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N.B. ISLAM, S.M.M. HOSSAIN, M.M. HASAN, S.M.E HASSAN AND M.M. ISLAM



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INCIDENCE AND SEVERITY OF BLACK POINT DISEASE OF WHEAT

N.B. ISLAM¹, S.M.M. HOSSAIN^{2*}, M.M. HASAN³, S.M.E HASSAN² AND M.M. ISLAM¹

¹MS Student, Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur;

²Professor, Department of Plant Pathology, HSTU, Dinajpur; ³Associate Professor, Department of Plant Pathology, HSTU, Dinajpur.

*Corresponding author & address: Dr. Sk. Md. Mobarak Hossain, E-mail: mobarakhstu@gmail.com

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ABSTRACT

Islam NB, Hossain SMM, Hasan MM, Hassan SME, Islam MM (2016) Incidence and severity of black point disease of wheat. *Int. J. Expt. Agric.* 6(2), 12-19.

Black point fungi associated with ten wheat varieties were studied. Seeds of ten wheat varieties of two growing season (time and late sowing) were collected from Wheat Research Center (WRC), Noshipur, Dinajpur. A significant variation was observed in disease incidence and severity in different varieties and sowing date where the highest disease incidence and severity was recorded in Kanchan and lowestr in BARI gam 25. The most common fungus observed from different wheat seeds was *Bipolaris* sp. However, *Alternaria* sp. *Curvularia* sp. *Fusarium* sp. *Aspergillus* sp. *Epicoccum* sp. *Rhizopus* sp. *Nigrospora* sp. and *Phoma* sp. were also associated with the wheat seed. Variety Kanchan showed maximum infection of *Bipolaris* sp. (54.50%), *Alternaria* sp. (24.50%), *Curvularia* sp. (9.00%), *Aspergillus* sp., (10.75%), *Epicoccum* sp. (12.75%) and *Rhizopus* sp. (3.50%). Beside this, variety Shatabdi showed maximum infection of *Fusarium* sp. (10.25%) where minimum infection of *Bipolaris* sp. (13.50%) was found in BARI gam 25, *Alternaria* sp. (9.75%) in BARI gam 28, *Curvularia* sp. (1.00%) in Prodip and BARI gam 28, *Fusarium* sp. (1.00%) in BARI gam 25, BARI gam 27, and Prodip, *Aspergillus* sp. (1.00%) in BARI gam 27, *Rhizopus* sp. (1.00%) in Prodip, Gourab, Shatabdi, BARI gam 25 and BARI gam 26 and finally minimum *Nigrospora* sp. was calculated in (3.50%) in Sourav variety, respectively. Black point incidence and severity was always observed higher in late sowing variety compared to time sowing variety. Fungal pathogen associated with wheat seeds also found higher in late sowing varieties than that of time sowing.

