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POPULATION DYNAMICS OF MYCOFLORA AND INCIDENCE OF BLACK POINT DISEASE IN WHEAT GRAINS

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Abstract

An attempt was made to monitor the prevalence of different fungi associated with floret lemma and developing grains of wheat and to assess the subsequent incidence of black point disease under different exposure periods of the spikes to airborne inocula of the causal fungi. Altogether 16 fungi representing 11 genera were detected from lemma and developing grains. The predominant fungi, in order of prevalence, were *A. alternata, B. sorokiniana, C. cladosporioides, C. lunata, Fusarium* spp. and *E. purpurascens.* Other fungi occurring less commonly were *A. triticina, C. pallescens, Nigrospora* sp., *Phoma* sp., *Chaetomium* sp., *B. tetramera, B. oryzae, Aspergillus flavus, A. niger* and *Doratomyces* sp. The incidence of all the fungi except *Fusarium* spp. was higher in lemma than in grains. In lemma, *A. alternata* occurred with the highest frequency, while the incidence of *B. sorokiniana* was found highest in grains. The population of *A. alternata, B. sorokiniana, C. lunata, E. purpurascens* and *Fusarium* spp. increased with the age of lemma and developing grains whereas the incidence of *C. cladosporioides* increased at early stages but declined at later stages of grain development. Other fungi did not follow any definite pattern in their incidence during grain development. The occurrence of black pointed grains per spike, percent black pointed grains and black point index were found increasing with the increase in exposure period of the spikes to airborne inocula of the causal fungi.

Keywords: Mycoflora, black point, wheat.

Introduction

Seeds are the carriers of various plant pathogenic fungi, bacteria, viruses and nematodes. When a pathogen is associated with seeds, in most of the cases, it produces a variety of symptoms on the seeds. These symptoms include seed rot, shrivelling of grain, seed necrosis and seed discolouration. Neergaard (1979) reported that many parasitic seedborne fungi infect the seed coat causing conspicuous black, brown or grey necrotic discolouration. Black point of wheat also known as kernel smudge, is characterized by brown to black discolouration mainly restricted to the embryonic end of the seed (Adlakha and Joshi, 1974). Occasionally, the symptom can be seen near the brush end, in the ventral crease,

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or on any other part of the kernel. In case of severe infection, the whole grain may be discoloured and shrivelled.

Black point of wheat caused by *Bipolaris sorokiniana* occurs almost all over the world wherever wheat crop is grown (Mathur and Cunfer, 1993). However, it is an important disease of wheat seed crop in Bangladesh (Fakir, 1998). Black point has adverse effects on seed weight, germination and grain yield. The reduction in 1000-grain weight was as large as 41.96% when the grains were severely infected with the black point fungi (Rahman and Islam, 1998). The germination of black point affected seeds was reduced to a great extent as compared to healthy grains (Khanum *et al.,* 1987). However, the reduction in germination was found to be related with the severity of black point infection (Dhruj, 1991).

The fungi commonly involved in the black point disease of wheat include *Alternaria alternata, Bipolaris sorokiniana, Cladosporium cladosporioides*, *Curvularia lunata* and *Fusarium* spp. (Fakir *et al.,* 1989; Dey *et al.,* 1992). Under the favourable conditions of disease development, the airborne inocula of the causal fungi infect the spikes and ultimately lead to the development of black point on the grains. However, the characteristic discolouration of the kernels usually appears during the soft dough to hard dough stages of grain development (Talukder and Fakir, 1993; Ahmed *et al.,* 1994).

The present study was undertaken to determine the prevalence of different fungi associated with floret lemma and developing grains and to assess the incidence of black point disease under different exposure periods of spikes to airborne inocula of the causal fungi.

Materials and Method

A black point susceptible wheat variety ‘Kanchan’ was grown in the experimental field of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during 2000-2001 crop season. Recommended doses of manures and fertilizers were used. The seeds were sown at the rate of 120 kg/ha in lines with 20 cm spacing between rows. Sowing was done in the week of November. Weeding, mulching and irrigation were done as and when necessary for maintaining normal crop growth.

After heading, samples of four hundred florets from the middle of randomly selected 100 spikes in four replicates were collected at weekly intervals for determining the incidence of the associated fungi. The sampling of florets was continued for eight weeks upto dead ripening stage. In addition, randomly selected 20 spikes in four replicates were covered with glassine paper bags during each sampling to create different exposure periods of the spikes to airborne inocula of the fungi involved in the development of black point on the

grains. Before bagging of the selected spikes, the awns were removed from the florets. The spikes were marked with tags mentioning the respective bagging dates indicating different exposure periods of the spikes to airborne fungal inocula.

