IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 11, Issue 1 Ver. II (January 2018), PP 80-92
www.iosrjournals.org

Seed Health Status of Selected Wheat Cultivars Available in Bangladesh

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Abstract: *This study has been planned to investigate the severity of black point disease on wheat variety available in Bangladesh. Seeds of twenty wheat varieties viz. Khri, Kahyanso, Sonora-64, Sonalika, Paron-76, Kanchan, Aghrani, Protiva, Sourav, Gourab, Shatabdi, Sufi, Bijoy, Prodip, BARI-25, BARI-26, BARI-27, BARI-28, BARI-29 and BARI-30 were sown in plastic trays containing sand medium to determine the incidence and severity of black point and associated fungi. Wheat seeds sown in peterdishes containing blotting paper to determine the incidence and severity of black point and fungi associated with wheat seed of different varieties. The highest black point diseases (22.25%) were found in the variety of Khri followed by Sonara-64, Bijoy, Kanchan, Sonalika, Shatabdi, Sourav and Kahyonso while the lowest (3.75%) was found in BARI Gom 30, followed by BARI Gom 27, BARI Gom 28, BARI Gom 29 and BARI Gom 26. The study revealed that BARI Gom 27 was moderately resistant to black point disease while Sonalika showed susceptibility. The result showed that the highest germination percentage 14 DAS (94.00%), the numbers of normal seedlings (79.00%), percent healthy seedling incidence (84%), shoot weight (0.19g), root length (34.78 cm), shoot length (0.86 g) was found in BARI Gom 27 but the highest root weight (24.61 cm) was found in BARI Gom 25, however, the lowest was found in Sonalika. This study gave valuable information regarding the incidence and severity of black point disease of wheat.*

Date of Submission: 9-01-2018 Date of acceptance: 26-01-2018

I. Introduction

Wheat (*Triticum aestivum* L.) is a cereal crop belonging to the family Gramineae. Total wheat production in all over the world in 2016 was 749 million tons, making it the third most produced cereal after maize and rice (FAO, 2016). Next to rice, it is second major cereal crop in Bangladesh, but the yield of wheat is too low in comparison to the world wheat production. In 2014-15, 436822.1338 hectors land was under cultivation and total yield of grains was 13, 47,926 M. ton (BBS, 2015). Edible portion (100g) of wheat contains 11.50% proteins, 59.40% carbohydrate, 9.10% fats, 10.60% crude fiber and 1.80% ash (Pathak et al., 2013). Wheat also contributes essential amino acids, minerals, vitamins beneficial phytochemicals and dietary fiber components to the human diet (Shewry, 2009). Grains of the wheat is used to make flour for leavened, flat and steamed breads, biscuits, cookies, cakes, pasta, noodles couscous (Stanley, 2003) and for fermentation to make beer (Palmer and John, 2001). In Bangladesh, the production of wheat is low due to the many reasons and among them abnormality in seed is a major constraint (Enikuomehin, 2005). Black point (kernel smudge or smudge) is a fungal disease that affects wheat, barley and rye which caused by various species of Alternaria, Fusarium, and Helminthosporium and possibly other fungal genera (Wise, 1987). Wheat is suffered by as much as 120 different diseases, among them, 42 are seed borne and fungi cause 35 diseases (Hasan et al., 2005). Pathogens associated with the black-point disease has become one of the most serious problems of wheat, causing great losses in both yield and quality of wheat grains (Bhandari et al., 2003; Fernandez and Conner, 2011; Draz et al., 2016) and characterized by a dark discoloration of the embryo sides of the wheat and barley grains (Mak et al., 2006). Diseased kernels are discolored, and are black at the ends of the seed. Embryos are often shriveled and brown to black in color. When seed moisture content exceeds 20%, coupled with the relative humidity above 90%, the amount of black point increases dramatically (Toklu et al., 2007). Diseased plants are often uniform in the fields or are associated with wetter, more humid areas of the field. Seed with black point is