Key words: black point, disease, wheat, bipolaris

INTRODUCTION

Wheat (*Triticum aestivum*) is a cereal crop belonging to the family Gramineae. Total production of wheat in the world in 2013 is 713 million tons considered as the third most harvested cereal crop following maize and rice (FAOSTAT 2015). In Bangladesh it is regarded as the second most staple food where annual production is 68 matric ton (BBS 2013). Production of wheat in Bangladesh is reduced due to many constrains in which abnormality of seed and seed borne diseases are major (Enikuomehin 2005). Wheat is suffered by as much as 120 different diseases, among them, 42 are seed borne (Hasan *et al.* 2005). Five are economically important for the reason of their nature of damage and wide incidence all over the wheat-growing region of the country (Malaker *et al.* 2009) where black point is frequently recognizing disease in all wheat growing region all over the world (Toklu *et al.* 2007). Infected seeds are characterize by dark brown to black discoloration which is mostly found in pericarp and testa, primarily at the embryo end of the seed (Fernandez and Conner, 2011) and gradually the kernels may shriveled. This infection is triggered by frequent rainfall or high relative humidity from milk to soft dough stage, lodging and late season irrigation; usually generate disease by seed borne fungi (Watkins 2004). In Bangladesh, the incidence of black point disease is ranged from 5-55% depending on varieties cultivated in wheat growing areas (Malaker *et al.* 2009). When moisture content of seed is more than 20% together with RH about 90%, the disease is increased dramatically (Toklu *et al.* 2007). Probable consequences of this disease is comprised of reduce seed weight, market grade and dockage by the elevator. It also reduces the rate of germination, number of embryonic roots and coleoptiles length and increased incidence of seedling blight (Watkins 2004; Toklu *et al.* 2007). Recent research on wheat also reported that black pointed seed is also responsible for some dangerous animal diseases like esophageal cancer (Busman *et al.* 2012). Other pathogens are also associated with wheat seed discoloration which are *Bipolaris sorokiniana*, *Alternaria alternata*, *Curvularia*, *Cladosporium*, *Epicoccum*, *Aspergillus*, *Rhizopus*, *Penicillum*, *Trichoderma* etc. (Kolawole *et al.* 2013; Islam *et al.* 2015; Pathak *et al.* 2013). Seed are rejected from different seed producing organizations specially BADC due to seed discoloration and lower germination rate resulting appreciable economic loss (Malaker *et al.* 2009). Black point is a problem to the wheat seed growers, specially to BADC for last few years. The organization has beed facing serious problem for procuring and distributing wheat seeds for discoloration of the grains as there was no fixed standard for the disease. Considering this problem, Bangladesh National Seed Board has recently fixed a seed standard of 15% for black point arbitrarily (Fakir 1998). Even though, the varieties released in Bangladesh for cultivation are infected by black point pathogen because of the incidence and severity of the disease in different released varieties in Bangladesh have been paid less attention. The present work is therefore, undertaken to assess disease incidence and disease severity of black point disease in different wheat varieties at different sowing dates and to find out the fungi associated with wheat seed.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during 2015. Ten time and late sowing wheat varieties namely Sourav, Kanchan, Prodip, Gourab, Shatabdi, Protiva, BARI gam 25, BARI gam 26, BARI gam

27 and BARI gam 28 were collected according to ISTA (2001) rule from Wheat Research Center (WRC), Noshiapur Dinajpur. The seeds were used for seed quality and health analysis mainly determination of black point incidence and detection of seed borne fungal pathogens associated with the seeds. Black pointed seeds were sorted out from the collected seed samples manually by physical sorting and expressed as percentage. In accordance with black pointed seeds, apparently healthy seed (best seed), undersized seed were also observed and stated as percentage. Health status of all seed samples were analyzed for detection of seed borne fungi. The blotter method as mentioned in the rules of ISTA (2001) was used. In this methods, four hundred seeds were randomly taken from each of the seed samples. Twenty-five seeds were placed on three layered moist blotter paper contained in each of 9 cm glass petridishes. The petridishes were placed on laboratory bench at room temperature (about 20-25°C) for incubation under 12/12 hours light-darkness cycle. After 7 days of incubation, the seeds were examined under a stereobionocular microscope for the growth and detection of fungi. In case of confusion, temporary water mounts slide were prepared and observed under a compound microscope for identification of associated fungi. The fungal genera and species were identified using standard keys (Booth 1972; Barnett and Hunter, 1972; Mathur and Kongsdal, 2003).

Black point incidence was determined by the following formula (Rai and Mamatha, 2005):

$$\text{Percent black point incidence} = \frac{\text{Total number of black pointed seeds}}{\text{Total number of seeds examined}} \times 100$$

The sorted black pointed seeds were separated into six different grades on the basis of severity of black point infection. The grading was done according to 0-5 rating scale as suggested by Gilchrist (1985); where,

Grade-0 = Grains free from any discoloration (apparently healthy);

Grade-1 = Tip of the embryo brown to blackish;

Grade-2 = Discoloration covering the whole embryo;

Grade-3 = Embryo with 1/4 of the grain discolored;

Grade-4 = Embryo with 1/2 of the grain discolored;

Grade-5 = Embryo with more than 1/2 of the grain discolored and shrivelled.

Severity of the disease was calculated by determining the percent disease index using following formula (Mian 1995).

$$\text{PDI} = \left[\frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade in scale}} \times 100 \right]$$

The experiment was conducted following complete randomized design (CRD) with four replications and two factor factorial experiment where wheat variety treated as factor one and sowing time as factor two. Data on various parameters were subjected to analysis of variance following MSTAT-C computer programme and the means were compared by Duncan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Apparently healthy seed, undersized seed, black point seed incidence and black point severity was significantly varied among all the ten wheat varieties (Table 1). The highest apparently healthy seed (90.92%) was recorded in BARI gam 25 which was statistically similar with the variety Gourab having 89.46% apparently healthy seed and the lowest (73.54%) apparently healthy seed was recorded in the variety Sourav.

