep pace with requirements of the growing population [1]. During recent studies, it has been reported that 100 g of edible portion of wheat grains contain 11.50% proteins, 59.40% carbohydrates, 9.70% fats, 10.60% crude fibers, and 1.80% ash [2]. Along with some abiotic limitations, this protein and starch rich cereal crop is also highly susceptible to various pests and diseases and a number of storage grain molds cause considerable losses [3]. Quality characters of wheat seeds, such as seed germination, moisture content, seed discolouration and seed-borne fungal prevalence have long been known to be influenced by various factors. The major mycotoxigenic fungi in rice are *Aspergillus* spp. [4], *Fusarium* spp. [5], and *Penicillium* spp [6] in cereal. The type and severity of seed abnormality may be dependent on the type and pathogenic potential of the associated fungi as well as the prevailing weather conditions [7]. The harmful effects of such fungal invasion are glume or grain discolouration. Other abnormalities such as deformation and damage in seeds are a major constraint in crop production in most of the developing countries. Seed abnormality due to the influence of seed-borne fungi is very common and often accounts for a large percentage of crop losses [8]. Seeds that showed distinct symptoms were selected for study and categorized in to three groups viz discoloured seeds, wrinkled seeds, and seeds with discoloured embryo. Seed abnormality could be in the form of shrunken seeds, seed necrosis, seed rot, reduction in seed size or total seed discolouration [9]. Discoloured seeds were further subgrouped, to isolate, identify and obtain information about the incidence of various seed-borne fungi associated with different types of discolouration and abnormalities of seeds related to crop wheat in Aligarh district.

2. MATERIAL AND METHODS

2.1. Dry Inspection of Seeds

Seeds of three varieties of wheat viz WH896, PBW-

373 and HD264 were obtained from the Quarsi farm, Aligarh. The seeds were subjected to visual observation and examination under stereoscopic microscope. Seeds that showed distinct symptoms and abnormalities were selected and categorized in to different groups viz, discoloured seeds, wrinkled seeds and seeds with discoloured embryo and blackened seeds. 100 seeds per cultivar were examined under four replicate.

2.2. Plating and Incubation of Seeds

Infected seeds of each cultivar were surface sterilized in 2% $HgCl_2$ for 15 min and then rinsed for 2 min each in three changes of sterile distilled water prior to plating. Ten seeds were plated in each petri dish containing 10 ml of potato dextrose agar (PDA) medium and moist blotter papers. For each of the categories and subgroupings, a total of 400 seed were plated in four replicates of 100 seeds per variety. These petri dishes were incubated at 22˚C ± 2˚C under alternating cycles of 12 h light and 12 h darkness. On the 8th day, incubated seeds were observed for fungal growth and identification under stereo binocular and compound microscopes. Identification was based on presence of physical characteristics of the structures such as conidia and hypha [10-12]. The number of seeds infected with each kind of fungus was counted and more than one fungal growth on the same seeds, was regarded as multiple infection.

2.3. Statistical Analysis

The data obtained from the seed health testing methods were subjected to statistical analysis to find out the significance of the results. The data collected were transformed prior to analysis using square root transformation method and analysis of variance and mean separation were performed using Statistical Analysis Software (SAS, 1994).Germinated seeds were counted and expressed as a measure of seed viability using the formula.

$$Sv = n/N \times 100$$

where *Sv* is % seed viability, *n* is the number of seeds germinated from each normal or abnormal seed type and *N* is the total number of seeds plated on blotter and PDA.