more likely to have seedling blight and root rot problems. In most countries where cereals are grown commonly, black point can result in reduced grain quality and value (Wang et al., 2003). Black point is frequently recognizing disease in all wheat growing region entire the world (Toklu et al., 2007) and delayed seedling emergence and reduced seedling vigour (Ozer, 2005). 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). 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 *B. sorokiniana, A. alternata, Curvularia, Cladosporium, Epicoccum, Aspergillus, Rhizopus, Penicillium, Trichodermaetc*. (Kolawole et al., 2013; Islam et al., 2015; Pathak et al., 2013). Seed treatments are not only controls seed borne diseases but also improve the seed health of plantsand crop yield. In the light of above discussion, this study aimed to investigate the severity of black point disease with different wheat culltivars grown in Bangladesh and also to find out the effect of black point disease on seed germination.

II. Materials and Methods

2.1 Variety used in the experiment

The most commonly cultivated 20 wheat varieties viz., Kheri, Kalyansone, Sonora 64, Sonalika, Pavon 76, Kanchan, Aghrani, Protiva, Sourav, Gourab, Shatabdi, Sufi, Bijoy, Prodip, BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 29, BARI Gom 30 were included in the study.

2.2 Collection of seed

Seed samples of all the 20 varieties were collected from wheat research centre, Dinajpur. From each sample, 0.5 kg wheat seeds were collected from their storage removing seed from the top 15 cm of the seed depth. The seed samples were collected following the rules of International Seed Testing Association (ISTA, 2001).

2.3 Preparation of working sample

The collected seed samples were registered maintaining the sample number and sowing time. After proper registration, the samples were preserved in a refrigerator in the laboratory. From each sample, 400 seeds were taken randomly for the checking of seed quality and health analysis viz., infected seeds, severity of black point, sorting of apparently healthy seed (best seed) and detection of seed borne fungal pathogens for germination, seedling vigor and other test.

2.4 Black point incidence of wheat

Collected seeds were analyzed in the laboratory for, prevalence and severity of black point, apparently healthy seed (best seed), seed germination and seed health. Black pointed seeds were sorted out from the collected seed samples manually by physical sorting.

Black point incidence was determined by the following formula:

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

2.5 Severity of black point infection of wheat

The collected seeds were separated into six different grades on the basis of severity of black point infection indicated in Fig. 1. 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 shriveled.

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

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

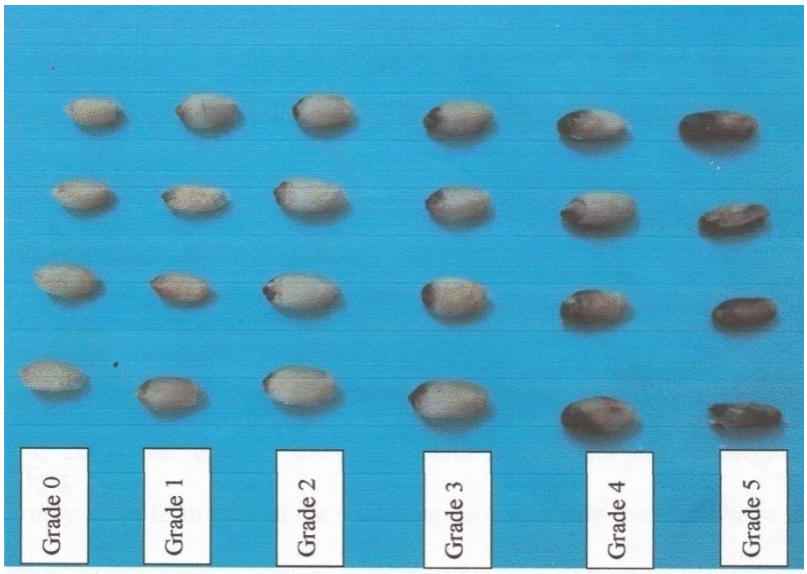

Figure 1. Black point infected wheat seeds of different grades (Afroz, 2012)

2.6 Design of the experiment

The experiment was conducted in complete randomized design(CRD) with four replications. Layout of the study was performed as single factor factorial experiment i.e. wheat variety.