Table 1. Apparently healthy seed, undersized seed, incidence of black pointed seed and percent disease index (PDI) of ten wheat varieties

Name of Variety	Healthy seed (%)	Undersize seed (%)	Black point incidence (%)	Black point severity/Percent disease index (PDI)
Sourav	73.54 g	20.71 a	5.75 d	4.35 b
Kanchan	83.00 e	7.63 c	9.38 a	4.85 a
Prodip	87.47 bc	6.61 cd	5.92 d	3.08 d
Gourab	89.46 ab	4.00 e	6.88 c	3.68 c
Shatabdi	83.42 e	11.93 b	4.66 e	4.85 a
Protiva	79.04 f	12.46 b	8.50 b	4.60 ab
BARI gam 25	90.92 a	5.21 de	3.88 f	2.28 e
BARI gam 26	84.83 de	6.83 cd	8.33 b	4.38 b
BARI gam 27	86.17 cd	6.58 cd	7.25 c	2.93 d
BARI gam 28	85.42 cde	8.71 c	5.88 d	2.43 e
Cv %	2.35	19.42	9.37	10.38

*Figures in a column having similar letter(s) do not differ significantly at 5% level of probability

On the other hand, the highest (20.71%) undersized seed was recorded in the variety Sourav followed by Protiva and Shatabdi (12.46% and 11.93%,) respectively. The lowest (4.00%) undersized seed was found in Gourab

which was statistically similar with BARI gam 25 (5.21%). The lowest disease incidence (3.88%) and severity (2.28%) was recorded in BARI gam 25 which was statistically similar with the variety BARI gam 28 having 2.43% PDI. On the other hand highest disease incidence (9.38%) and severity (4.85%) was recorded in the variety Kanchan. Similar type of disease severity was also recorded in Shatabdi.

Sowing time of wheat found interfere the production of apparently healthy, undersized and black pointed seed. The highest apparently healthy seed (87.20%) was recorded in time sowing wheat than that of late sowing (81.56%). Minimum (7.50%) undersize seed was recorded in time sowing variety whereas, maximum (10.64%) found in late sowing variety. Similarly 5.31% black pointed seed was recorded in time sowing and 8.00% was recorded in late sowing wheat seed. Percent disease index (PDI) was recorded 2.21% in time sowing and 5.03% in late sowing of wheat varieties (Table 2).

Table 2. Apparently healthy seed, undersized seed, incidence of black pointed seed and percent disease index (PDI) of wheat varieties at different sowing date

Sowing time	Apparently healthy seed (%)	Undersize seed (%)	Black point incidence (%)	Black point severity /Percent disease index (PDI)
Time sowing	87.20	7.50	5.31	2.21
Late sowing	81.56	10.64	8.00	5.03
Cv%	2.35	19.42	9.37	10.38

Significant interaction effect was found between variety and sowing time in respect of apparently healthy seed undersized seed, black point seed incidence and black point severity. Among all the wheat varieties studied, the highest (92.83% and 91.67%) apparently healthy seed was recorded in both time and late sowing variety of Gourab. However, statistically similar apparently healthy seed was recorded in time and late sowing variety of BARI gam 25 (92.45% and 91.08%). On the other hand the lowest (72.00% and 75.33%) apparently healthy seed was found in late sowing variety of Sourav and time sowing variety of Kanchan which was statistically similar (75.08%) with the time sowing variety of protiva (Table 3).

Table 3. Apparently healthy seed, undersized seed, incidence of black pointed seed and percent disease index (PDI) of ten wheat varieties at two different sowing date