3. RESULTS

Inspection of dry seeds of wheat varieties WH896, PBW373, and HD264 indicated that certain seeds were damaged, deformed, discoloured and contaminated with inert matter and infected with mycelial fragments. In all the three varieties, colour of pericarp was found Golden, Golden Brown and Light Golden Brown respectively. Inspection of seeds revealed that normal seeds were 12.57% - 28.7% while among the different abnormal seed types. Deformed seeds were (21.25%), Broken seeds (7.25%), and Malformed seeds (32.8%) were found across all the three varieties tested. (**Table 1**, **Figure 1**) show the percentage of normal and abnormal seeds after dry inspection of seeds. There were more abnormal than normal seeds. Wrinkled seed type was the most common form of abnormality with 64.4% - 72.2%. Incidence of entirely discoloured seed was 12.50 and 8.50 on variety PBW-373 and HD264 respectively (**Table 1**). Incidence of seeds with discoloured embryo and brush end was 1.25 to 1.80% and 1.75% - 6.0%. *Alternaria alternata* and *A. clamydophor* and *Fusarium moniliforme* were isolated from both normal and abnormal seeds (**Table 2**). However, with a higher incidence in abnormal than normal seeds (**Table 2**). *Rizopus* spp. (12.5%) and *Penicillium* spp. (4.2%) were also isolated additionally from entirely discoloured seeds. Incidence of *Alternaria alternata* was 2.2% on normal seeds while it was 2.25% - 78.0% on the abnormal seeds. Incidence of *Fusarium moniliforme* (30.2%) on seed with discoloured embryo end (**Table 2**). Seed abnormality that are associated with seed infection and discoloration had a higher incidence of fungal infection of seeds (**Table 2**). Germination percentage of normal seeds was 80% and this was higher than that (2.2% - 42.0%) of the abnormal seeds (**Table 2**).

Fusarium moniliforme, *Alternaria alternata*, and *Aspergillus flavus* were associated with white streaks from the all three varieties of wheat. However the incidence of *F. moniliforme* (94.0) was significantly higher than of any of the other fungi in all the three varieties tested

Figure 1. Seed discoloration and isolation of fungus on Blotter Method; ➡ Indicates the infection of *Fusarium* spp. on Blotter.

(**Table 3**). On variety WH896, *F. moniliforme* was identify from 94% of seeds that showed white streaks. The incidence of *F. moniliforme* on seeds that showed purple/pink discolouration was significantly higher than any other fungi isolated on variety PBW373 (56%) and HD264 (42%). *Fusarium moniliforme*, *Drechslera australiensis* and *Curvularia lunata* were observed on seeds with brown spots. The incidence of *D. australiensis* (62.8%) on variety HD264 was significantly higher than that of other fungi. *Aspergillus flavus*. *Cladosporium*, *Drechslera* were mostly associated with blackened seeds of three varieties tested. The percentage incidence of *Aspergillus flavus* on the blackened seed of the three varieties incubated (31%, 28%, and 25%) on WH896, HD264 and PBW372 respectively were significantly higher than any other observed fungi. **Table 2** also shows that the incidences of *Alternaria alternata* and *Fusarium moniliforme* were significantly high on seeds of all the varieties regardless of the type of discolouration (**Table 3**, **Figure 2**).

4. DISCUSSION

Seed-borne pathogens affect directly and indirectly the quality of seeds in commerce. The germination of seed infected with seed-borne pathogens may be reduced because the pathogens attack and kill the seedling. Seed-borne diseases have been found to affect the growth and productivity of crop plants [13-15]. Seeds are regarded as highly effective means for transferring plant pathogens over the years. Seed-borne diseases have been found to affect the growth and productivity of crop plants. Some of the fungi infect the seed and cause discolouration of the seed and also some other seed borne pathogens are known to be associated with wheat seed which are responsible for deterioration of seed quality during storage [16]. Detecting seed-borne fungi on seeds is accomplished by a number of methods. Examination of dry seeds with the naked eye and at magnifications of 10 to 30 times reveals a number of plant pathogens that occur mixed with the seeds as fungus bodies or have converted the seed into fungus structure. The spores of some pathogens may be associated in such great numbers with individuals seeds that merely mounting the seeds in drops of water, tearing the seeds apart, in such condition and then examining the exuding spores microscopically are sufficient for fungal detection. The dry inspection of seeds

Table 1. Incidence of normal and abnormal wheat seeds from dry inspection of seeds.

Variety No	Normal seeds (%)	Wrinkled seeds (%)	Seeds with discoloured embryo end (%)	Entirely discoloured seeds (%)	Seeds with discoloured brush end (%)
WH896	15.25	72.20	1.80	4.0	6.0
HD264	12.57	64.4	0.00	8.50	0.25
PBW373	26.57	38.2	1.25	12.50	1.75

Table 2. Viability and mycoflora of normal and abnormal seeds of all three wheat varieties.