2.7 Health status of collected wheat seed

Health status of all seed samples were analyzed for detection of seed borne fungi. The blotter method as mentioned by International Seed Testing Association (ISTA, 2001) was used for this study. 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 petridish. The petridishes were placed on laboratory bench at room temperature for incubation under 12/12 h light-darkness cycle. 7 DASof incubation, the seeds were examined under a stereobinocular microscope for detection of the growth of fungi. In case of confusion, temporary water mounts were prepared and were observed under a compound microscope for identification of associated fungi. The fungal genera and species were identified using standard keys (Ellis, 1960; Benoit and Mathur, 1970; Booth 1971; Chidambaram et al. 1973 and Mathur and Kongsdal, 2003).

2.8 Seed germination

Germination test was done in plastic trays filled with sterilized sand obtained from the riverbank of Punarvaba. Four hundred seeds were randomly taken from each sample for petridish. For germination test fifty seeds were sown in each tray and the trays were then kept on the roof of academic building for 14 DAS. Care was taken to ensure the proper sunlight and moisture in soil of tray. Then data on percent seed germination, normal, abnormal, healthy and diseased seedlings were recorded according to International Seed Testing Association Rules (ISTA, 2001).

2.8.1 Categories of normal and abnormal seedlings

2.8.1.1 Normal seedlings

Normal seedlings were categorized by following points:

Intact seedling with all essential structures, well developed, complete in proportion and healthy.Seedlings with slight defects are (a) primary root defective but with limited damage or slight growth reduction (b) primary root defective but with sufficient well developed.Seedlings with secondary infection that would have fallen into category 2 or 1 but for infection by fungi or bacteria from sources other than parent seeds.

2.8.1.2. Abnormal seedlings

During recording the abnormalities of germinating seeds and seedlings, the following points were considered:

1. Seminal roots missing/stunted or broken and decayed due to primary infection.
2. Coleoptiles missing /split deformed or bent over.
3. Shoot system (the mesocotyl if developed) broken/decayed
4. Leaf missing /extending less than halfway of the coleoptile, shredded or deformed
5. Seedling as a whole deformed spindly, discolored or decayed as a result of primary infection.
6. Seedling with slight defects and seedlings with secondary infection.

7. Blackened dead or decayed seed.
8. The number of seeds that produced normal seedlings were counted and the percentage was calculated over the number of seeds placed for the test.

2.8.2 Measurement of plant shoots and root growth and weight

At first, the soil of the tray was watered to make it moist for easy uprooting of the plant. Then the plant along with root was lifted from the tray and dipped in a bucket of water. The root of each seedling was washed and cleaned carefully with gentle running tap water. The root portion was separated from shoot portion with sharp knife. Ten (10) seedlings were randomly selected from each tray and their individual shoot length was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length of root was measured from the starting point of root to the largest available lateral root apex, fresh weight shoot and root was also determined with the help of digital balance.

2.8.3 Determination of vigour index

Vigour index of the seedling was determined by following formula (Baki and Anderson, 1972).
Vigour index = (mean of root length + mean of shoot length) × % seed germination

2.9 Statistical analysis

Data on various parameters were subjected to analysis of variance following MSTAT-C computer programme and the means were compared by Duncan's Multiple Range Test (DMRT).

III. Results and Discussion

3.1 Seed germination of different wheat genotypes

3.1.1 Germination 7 DAS(%)

A significant variation in germination percentage was observed among all the wheat varieties used in this study (Table 1). The highest germination percentage 7 DAS (91.00%) was recorded in the variety of Kanchan which is statistically similar to the varieties of BARI Gom 27 (89.00%) but the lowest germination percentage 7 DAS (41.00%) was recorded in the variety of Sonalika. The variation in the germination percentage may be occurred due to variation in genetic makeup or the association of seed borne pathogens. However, reduced germination percentage was found to be related with the severity of black point infection. Similar to our findings, many reports also showed the reduced germination of wheat seeds is related to the severity of black point infection (Chowdhury, 2008; Hossain, 2000). The germination of seed infected with seed-borne pathogens may be caused the pathogens attack and kill the seedling. Seed-borne diseases have been found to affect the growth and productivity of crop plants.