Variety	Sowing time	Apparently healthy seed (%)	Undersize seed (%)	Black point incidence (%)	Black point severity/percent disease index (PDI)
Sourave	Time sowing	86.08 def	8.42 def	5.50 gh	2.15 hi
	Late sowing	72.00 j	19.00 b	9.00 c	6.55 b
Kanchan	Time sowing	75.33 ij	15.17 c	9.50 c	2.30 ghi
	Late sowing	79.92 gh	6.83 efg	13.25 a	7.40 a
Prodip	Time sowing	87.58 cde	6.42 fg	6.00 efg	2.25 hi
	Late sowing	82.75 fg	9.75 de	7.50 d	3.90 e
Gourab	Time sowing	92.83 a	4.42 ghi	2.75 k	1.65 ij
	Late sowing	91.67 ab	3.58 ghi	4.75 hi	5.70 c
Shatabdi	Time sowing	87.67 cde	4.83 ghi	7.50 d	2.95 fg
	Late sowing	84.75 ef	8.50 def	6.75 def	4.35 de
Protiva	Time sowing	75.08 ij	22.42 a	2.50 k	3.90 e
	Late sowing	79.25 gh	17.43 bc	3.32 jk	4.85 d
BARI gam 25	Time sowing	92.45 ab	1.72 i	5.83 fgh	1.35 jk
	Late sowing	91.08 abc	4.92 ghi	4.00 ij	3.20 f
BARI gam 26	Time sowing	82.50 fg	11.50 d	6.00 efg	2.05 hi
	Late sowing	87.83 cde	3.08 hi	9.75 c	7.15 ab
BARI gam 27	Time sowing	89.00 bcd	6.00 fgh	5.00 ghi	0.85 k
	Late sowing	78.00 hi	10.08 de	11.92 b	5.00 d
BARI gam 28	Time sowing	84.67 ef	8.33 def	7.00 de	2.65 fgh
	Late sowing	86.08 def	8.92 def	5.00 ghi	2.20 hi
Cv %		2.35	19.42	9.37	10.38

*Figures in a column having similar letter(s) do not differ significantly at 5% level of probability

Among the ten wheat varieties at two different sowing date Protiva gave the highest (22.42% and 17.43%) undersized seed which was statistically significant than any other wheat variety. The lowest (1.72%) undersized seed was recorded in BARI gam 25 which was statistically similar with late sowing variety of BARI gam 26 (3.08%), BARI gam 25 (4.92%), time and late sowing variety of Gourab (4.42% and 3.58%) and time sowing variety of Shatabdi (4.83%). Black point incidence was found highest (13.25%) in late sowing variety of Kanchan and lowest in time and late sowing variety of Protiva (2.50% and 3.32%), time sowing variety of Gourab (2.75%), followed by late sowing variety of BARI gam 25 (3.32%) black pointed seed. Maximum percent disease index (7.40%) was found in the Kanchan variety of late sowing which was statistically similar

with the late sowing variety of BARI gam 26 (7.15%). Lowest PDI (0.85%) was recorded in the time sowing variety of BARI gam 27 which was statistically similar with the BARI gam 25 and Gourab variety of time sowing having 1.35% and 1.65% PDI, respectively.

Fungi associated with wheat seed

Nine different fungal genera namely *Bipolaris*, *Alternaria*, *Curvularia*, *Fusarium*, *Aspergillus*, *Epicoccum*, *Rhizopus*, *Nigrospora*, *Phoma* were recorded in ten different varieties of wheat seed. Among them *Bipolaris*, *Alternaria*, *Curvularia*, *Fusarium*, *Aspergillus* were found predominant. Almost all the varieties were infected with the fungal pathogen. The highest (54.50%) *Bipolaris* sp. was found in the variety kanchan followed by Prodip (34.50%), Sourav (31.00%) and BARI gam 26 (30.75%). Whereas, the lowest (13.50%) *Bipolaris* was observed in BARI gam 25 which was statistically similar with the variety Protiva (13.75%) and BARI gam 28 (14.00%), respectively (Table 4). Significant variation of *Alternaria* sp. was observed among all the variety used for the study. The highest (24.50%) *Alternaria* sp. was recorded in the variety Kanchan. However, statistically similar result was also found in Gourab (23.50%), Prodip (23.00%), Protiva (23.00%) and Shatabdi (21.50%). Minimum (9.75%) association of *Alternaria* sp. was recorded in BARI gam 28. Among all the varieties, highest association of *Curvularia* sp. (9.00%) was found in Protiva and Kanchan and the lowest (1.00%) in BARI gam 28 and prodip variety which was statistically similar with the variety Sourav.