The florets collected at each sampling time were brought to the laboratory and separated into lemma and grains. The awns were then removed from the individual lemma. The association of fungi with lemma and grains and their percentages of incidence were determined by blotter method according to ISTA rules (Anon., 1996). The lemma and grains were surface sterilized with 1% chlorox for 2 minutes followed by three times rinsing with sterile distilled water. The lemma and grains were plated separately on three layered moistened blotter papers contained in 9 cm petridishes. Each plate received 25 lemmas or the same number of grains. The plates with lemma and grains were incubated at 25 ± 1 ^{0}C for 7 days under 12/12 hr light and darkness cycle. The incubated lemma and grains were examined under a stereobinocular microscope and the associated fungi were identified following the appropriate keys (Ellis, 1971, 1976; Barnett and Hunter, 1998). When necessary, temporary slides were prepared and identification was done under compound microscope.

The spikes bagged at different days after heading were harvested at the end of dead ripening stage. The harvested spikes were sun-dried and threshed by hands separately for different exposure periods. Black pointed grains were counted by visual observation of grains under a hand lens (l0X). Data on number of black pointed grains per spike and percent incidence of black pointed grains were recorded. The grains were indexed for severity of black point infection following 0-5 scale of CIMMYT (Gilchrist, l985) and the percent black point indices were calculated.

Data on fungal incidence and different parameters of black point infection were analysed statistically following completely randomized design with four replications. The percentage data were transformed following square root transformation method before analysis. The mean values were compared by DMRT.

Results and Discussion

Association of fungi with floret lemma and grains

Sixteen different fungi representing 11 genera were detected from the floret lemma and grains of wheat during different stages of grain development. In order of prevalence, the predominant fungi were *Alternaria alternata, Bipolaris sorokiniana, Cladosporium cladosporioides, Curvularia lunata, Fusarium* spp. and *Epicoccum purpurascens.* The mean prevalence of the six fungi ranged from 5.57 to 33.68%. Other fungi occurring less commonly were *Alternaria triticina,*

Curvularia pallescens, Nigrospora sp., *Phoma* sp., *Chaetomium* sp., *Bipolaris tetramera, Bipolaris oryzae, Aspergillus flavus, Aspergillus niger* and *Doratomyces* sp. The average incidence of these fungi ranged from 0.04 to 3.22%. The incidence of all the fungi except *Fusarium* spp. was higher in lemma than in grains. The fungi *A. flavus, A. niger* and *Doratomyces* sp. were found associated only with the grains (Table 1).

Table 1. Prevalence of fungi associated with lemma and developing grains of wheat.

Fungi	Prevalence (%)		Average (%)
	Lemma[a]	Grains[b]	
Alternaria alternata	43.19	24.17	33.68
Alternaria triticina	7.31	3.83	5.57
Aspergillu sfiavus	0.00	0.21	0.11
Aspergillus niger	0.00	0.07	0.04
Bipolaris oryzae	0.20	0.04	0.12
Bipolaris sorokiniana	32.19	27.91	30.05
Bipolaris tetramera	0.60	0.38	0.49
Chaetomium sp.	1.09	0.52	0.81
Cladosporium cladosporioides	36.29	16.67	26.48
Curvularia lunata	19.39	10.50	14.95
Curvularia pallescens	3.83	2.61	3.22
Doratomyces sp.	0.00	0.07	0.04
Epicoccum purpurascens	8.26	4.62	6.44
Fusarium spp.	4.94	8.33	6.64
Nigrospora sp.	2.63	2.29	2.46
Phoma sp.	1.72	0.28	1.00

[a]Mean of 32 samplings from heading to dead ripening stage.
[b]Mean of 28 samplings from seven days after heading to dead ripening stage.

Weekly incidence of fungi on floret lemma

Weekly determination of fungal population associated with lemma collected from heading to dead ripening stage revealed that the occurrence of different fungi varied with the age of lemma. Infestation of lemma with *A. alternata, B. sorokiniana, C. lunata, E. purpurascens* and *Fusarium* spp. was found to increase with the increasing stages of crop development after heading. In case of *A. triticina*, the incidence was first observed at 7 days after heading, which increased significantly at 14 days but remained statistically similar upto dead ripening stage. The population of *C. cladosporioides*, on the other hand, increased significantly in the early stages after heading but declined gradually thereafter. However, no specific trend in prevalence was observed in other fungi (Table 2).