3.1.2 Germination 14 DAS (%)

The wheat seed germination 14 days was different from the 7 DAS, however, the trend was similar (Table 1). The highest germination percentage 14 DAS (94.00%) was recorded in the variety of BARI Gom 27 whereas the lowest germination percentage after 14 days (41.00%) was recorded in the variety of Sonalika. However, reduced seed germination is found to relate with the association of black point infection. Previous studies also showed that black point infection cause severe reduction in wheat seed germination, seedling emergence etc. (Chowdhury, 2008; Hossain, 2000).

3.2 Seedlings emergence of different wheat genotypes

3.2.1 Normal Seedlings 14 DAS(%)

Percentage of normal seedlings 14 DAS was significantly varied among varieties (Table1). The numbers of normal seedlings were found higher in BARI Gom 27 (79.00%) which was statistically similar to the variety of Protiva (78.00%) and Bijoy (78.00%) while the lowest number recored in the variety of Sonalika (24.00%), Khri (30.00%) and Kahyanso (32.00%). However, gradual decline of seedling emergence was observed with the increase of black point infected seed. Similar to our findings, Hossain (2000) observed that, black point infection greatly affected seed germination and seedling emergence of wheat and percentage reduction in germination become higher with the increasing level of black pointed seed.

3.2.2 Abnormal Seedlings 14 DAS (%)

In our study, all wheat varieties showedvarying degree of abnormal seedlings 14 DAS of germination (Table 1). Paron-76 showed the maximum abnormal seedling (33.00%) which was followed by the variety of Aghrani (31.00%). The minimum abnormal seedling 14 DAS (3.00%) was observed in BARI Gom 27 which was statistically similar with Sourav (5.00%). The discoloration of most seeds revealed black-point symptom as described by Varshney (1990).

Table 1: The Effect of Black Point Disease on Wheat Varieties

Variety	Germination 7DAS	Germination 14 DAS	Normal 14 DAS	Abnormal 14 DAS
Kheri	48.00 ij	48.00 fg	30.00 fg	18.00 e
Kalyansona	52.00 hi	53.00 eg	32.00 fg	21.00 d
Sonora 64	82.00 af	85.00 ac	60.00 bd	25.00 c
Sonalika	41.00 j	41.00 g	24.00 g	17.00 e
Pavon 76	76.00 cg	77.00 ad	43.00 ef	33.00 a
Kanchan	91.00 a	93.00 ab	75.00 ac	18.00 e
Aghrani	87.00 ac	87.00 ac	66.00 ad	31.00 ab
Protiva	87.00 ac	89.00 ac	79.00 a	10.00 gi
Sourav	73.00 eg	81.00 ad	76.00 ab	5.00 lm
Gourab	59.00 h	64.00 df	60.00 bd	9.00 hj
Shatabdi	76.00 cg	78.00 ad	63.00 ad	10.00 gi
Sufi	79.00 bg	79.00 ad	67.00 ad	12.00 fg
Bijoy	84.00 ae	85.00 ac	78.00 a	7.00 jl
Prodip	72.00 fg	72.00 cd	58.00 ce	14.00 f
Bari Gom 25	74.00 dg	74.00 bd	59.00 be	9.00 hj
Bari Gom 26	70.00 g	70.00 ce	60.00 bd	29.00 b
Bari Gom 27	89.00 ab	94.00 a	78.00 a	3.00 m
Bari Gom 28	84.00 ae	84.00 ac	73.00 ad	11.00 gh
Bari Gom 29	79.00 bg	80.00 ad	74.00 ac	6.00 kl
Bari Gom 30	85.00 ad	85.00 ac	56.00 de	8.00 ik
Level of significance	**	**	**	**
CV %	6.50	10.29	11.86	7.09

Same letter(s) within the same column do not differ significantly at 5% levelof significance.
** Highly significance (p≤ 1%)

3.3 Health status of the emerged seedlings

3.3.1 Healthy seedling

Percent healthy seed varied significantly depending on wheat variety (Table 2). The highest healthy seedling (84%) was recorded in BARI Gom 27 and statistically similar results were also observed in BARI Gom 30(73%), while the lowest healthy seedling (20%) was recorded in the variety of Sonalika. A report from Wheat Research Center 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).