Highest (10.25%) *Fusarium* sp. was recorded in the variety Shatabdi and lowest (1.00%) in the variety Prodip, BARI gam 27 and BARI gam 25 which was statistically similar with the variety BARI gam 26 (1.75%) and Gourab (2.00%). In Kanchan, *Aspergillus* sp. was found as the most predominant (10.75%) followed by BARI gam 25 having 7.50% while the least (1.00%) infection observed in BARI gam 27 which was statistically similar with Sourav (2.00%) and Gourab (2.00%). BARI gam 28 and Protiva were not infected with *Epicoccum* sp. but other varieties were infected with *Epicoccum* sp. Highest (12.75%) seed infection with *Epicoccum* sp. was found in Kanchan followed by Prodip (7.25%), Shatabdi (7.75%) and BARI gam 26 (7.50%). Lowest (2.75%) seed infection was recorded in the variety Sourav which was almost similar to the variety BARI gam 25 having 3.00% infection of *Epicoccum* sp. Interestingly, *Rhizopus* sp. infection was not found in the variety Sourav but in the other varieties were observed at minimum ranged (1.00% to 3.50%) where highest (3.50%) *Rhizopus* sp was recorded in the variety Kanchan and lowest (1.00%) was recorded in the variety Protiva, Prodip, Gourab, Shatabdi and BARI gam 26. The wheat varieties Protiva, BARI gam 27 and BARI gam 28 were found free from the infection of *Nigrospora* sp. However, maximum (5.25%) infection of *Nigrospora* sp. was found in the variety BARI gam 25 and lowest (3.50%) in Sourav which was statistically similar with BARI gam 26 having 3.75% infection of *Nigrospora* sp. BARI gam 26 having higher (1.25%) *Phoma* infection which was statistically similar with BARI gam 25 (1.00%) and the rest of the varieties were found free from the infection of *Phoma* sp.

Table 4. Fungi associated with wheat seed discoloration

Variety	Name of fungi								
	<i>Bipolaris</i>	<i>Alternaria</i>	<i>Curvularia</i>	<i>Fusarium</i>	<i>Aspergillus</i>	<i>Epicoccum</i>	<i>Rhizopus</i>	<i>Nigrospora</i>	<i>Phoma</i>
Sourav	31.00 bc	14.75 c	1.75 cd	2.00 cd	2.00 de	2.75 d	0.00 d	3.50 c	0.00 b
Kanchan	54.50 a	24.50 a	9.00 a	2.50 c	10.75 a	12.75 a	3.50 a	5.00 ab	0.00 b
Prodip	34.50 b	23.00 a	1.00 d	1.00 d	2.50 d	7.25 b	1.00 c	4.75 abc	0.00 b
Gourab	26.75 cd	23.50 a	6.25 b	2.00 cd	2.00 de	5.00 c	1.00 c	3.75 bc	0.00 b
Shatabdi	22.25 de	21.50 ab	5.75 b	10.25 a	2.75 d	7.75 b	1.00 c	5.00 ab	0.00 b
Protiva	13.75 f	23.00 a	9.00 a	8.75 b	3.00 cd	0.00 e	1.00 c	5.25 a	0.00 b
BARI gam 25	13.50 f	18.00 bc	6.00 b	1.00 d	7.50 b	3.00 d	2.00 b	0.00 d	1.00 a
BARI gam 26	30.75 bc	15.00 c	2.75 c	1.75 cd	4.00 c	7.50 b	1.00 c	3.75 bc	1.25 a
BARI gam 27	19.75 e	16.25 c	6.00 b	1.00 d	1.00 e	5.25 c	2.50 b	0.00 d	0.00 b
BARI gam 28	14.00 f	9.75 d	1.00 d	8.75 b	2.50 d	0.00 e	2.25 b	0.00 d	0.00 b
Cv%	12.69	14.45	22.59	19.86	20.94	22.95	28.08	28.25	99.38

*Figures in a column having similar letter(s) do not differ significantly at 5% level of probability

Significant variation of fungal infection at different sowing date was recorded (Table 5). The infection of *Bipolaris*, *Fusarium* and *Nigrospora* were recorded higher at late sowing wheat than that of time sowing. However, infection of *Alternaria*, *Curvularia*, *Aspergillus*, *Epicoccum*, *Rhizopus* and *Phoma* were recorded higher in time sowing and lower in late sowing.

Table 5. Fungi associated with wheat seed at different sowing date

Sowing time	Name of the fungi								
	<i>Bipolaris</i>	<i>Alternaria</i>	<i>Curvularia</i>	<i>Fusarium</i>	<i>Aspergillus</i>	<i>Epicoccum</i>	<i>Rhizopus</i>	<i>Nigrospora</i>	<i>Phoma</i>
Time sowing	11.60	19.75	5.90	2.65	3.85	5.75	2.55	3.00	0.25
Late sowing	40.55	18.10	3.80	5.15	3.75	4.50	0.50	3.20	0.20
Cv %	12.69	14.45	22.59	19.86	20.94	22.95	28.08	28.25	99.38