Table 2. Variation in prevalence of fungi associated with lemma of wheat as recorded at weekly intervals beginning from heading to dead ripening stage.

Days after heading	Prevalence of fungi (%)												
	A. a.	A. t.	B. o.	B. s.	B. t.	C. sp.	C. c.	C. l.	C. p.	E. p.	F. spp.	N. sp.	P. sp.
0	5.06e	0.00c	0.00b	0.00e	0.00d	0.00c	5.31e	4.06d	0.00e	1.00d	1.06e	0.00c	0.0d
	(2.35)	(0.71)	(0.71)	(0.71)	(0.71)	(0.71)	(2.40)	(2.12)	(0.71)	(1.18)	(1.20)	(0.71)	(0.71)
7	15.25d	2.31b	0.00b	3.56d	0.00d	0.50bc	10.00e	7.50d	1.13d	1.00cd	l.06e	0.00c	0.00d
	(3.96)	(1.67)	(0.71)	(2.01)	(0.71)	(0.97)	(3.23)	(2.80)	(1.24)	(1.23)	(1.22)	(0.71)	(0.71)
14	35.69c	8.44a	0.00b	14.69c	0.00d	0.50bc	26.81d	15.13c	4.00bc	2.50c	3.56d	2.13b	2.06b
	(6.00)	(2.98)	(0.71)	(3.89)	(0.71)	(0.93)	(5.14)	(3.92)	(2.08)	(1.73)	(2.01)	(1.62)	(1.59)
21	43.19c	9.06a	0.63a	30.13b	0.00d	0.94abc	50.06bc	19.94bc	8.13a	8.00b	4.50cd	2.13b	3.00b
	(6.59)	(3.08)	(1.05)	(5.53)	(0.71)	(1.19)	(7.09)	(4.50)	(2.90)	(2.90)	(2.21)	(1.60)	(1.86)
28	54.94b	10.19a	1.00a	50.13a	2.19a	0.56bc	69.75a	29.94a	4.63bc	10.00b	6.25bc	4.00a	3.00b
	(7.44)	(3.26)	(1.18)	(7.09)	(1.63)	(0.99)	(8.37)	(5.49)	(2.25)	(3.22)	(2.59)	(2.11)	(1.86)
35	62.00ab	8.06a	0.00b	50.94a	0.69c	2.l9ab	61.l9ab	31.00a	2.75c	8.19b	7.50ab	5.56a	4.56a
	(7.90)	(2.91)	(0.71)	(7.16)	(1.07)	(1.55)	(7.84)	(5.57)	(1.79)	(2.94)	(2.82)	(2.44)	(2.23)
42	70.00a	10.00a	0.00b	56.06a	0.44cd	1.50ab	41.88c	22.38abc	4.63bc	11.44b	5.56bcd	5.00a	1.13c
	(8.38)	(3.23)	(0.71)	(7.51)	(0.95)	(1.35)	(6.49)	(4.76)	(2.25)	(3.43)	(2.45)	(2.34)	(1.27)
49	59.38ab	10.38a	0.00b	52.00a	1.50b	2.50a	25.31d	25.l9ab	5.38ab	23.94a	10.00a	2.25b	0.00d
	(7.72)	(3.28)	(0.71)	(7.23)	(1.40)	(1.73)	(5.06)	(5.04)	(2.40)	(4.92)	(3.22)	(1.64)	(0.71)
CV(%)	7.20	9.66	17.34	8.82	15.57	32.32	11.16	13.28	17.83	12.88	13.37	14.00	14.03

Means within a column followed by a common letter do not differ significantly (p = 0.05).

Data within parentheses are square root (x + 0.5) transformed values.

A. a. = *A. alternata*, A. t. = *A. triticina*, B. o. = *B. oiyzae*, B. s. = *B. sorokiniana*, B. t. = *B. tetramera*, C. sp. = *Chaetomium* sp., C. c. = *C. cladosporioides*, C. 1. = *C. lunata*, C. p. = *C. pallescens*, D. sp. = *Doratomyces* sp., E. p. = *E. purpurascens*, F. spp. = *Fusarium* spp., N. sp. = *Nigrospora* sp., P. sp. = *Phoma* sp.

Table 3. Variation in prevalence of fungi associated with developing grains of wheat as recorded at weekly intervals beginning from seven days after heading to dead ripening stage.