3.3.2 Diseased seedlings

The diseased seedling showed significant variation among the treatment (Table 2). The highest diseased seedling (47%) was recorded the variety of Sonora-64 where the lowest (10%) was observed in the variety of BARI Gom 27. The susceptibility of different wheat cultivars to the black point diseasemay be occured due to the genetically variation (BARI 2004). In a few cases, a negative effect of black point on germination and seedling emergence was observed in bread wheat cultivars (Conner et al., 1996; Toklu et al., 1999). Zhimin et al. (1998) reported that seed germination and seedling growth decreased with the increase in susceptibility to a variety of infection.

3.3.3 Percent Disease Index of Black point incidence

As indicated in Table 2, the maximum percentage of black point incidence was recorded in variety of Khri (197.5%) which was significantly similar to the Kahyanso (191.3%), whereas, the minimum percentage of black point incidence was observed in the variety of BARI Gom 30 (47.50%), which was statistically similar to BARI Gom 29 (55.00%), BARI Gom 28 (62.50%), BARI Gom 27 (68.75%), and BARI Gom 26 (68.75%).Previously, the highest black point incidence was found in Sourav (21%) followed by Sonalika (17%) and lower in Shatabdi (13%) and Prodip (13%) (BARI, 2004; Afroz, 2012). However, black point incidence may be related to the groing season, environmental conditions, and genetic makeup of the plant (Chakarabarti et al., 2011; BARI 2007; BARI 2009). BARI (2004) estimated overall black point incidence abount 15% in wheat seeds of different varieties grown in different locations of Bangladesh.

Table 2: The Incidence of Black Point Disease on Healthy, Diseased Seedling and PDI.

Varieties	Healthy seedling	Diseased seedling	PDI
Kheri	25.00gh	23.00 df	197.50 a
Kalyansona	21.00 gh	32.00 bd	191.30 a
Sonora 64	38.00 eg	47.00 a	163.80 b
Sonalika	20.00 h	21.00 df	141.30 c
Pavon 76	37.00 eh	40.00 ab	137.50 cd
Kanchan	57.00 bd	36.00 ac	128.80 ce
Aghrani	47.00 df	40.00 ab	126.30 cf
Protiva	64.00 bd	25.00 ce	125.00 df
Sourav	68.00 ac	13.00 ef	117.50 ef
Gourab	35.00 eh	17.00 ef	112.50 f
Shatabdi	62.00 bd	16.00 ef	80.00 g
Sufi	37.00 eh	42.00 ab	78.58 g
Bijoy	71.00 ab	14.00 ef	77.50 gh
Prodip	48.00 df	39.00 ab	75.42 gh
Bari Gom 25	51.00 ce	23.00 df	73.75 gh
Bari Gom 26	33.00 fh	37.00 ac	68.75 gi
Bari Gom 27	84.00 a	10.00 f	68.75 gi
Bari Gom 28	67.00 ac	17.00 ef	62.50 hj
Bari Gom 29	67.00 ac	13.00 ef	55.00 ij
Bari Gom 30	73.00 ab	12.00 ef	47.50 j
Level of significance	**	**	**
CV %	14.75	21.71	8.64%

Same letter(s) within the same column do not differ significantly at 5% levelof significance.
** Highly significance (p≤ 1%)