Seed type	Germination %	Associated fungi	Seed infections %	Incidence of fungi %
Normal seeds	80.0 ± 0.15	*A.alternata*,	2.25 ± 0.25	0.60%
		F. moniliforme,	0.50 ± 0.10	
Wrinkled seed	42.0 ± 2.4	*Alternaria*,	20.5 ± 1.50	6.25%
		Fusarium,	42.0 ± 4.50	
		Aspergillus	34.0 ± 5.50	
Entirely discoloured seed	8.0 ± 6.0	*A. clamydophore*,	57.5 ± 0.30	30.6%
		A. flavus,	30.0 ± 1.0	
		D. australiensis	18.40 ± 2.24	
Seed with discoloured embryo end	2.5 ± 10.3	*A. alternata*,	18.25 ± 0.50	40.2%
		D. australiensis,	78.0 ± 1.52	
		C. lunata	60.50 ± 0.20	
		Fusarium	30.20 ± 1.20	
Seed with discoloured brush end	4.7 ± 3.0	*A. clamydophore*,	10.25 ± 2.10	28.18%
		Penicillium spp.,	24.0 ± 4.10	
		Cladosporium spp.	15.4 ± 2.50	

Values are means from four varieties of wheat. *±S.E.—Standard error.

Table 3. Seed borne fungi associated with different types of discolouration on seed of three wheat varieties.

	White streaks			Pink/Purple			Brown spots			Blackened seeds		
Fungus	*1	2	3	1	2	3	1	2	3	1	2	3
Alternaria alternata	49.2 a	82.0 a	56.0 a	10.6 c	15.0 b	120 b	8.5 c	26.0 b	18.5 b	0.2 c	4.6 c	8.0 c
Aspergillus niger	21.0 b	42.5 a	45.2 a	13.3 c	16.5 b	30.5 b	81.1 a	31.2 b	17.0 b	8.5 c	0.0 c	2.6 c
Alternaria clamydophor	42.6 b	27.0 b	36.8 a	32.2 b	28.2 b	10.5 b	36.2 b	56.0 b	45.2 b	24.2 b	0.0 c	8.2 c
Aspergillus flavus	32.1 b	14.6 b	10.3 b	18.5 b	26.2 b	13.6 b	28.6 b	38.0 b	26.0 b	31.0 b	28.5 b	25.6 b
Fusarium moniliforme	94.0 b	74.2 a	52.0 a	38.5 b	42.5 b	56.0 a	36.2 b	78.5 b	45.0 c	8.6 c	17.0 b	15.4 b
Mucor	0.0 c	0.2 c	4.0 c	2.0 c	8.2 c	6.5 c	10.2 b	20.2 b	8.4 c	0.0 c	0.0 c	0.0 c
Rhizopus oryzae	1.5 c	0.0 c	6.2 c	0.0 c	0.0 c	0.0 c	13.8 b	18.1 b	5.0 c	4.0	10.0 c	2.8 c
Drechsleria australiensis	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	46.6 b	62.8 b	26.2 b	16.0 b	24.o b	20.4 b
Rhizopus spp.	0.0 c	0.1 c	2.1 c	0.2 c	4.0 c	10.4 b	0.0 c	13.2 b	5.5 c	5.6 c	8.0 c	10.4 c
Penicillium spp.	0.5 c	1.9 c	4.5 c	0.0 c	0.0 c	0.0 c	10.1 c	26.4 b	30.2 b	0.0 c	0.0 c	0.0 c
Curvularia lunata	0.0 c	0.0 c	0.0 c	8.0 c	6.0 c	10.0 c	25.8 b	37.4 b	28.4 b	0.0 c	0.0 c	0.0 c
Cladosporium	0.0 c	0.0 c	0.0 c	2.0 c	1.0 c	11.7 b	18.5 b	15.2 b	12.6 b	24.6 b	20.0 b	16.8 b

*1) Variety WH896, 2) HD264, 3) PBW373; Each value is a mean of four replicates.