Interaction effect of variety and sowing time was also found significant in respect of fungal pathogen associated with wheat seed (Table 6). All the wheat varieties used in this experiment were found more or less infected by *Bipolaris* sp. and the infection was higher (72.50%) in late sowing variety of Kanchan which was statistically different from any other varieties and lower (2.00%) *Bipolaris* sp. infection was in BARI gam 27 of time sowing. The time sowing variety of BARI gam 25 and Shatabdi showed statistically similar result (4.00% and 5.50%) of infection. In case of *Alternaria* sp. late sowing variety of Kanchan showed maximum (28.50%) infection which was statistically similar with late sowing variety of Shatabdi (27%), Prodip (28.50%) and Protiva (26.50%) and minimum (8.00%) infection was recorded in time sowing variety of BARI gam 25.

Table 6. Fungi associated with wheat seeds at different sowing date

Variety	Sowing time	Name of the fungi								
		<i>Bipolaris</i>	<i>Alternaria</i>	<i>Curvularia</i>	<i>Fusarium</i>	<i>Aspergillus</i>	<i>Epicoccum</i>	<i>Rhizopus</i>	<i>Nigrospora</i>	<i>Phoma</i>
Sourave	Time	14.00 hi	14.00 efg	3.50 de	2.00 e	2.00 e	0.00 f	0.00 e	2.00 e	0.00 c
	Late	48.00 cd	15.50 def	0.00 f	2.00 e	2.00 e	5.50 d	0.00 e	5.00 b	0.00 c
Kanchan	Time	36.50 f	20.00 bc	4.00 d	2.00 e	5.00 c	11.00 bc	0.00 e	3.00 de	0.00 c
	Late	72.50 a	28.50 a	14.00 a	3.00 de	13.00 a	14.50 a	7.00 a	7.00 a	0.00 c
Prodip	Time	12.50 hij	20.50 b	0.00 f	0.00 f	2.00 e	3.00 e	0.00 e	4.50 bc	0.00 c
	Late	56.50 b	28.50 a	2.00 e	2.00 e	3.00 de	11.50 bc	2.00 d	5.00 b	0.00 c
Gourab	Time	8.50 jkl	11.50 fgh	10.00bc	2.00 e	2.00 e	3.50 e	0.00 e	3.00 de	0.00 c
	Late	45.00 de	18.50 bcd	2.50 de	2.00 e	2.00 e	6.50 d	2.00 d	4.50 bc	0.00 c
Shatabdi	Time	5.50 klm	16.00 cde	0.00 f	0.00 f	2.00 e	10.00 c	2.00 d	2.50 de	0.00 c
	Late	42.50 e	27.00 a	11.50 b	17.00 a	3.50 d	5.50 d	2.00 d	7.50 a	0.00 c
Protiva	Time	11.50 hij	19.50 bcd	4.00 d	7.50 c	0.00 f	0.00 f	0.00 e	0.00 f	0.00 c
	Late	16.00 h	26.50 a	14.00 a	10.00 b	6.00 c	0.00 f	2.00 d	0.00 f	0.00 c
BARI gam 25	Time	4.00 lm	8.00 h	2.00 e	0.00 f	2.00 e	2.50 e	0.00 e	3.50 cd	0.00 c
	Late	23.00 g	16.00 cde	10.00 bc	2.00 e	3.00 de	3.50 e	2.00 d	7.00 a	2.00 b
BARI gam 26	Time	10.00 ijk	19.00 bcd	3.00 de	0.00 f	10.50 b	3.00 e	0.00 e	2.50 de	2.50 a
	Late	51.50 c	11.00 gh	2.50 de	3.50 d	11.00 b	12.00 b	2.00 d	5.00 b	0.00 c
BARI gam 27	Time	2.00 m	21.00 b	9.00 c	2.00 e	0.00 f	3.50 e	0.00 e	0.00 f	0.00 c
	Late	34.00 f	11.50 fgh	3.00 de	3.50 d	2.00 e	7.00 d	3.00 c	0.00 f	0.00 c
BARI gam 28	Time	11.50 hij	19.50 bcd	0.00 f	7.50 c	0.00 f	0.00 f	0.00 e	0.00 f	0.00 c
	Late	16.50 h	26.50 a	2.00 e	10.00b	5.00 c	0.00 f	4.20 b	0.00 f	0.00 c
Cv%		12.69	14.45	22.59	19.86	20.94	22.95	28.08	28.25	99.38

*Figures in a column having similar letter(s) do not differ significantly at 5% level of probability