Days after heading	Prevalence of fungi (%)															
	A. a.	A. t.	A. f.	A. n.	B. o.	B. s.	B. t.	C. sp.	C. c.	C. l.	C. p.	D. sp.	E. p.	F. spp.	N. sp.	P. sp.
7	5.31c	1.31e	0.00b	0.00b	0.00b	1.00d	0.00b	0.44ab	3.56d	4.00e	0.56e	0.00b	0.00d	1.19d	0.00c	0.00c
	(2.39)	(1.34)	(0.71)	(0.71)	(0.71)	(1.23)	(0.71)	(0.92)	(2.00)	(2.11)	(1.01)	(0.71)	(0.71)	(1.28)	(0.71)	(0.71)
14	14.13b	4.00bcd	0.00b	0.00b	0.00b	7.25c	0.00b	0.00b	6.38cd	6.06de	3.50ab	0.00b	0.00d	6.56c	1.94b	0.00c
	(3.76)	(2.12)	(0.71)	(0.71)	(0.71)	(2.76)	(0.71)	(0.71)	(2.61)	(2.55)	(1.99)	0.71)	(0.71)	(2.65)	(1.54)	(0.71)
21	19.63b	5.06ab	0.00b	0.00b	0.00b	22.88b	0.00b	0.56ab	25.00a	8.l9cd	4.94a	0.00b	3.88c	7.56bc	3.06ab	0.00c
	(4.46)	(2.35)	(0.71)	(0.71)	(0.71)	(4.82)	(0.71)	(0.99)	(5.03)	(2.93)	(2.32)	(0.71)	(2.09)	(2.82)	(1.88)	(0.71)
28	29.56a	4.50abc	0.00b	0.00b	0.31a	38.31a	1.00a	0.00b	31.06a	10.88bc	2.56bc	0.00b	7.44ab	9.8lbc	2.44ab	0.56ab
	(5.45)	(2.22)	(0.71)	(0.71)	(0.88)	(6.18)	(1.23)	(0.71)	(5.60)	(3.37)	(1.72)	(0.71)	(2.80)	(3.19)	(1.71)	(1.02)
35	33.44a	5.50a	0.00b	0.00b	0.00b	40.00a	0.25b	1.25a	26.06a	12.50ab	1.25de	0.00b	5.56b	10.69ab	2.94ab	1.00a
	(5.80)	(2.44)	(0.71)	(0.71)	(0.71)	(6.33)	(0.84)	(1.26)	(5.14)	(3.59)	(1.31)	(0.71)	(2.45)	(3.33)	(1.84)	(1.20)
42	35.13a	3.00d	0.00b	0.00b	0.00b	40.94a	0.25b	0.25ab	15.50b	15.81a	2.06cd	0.00b	7.44ab	8.56bc	3.56a	0.38bc
	(5.93)	(2.86)	(0.71)	(0.71)	(0.71)	(6.42)	(0.84)	(0.84)	(3.98)	(4.02)	(1.59)	(0.71)	(2.81)	(3.00)	(2.00)	(0.92)
49	32.00a	3.44cd	1.50a	0.50a	0.00b	45.00a	1.13a	1.l3ab	9.13c	16.06a	3.38ab	0.50a	8.00a	13.94a	2.06b	0.00c
	(5.69)	(1.98)	(1.40)	(1.00)	(0.71)	(6.72)	(1.26)	(1.23)	(3.09)	(4.03)	(1.97)	(1.00)	(2.90)	(3.78)	(1.59)	(0.71)
CV(%)	11.65	9.65	9.64	5.20	12.62	12.90	17.54	33.45	10.63	12.25	13.24	5.20	11.88	11.96	13.99	17.92

Means within a column followed by a common letter do not differ significantly (p = *0.05).*

Data within parentheses are square root (x + 0.5) transformed values.

A. a. = *A. alternata,* A. **t.** = *A. triticina,* A. f. = *A. flavus,* A. n. = *A. niger,* B. o. = *B. oryzae,* B. s. = *B. sorokiniana,* B. **t.** = *B. tetramera,* C. sp. = *Chaetomium* sp., C. c. = *C. cladosporioides,* C. 1. = *C. lunata,* C. p. = *C. pallescens,* D. sp. = *Doratomyces* sp., E. p. = *E. purpurascens,* F. spp. = *Fusarium* spp., N. sp. = *Nigrospora* sp., P. sp. = *Phoma* sp.