Figure 2. Culture of *Alternaria alternata* on PDA Method.

revealed a higher incidence of wrinkled and discoloured than normal seeds. Grain weathering manifests as discolouration, rough appearance, shriveling, loss of texture or reduced size [17]. The association of *Alternaria alternata* and *Fusarium moniliforme* with all the different types of seed discolouration and abnormalities, and its high incidence may be due to the susceptibility of the wheat crop to attack by both fungi. The isolation of different microorganism associated with seeds of wheat as detected by different techniques compared in terms of percentage frequencies indicated that the Standard Blotter method yielded quantitatively high percent frequency of *Aspergillus spp.* and *Alternaria spp.* [18]. [19] Thomas and Buddenhagen (1980) and [20] Zummo and Scott (1992) observed higher incidence of *F. moniliforne* than other fungi isolated from maize seeds. [21] Adlkha and Joshi (1974) reported that severe infection of wheat heads caused discolouration and shriveling of the seeds. The association of *Alternaria alternata* and *Fusarium moniliforme* with all forms of seed abnormalities and its relatively high incidence on discoloured seeds indicates the susceptibility of the wheat crop to these fungal species, similar to the reports of [8] Varshney (1990). [22] Parry *et al.* (1995) had listed *F. grammearum* as one of the several *Fusarium spp.* associated with the head blight of wheat. *Fusarium moniliforme* was found to be associated with pink/purple seeds. Similar observation was reported by [23] Neergaard (1979). Brown spots on wheat seeds were associated with *F. moniliforme*, *Drechslera australiensis* and *Curvularia lunata*. Blackened wheat seeds were infected with *Aspergillus flavus*, *Alternaria clamydophore*, and *Cladosporium*. *Alternaria tenuis* isolated from entirely discoloration seeds is also a reputed black-point pathogen [8,24]. The reduced viability of abnormal seeds is due to the influence of fungi. [25] Fakir (1988) reported significant reduction in the germination of infected seeds while [26] Rena and Gupta (1982) reported that localized discoloured areas, usually around the embryo end of seeds are often responsible for reduced germ inability. The isolation of some economically important seed-borne fungi of wheat from the discoloured embryo ends may be due to the fact that the embryo contains abundant protein materials. The find-

ings in this investigation suggest that wheat seeds with various types of discolouration and abnormalities have the potential to cause seed deterioration in storage and in the field too.

REFERENCES

[1] Ali, M. and Kumar, S. (2000) Problems and prospects of pulses research in India. *Indian Farming*, **50**, 4-13.

[2] Akhtar, L. H. and Siddiqui, S.Z. (2003) Current status of research and development on pulses in Pakisthan. *Science, Tecnology and Development*, **22**, 16-28.

[3] Subramanian, M. (2000) Pulses production strategies in Tamil Nadu. Centre for Plant Breeding and Genetics Tamil Nadu Agricultural University, Coimbatore.

[4] Reddy, C.S., Reddy, K.R.N., Kumar, R.N., Laha, G.S. and Muralidharan, K. (2004) Exploration of aflatoxia contamination and its management in rice. *Journal of Mycology and Plant Pathology*, **34**, 816-820.

[5] Abbas, H.K., Cartwright, R.D., Xie, W., Mirocha, C.J., Richard, J.L., Dvorak, J.J., Sciumbato, G.L. and Shier, W.T. (1999) Mycotoxin production by Fusariumproliferatum isolates from rice with *Fusarium sheath* rot disease. *Mycopathologia*, **147**, 97-104. doi:10.1023/A:1007147813326

[6] Makun, H.A., Gbodi, T.A., Akanya, H.O., Sakalo, A.E. and Ogbadu, H.G. (2007) Fungi and some mycotoxins contamination rice (*Oryza sativa*) in Niger State, Nigeria. *African Journal of Biotechnology*, **6**, 99-108.

[7] Owolade, B.F., Fawole, B. and Osinkanlu, Y.O.K. (2001) Fungi associated with maize seed discolouration and abnormalities in South Western Nigeria. *African Crop Science Journal*, **9**, 693-697.