All the wheat varieties of time and late sowing showed significant result in case of *Curvularia* infection. Late sowing variety of Kanchan and Protiva had highest (14.00%) infection of *Curvularia* and lowest (2.00%) was recorded in the late sowing variety of Prodip and time sowing variety of BARI gam 25 and late sowing variety of BARI gam 28 having 2.00% infection. Among the ten wheat varieties used, time sowing variety of Prodip, Shatabdi, BARI gam 25 and BARI gam 26 were found free from *Fusarium* infection where highest (17.00%) *Fusarium* was recorded in the late sowing variety of Shatabdi and lowest (2.00%) infection in time and late sowing variety of Sourav, time sowing variety of Kanchan, Gourab, BARI gam 27 and late sowing variety of BARI gam 25. Highest (13.00%) infection of *Aspergillus* sp. was recorded in the late sowing variety of Kanchan and lowest (2.00%) infection in time and late sowing variety of Sourav, Gourab, time sowing variety of Shatabdi, BARI gam 25, BARI gam 28 and late sowing variety of BARI gam 27 all having 2.00% infection, respectively. Time sowing variety of Protiva and BARI gam 27 were found free from *Aspergillus* sp infection. *Epicoccum* sp. was not found in time sowing variety of Sourav, time and late sowing variety of Protiva and BARI gam 28. Highest (14.50%) infection was found in late sowing variety of Kanchan and lowest infection in the time sowing variety of BARI gam 25 having 2.50%, BARI gam 26 having 3.00%, Prodip, having 3.00% Gourab having 3.50% and BARI gam 27 having 3.50%. Maximum (7.00%) *Rhizopus* sp. was recorded in the late sowing variety of Kanchan and minimum (2.00%) in the late sowing variety of Protiva, Prodip, Gourab, Shatabdi, BARI gam 26, BARI gam 28 and BARI gam 25. On the other hand, time sowing variety of BARI gam 26, BARI gam 27 and BARI gam 28 were free of *Rhizopus* infection. Surprisingly, *Nigrospora* sp. were not found in time and late sowing varieties of Protiva, BARI gam 27 and BARI gam 28. Shatabdi having uppermost (7.50%) infection of *Nigrospora* sp., which was statistically similar with late sowing variety of Kanchan and BARI gam 25. Lower most (2.00%) infection was recorded in time sowing variety of Sourav, which was statistically similar with time sowing variety of Kanchan, Gourav having 3.00% and time sowing variety of BARI gam 26 and Shatabdi having 2.50% infection, respectively. Time sowing variety of BARI gam 26 and late sowing variety of BARI gam 25 were only infected by *Phoma* sp.

Blank point disease Incidence, severity, fungi associated with seed were studied of wheat varieties at different sowing date. The variety taken for this study were of time and late sowing Sourav, Kanchan, Prodip, Gourab, Shatabdi, Protiva, BARI gam 25, BARI gam 26, BARI gam 27 and BARI gam 28. Among the ten varieties studied, none of the variety was found free from black point infection but depend on the variety and time of sowing, disease incidence varied significantly. Highest 9.38% black point incidence was recorded in Kanchan