Weekly incidence of fungi on developing grains

The incidence patterns of *A. alternata*, *B. sorokiniana*, *C. lunata*, *E. purpurascens* and *Fusarium* spp. on developing grains were almost similar to those as observed on the floret lemma. A gradual increasing trend in population of these fungi was observed as the grain approached towards dead ripening stage. Seedborne incidence of *A. triticina* and *C. cladosporioides* increased in the early stages but declined significantly in the later stages of grain development. The other fungi did not show any definite pattern in their weekly prevalence on the developing grains (Table 3).

Incidence of black point on wheat grains

Occurrence of black point infection in wheat grains varied with the variation in exposure periods of the spikes to airborne inocula of the fungi after heading to dead ripening stage. A gradual increasing trend in number of black pointed grains per spike, percent black pointed grains and percent black point index was observed with the increase in exposure period of the spikes in days after heading (DAH). Number of black pointed grains per spike ranged from 0.56 to 8.74 depending on the length of exposure period. The lowest number of black pointed grains per spike was recorded under 0 day exposure after heading, which was statistically similar to the exposure period of 7 DAH. Exposure of spikes for 14 DAH caused significant increase in number of black pointed grains per spike as compared to those recorded under the expoure periods of 0 and 7 DAH, and the increasing trend remained statistically similar upto the expoure of 35 DAH. The highest number of black pointed grains per spike was recorded when the spikes were exposed for 49 DAH, which was statistically similar to exposure of 42 DAH but significantly higher as compared to other exposure periods. Almost similar increasing trend was observed in case of percent black pointed grains. The lowest incidence of 1.31% black pointed grains was observed under the exposure of 0 DAH, while the highest of 21.50% was recorded at the exposure of 49 DAH. The percentages of black pointed grains recorded at the exposures of 49 and 42 DAB were statistically similar to one another but significantly higher than those recorded under the other exposure periods. Percent black point index was found maximum when the spikes were exposed for 49 DAH, and it was statistically similar to the exposure of 42 DAH. The lowest black point index was recorded at the exposure of 0 DAH, which was significantly lower compared to other periods of exposure (Table 4).

In the present investigation, altogether 16 fungi belonging to 11 genera were found to be associated with lemma and developing grains of wheat during the period from heading to dead ripening stage. The most frequently occurring fungi were *A. alternata* and *B. sorokiniana*. The incidence of total fungi increased with the increase in days from heading to dead ripening stage. Association of these

fungi with wheat grains were also reported by other researchers (Ali and Fakir, 1992; Bazlur Rashid *et al.*, 1995; Mahmud, 2005). Magan and Lacey (1986) reported that the population of A. *alternata*, *Cladosporium* spp., *Fusarium* spp. and *E. nigrum* increased rapidly between anthesis and harvest. Talukder and Fakir (1991) found that A. *tenuis* occurred most frequently at full ripe stage and *E. purpurascens* at dead ripe stage, whereas *C. cladosporioides* was the most prevalent fungus between flowering and anthesis, and between soft and hard dough stages.

Table 4. Incidence of black point disease of wheat grains under different exposure periods of the spikes to airborne inocula of the causal fungi beginning from heading to dead ripening stage.

Exposure periods of spikes (days after heading)	Black pointed grains/spike	Percent black pointed grains	Percent black point index
0	0.56d	l.31c (1.32)	0.63e (1.05)
7	1.34d	3.19c (1.89)	1.65d (1.45)
14	4.69c	10.88b (3.36)	6.18c (2.56)
21	5.75c	12.31b (3.57)	6.89c (2.72)
28	6.16c	14.75b (3.88)	7.51c (2.83)
35	6.69bc	15.25b (3.94)	7.84bc (2.88)
42	8.26ab	21.06a (4.62)	9.79ab (3.20)
49	8.74a	21.50a (4.66)	9.98a (3.23)
CV(%)	23.81	13.21	8.97

Means within a column followed by a common letter do not differ significantly (p= 0.05). Data within parentheses are square root (x + *0.5)* transformed values.

Visual observation of wheat grains after harvest indicated that black point incidence increased with the increase in exposure period of the spikes to airborne inocula of the causal fungi. The increase in black point incidence might be due to increasing prevalence of black point fungi occurred between heading and dead ripening stage. Ahmed (1989) reported that black point symptom appeared at the late stage of dough development and became conspicuous towards ripening of the grain. In a similar study, Talukder and Fakir (1993) observed that the disease appeared on the floret grains at hard dough stage and it became pronounced at the dead ripening stage. From the results of the present study, it may be concluded that the population of fungi associated with floret lemma and developing grains of wheat increased with their age and the subsequent incidence of black point disease increased with the exposure period of the spikes to airborne inocula of the fungi.

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