[8] Varshney, J.L. (1990) Seed-borne diseases of wheat—Their impact in relation to production and productivity. *International Journal Of Tropical Plant Diseases*, **8**, 173-192.

[9] Shetty, H.S. (1993) Different types of damages in seeds caused by seedborne fungi. In: Mathur, S.B. and Jorgensen, J., Eds., *Seed Pathology*: *Proceedings of CTA Seminar*, Copenhagen, 20-25 June 1988, p. 53.

[10] Benoit, H.C. and Mathur, S.B. (1970) Identification of species of Curvularia on rice seed. *Proceedings International Seed Testing Association*, **35**, 99-119.

[11] Chidambaran, P., Mathur, S.B. and Neergaard, P. (1973) Identification of seed-borne Drechslera species. *Friersia*, **10**, 165-207.

[12] Barnett, H.C. and Hunter, B.B. (1972) Illustrated genera of imperfect fungi. 3rd Edition, Burgress Publishing Co., Minneapolis, 20 p.

[13] Kubiak, K. and Korbas, M. (1999) Occurrence of fungal diseases on selected winter wheat cultivars. *Postepy w Ochronie Roslin*, **39**, 801-804.

[14] Weber, R., Hrynczuk, B., Runowska-Hrynczuk, B. and Kita, W. (2001) Influence of the mode of tillage on diseases of culm base in some winter wheat varieties, oats and spring wheat. *Journal of Phytopathology*, **149**, 185-188. doi:10.1046/j.1439-0434.2001.00592.x

[15] Dawson, W.A.J.M. and Bateman, G.L. (2001) Fungal communities on roots of wheat and barley and effects of seed treatments containing fluquinconazole applied to control take-all. *Plant Pathology*, **50**, 75-82. doi:10.1046/j.1365-3059.2001.00538.x

[16] Pathak, N. and Zaidi, R.K. (2013) Comparative study of seed dressing fungicides and *Calotropis Procera* latex for the control of seed-borne mycoflora of wheat. *Annals of Biological Research*, **4**, 1-6.

[17] Tyagi, P.D. and Olugbemi, L.B. (1980) Rain-fed wheat in Nigeria as influenced by fungal pathogens and adverse weather conditions. *Samaru Miscellaneous Paper*, **91**, 1-15.

[18] Pathak, N. and Zaidi, R.K. (2013) Studies on seed-borne fungi of wheat in seed health testing programme. *Archives of Phytopathology and Plant Protection*, **46**, 389-401. doi:10.1080/03235408.2012.741978

[19] Thomas, M.D. and Buddenhagen, I.W. (1980) Incidence and persistence of Fusarium moniliforme in symptomless maize kernels and seedlings in Nigeria. *Mycologia*, **72**, 882-887. doi:10.2307/3759731

[20] Zummon, N. and Scott, G.E. (1992) Interaction of Fusariummoniliforme and Aspergillusflavus on kernel infection and aflatoxin contamination in maize ears. *Plant Disease*, **76**, 771-773. doi:10.1094/PD-76-0771

[21] Adlakha, K.L. and Joshi, L.M. (1974) Black point of wheat. *Indian Phytopath*, **27**, 41- 44.

[22] Parry, D.W., Jenkinson, P. and McLeod, L. (1995) Fusarium ear blight (scab) in small grain cereals. *Review of Plant Pathology*, **44**, 207-238. doi:10.1111/j.1365-3059.1995.tb02773.x

[23] Neergaard, P. (1981) Introduction to methods of seed-health testing. *Seed Science and Technology*, **7**, 601-635.

[24] Parashar, R.D. and Chohan, J.S. (1967) Effect of black point, both *Alternania sp*. and *Helminthosporium sativum* on seed germination under laboratory and field conditions and on yield. *Journal of Research in Ludhiana*, **4**, 73-75.

[25] Fakir, G.L. (1988) Report on investigation into black point disease of wheat in Bangladesh. Bangladesh Agricultural University, Mymensingh.

[26] Rena, J.P. and Gupta, P.K.S. (1982) Occurrence of black point disease of wheat in West Bengal. *Phytopathology*, **35**, 700-702.