and 3.88% in BARI gam 25. Other varieties showed different level of black point infection where Sourav, Prodip and BARI gam 28 showed lower level of infection, which was around 6.00%. The variety Gourav and BARI gam 27 showed 6.88% and 7.25% black pointed seed, respectively. More than 80% apparently healthy seeds were observed in all the varieties except Sourav. Presence of more than 80% healthy seed is the indication of good quality seed and minimum carryover of black point pathogen in the next season. A report from Wheat Research Centre revealed the highest black point incidence in Sourav 21% followed by Sonalika while the lowest observed in Shatabdi 13% and Prodip 13%. The report also noted that overall black point incidence in different varieties were around 15% (BARI 2004). In another report of BARI (2009) noted that the line RAW-1059 showed the lowest percent of black point infection, while the highest prevalence of black point was observed in variety Sourav. Among 32 cultivars of wheat, none of the variety was found resistant to black point disease (Cappelli *et al.* 1993). Black point incidence was found higher in late sowing varieties compared to time sowing. Susceptible late sowing variety Kanchan showed the highest 13.25% incidence of black point and least affected variety Protiva had lower incidence in both time and late sowing. The differences of black point incidence in different sowing date may related to the environmental conditions of two seasons. However, low temperature is one of the most important environmental parameters for the black point incidence in late sown wheat in subtropical environments. (Chakrabarti *et al.* 2011) Black point incidence was higher in late sowing variety of wheat compared to time was also reported by different research organization in Bangladesh (BARI 2007; BARI 2009). After separation of black pointed seeds into different grades, it was observed that the variety Kanchan had the highest percent of black pointed seeds. Percent disease index (PDI) was also found highest in the variety Kanchan in compare with other variety used. The Kanchan variety was released in 1983 and observed more disease susceptible to the infection of *Bipolaris* leaf blight; consequently low yield (Baksh *et al.* 2009). Highest percentage of black pointed seed under different grades and PDI in the variety Kanchan was also reported by Malaker (2003). PDI was also found higher in late sowing season compared to time sowing. Significant yield losses were reported in late sown wheat when terminal heat stress aggravates the disease severity during grain filling stages of the crop. However, the degree of disease severity and yield losses were depends on variety and growing condition (BARI 2013). Wheat Research Center of Bangladesh (WRC) of Agricultural Research Institute (BARI) has released 24 varieties developed by traditional breeding approach and many of those are better than Kanchan (Rashid *et al.* 2004) in respect to yield and other characters under control environment. Nine different seed borne fungi were detected from all the used wheat seeds in laboratory study. The following fungi were detected from wheat seeds *viz.* *Bipolaris* sp. *Alternaria* sp. *Curvularia* sp. *Fusarium* sp. *Aspergillus* sp. *Epicoccum* sp. *Rhizopus* sp. *Nigrospora* sp. and *Phoma* sp. Among all the detected fungi, the lowest incidence of *Bipolaris* sp. 13.50% was observed in BARI gam 25, and the varieties Protiva and BARI gam 28 also showed lower level of infection 14.00% while the highest 54.50% was found in Kanchan. Incidence of other seed borne fungi were varied depend on the variety and sowing time. In overall observation, the variety Kanchan had maximum association of seed borne fungi. Maximum number of fungal conidia was recorded in the variety Sonalika (320) which was closely followed by variety Kanchan (280) (Laila *et al.* 2010). We observed the lowest association of different fungi in BARI gam 25 which is in agreement with the previous study where the variety Kanchan showed higher incidence and severity of black point infection (Laila *et al.* 2010). Many authors identified *A. tenuis*, *B. sorokiniana*, *C. lunata* and *Fusarium* sp. as causal organism of black point of wheat and also found that the incidence of *B. sorokiniana* increases with level of infestation and severity of black point infection (Hossain 2000). *Alternaria alternate*, *Aspergillus flavus*, *Aspergillus niger*, *Bipolaris sorokiniana*, *Curvularia lunata*, *Chaetomium globosum*, *Diplodia* spp. *Fusarium oxysporum*, *Macrophomina phaseolina*, *Penicillium* spp. *Rhizopus* spp. were found as seed borne mycoflora of some commercial wheat varieties cultivated in Punjab that reduces the germination of wheat in laboratory condition (Hussain *et al.* 2013). Whereas Kolawole *et al.* (2013) isolated eight post-harvest fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates*, *Penicillium* spp., *Fusarium solani* *Rhizopus* spp., *Alternaria* spp., *Trichoderma* spp. from wheat in Lagos state, Nigeria. Different research organization reported incidence of black point infection of wheat seed on recently released varieties and isolated the fungi associated with wheat seeds were 19, 22, 28 during the year of 2006, 2007 and 2009, respectively (BARI 2006, 2007 and 2009). Among all the detected fungi, *B. sorokiniana* and *A. alternata* were found predominant with lowest 24.00% *B. sorokiniana* in Shatabdi and highest 44.00% in Sonalika. On the other hand, during 2007 Shatabdi and Gourab showed lowest and Sonalika showed highest level of infection but during 2009, the line BAW-1059 showed lowest infection of *B. sorokiniana* and highest in Sourav (BARI 2006, 2007, 2009). *Alternaria alternate* was a frequently isolated fungus and sometimes isolated as most dominant in black point disease of wheat (Ozer 2005) and *Fusarium culmorum* also found as average 15.5% infection level with wheat seeds (Zara *et al.* 2006).

CONCLUSION

From the above study, it may be concluded that the variety Kanchan possesses highest incidence and severity of black pointed seed and maximum seed borne pathogen like *Bipolaris* sp., *Alternaria* sp. and *Curvularia* sp. On the other hand BARI gam 27 had least infection of the disease that can minimize the black point disease of wheat.

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