3.4 Plant growth parameters of different wheat genotypes

3.4.1 Shoot length (cm)

Highly significant differences were obeseerved among all the wheat cultivar used in this study in regards to shoot length(Table3). The highest shoot length (24.61cm) was recorded in the variety of BARI Gom 25 which was statistically similar toBARI Gom 27 (23.90cm).In contrast, the lowest shoot length (12.86cm) was observed the variety of Paron-76.Similar to our fingings, Rahman and Islam (1998) also observed varying seedling vigor, germination, shoot length and root height with the increase of black point infection.Karwasraet al. (2007) reported that the effect of black point disease caused by D.sorokinianaor A. tenuison, the seed vigor-related parameters (1000-grain weight, germination, and shoot and root growth of bold-seeded (Sonalika) wheat cultivars decreaed markedly with the increase in the severity of infection. .

3.4.2 Root length (cm)

Significant variations of root length among varieties were also observed in our study (Table3). The highest root length (34.78cm) was found in the variety of BARI Gom 27 and the lowest (5.73cm) was recorded in the variety of Kahyonso. However, root length may be decreased with the increase of black point infection grade where seedlings developed from poor vigoured seeds (Rashid, 1997).Rana and Gupta (1982) found that wheat seed infected with black point pathogens greatly affect not only seed germination but also root and shoot growth of the seedlings, the effect being very prominent on root growth.

3.4.3 Shoot weight (g)

Table 3 reveals that the highest shoot weight (0.18g) was recorded in the variety of BARI Gom 27 which was statistically similar the varieties of Shatabdi (0.18g), BARI Gom 29 (0.17g), BARI Gom 25 (0.16g), BARI Gom 30 (0.16 g), BARI Gom 28 (0.16g) and BARI Gom 26 (0.15g) and the lowest (0.05g) was observed in the variety of Sonalika.Rana and Gupta (1982) reported that, black point infection greatly affected root and shoot growth of seedlings; Also (Hossain 2000) observed that shoot and root weight, length and vigor index were decreased with the increase of black point infection level in the seed sample.

3.4.4 Root weight (g)

Significantly root weight varied among varieties (Table3). The highest root weight (0.85g) was recorded in the variety of BARI gam-27 while the lowest root weight (0.04g) was recorded in the variety of Sonalika which was statistically similar to Shatabdi (0.04g), Khri (0.06g), Sourav (0.06g), Sonora-64 (0.06g), Kahyonso (0.06g), Paron-76 (0.07g) and Kanchan (0.07g). Karwasra et al. (2007) reported that the effect of black point disease caused by *D. sorokiniana* or *A. tenuis* on the seed vigor- related parameters (1000-grain

weight, germination, and shoot and root growth). Shoot and root growth also decreased as the level of black point infection increased (Karwasra et al., 2007).

Table 3: The effect of black point disease on shoot, root length and shoot, root weight

Variety	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)
Kheri	14.57 hi	14.03 eg	0.08 bd	0.07 gi
Kalyansona	14.87 gi	5.73 k	0.14 ac	0.07 gi
Sonora 64	17.67 fh	8.87 hk	0.07 cd	0.07 gi
Sonalika	14.48 hi	7.45 ik	0.06 d	0.04 i
Pavon 76	12.86 i	6.96 jk	0.06 cd	0.07 gi
Kanchan	16.33 fh	9.545hj	0.06 cd	0.07 gi
Aghrani	18.54 df	25.50 b	0.11 ad	0.48 b
Protiva	19.44 cf	19.44 c	0.13 ad	0.11 di
Sourav	22.28 ac	17.10 ce	0.11 ad	0.07 gi
Gourab	17.27 fh	11.22 gh	0.11 ad	0.09 fi
Shatabdi	21.94 ac	18.33 cd	0.19 a	0.04 hi
Sufi	21.13 be	19.56 c	0.13 ad	0.10 ei
Bijoy	23.80 ab	14.93 df	0.15 ab	0.12 dh
Prodip	21.92 ac	20.13 c	0.13 ad	0.12 dg
Bari Gom 25	24.61 a	15.18 df	0.17 a	0.18 d
Bari Gom 26	22.28 ac	11.19 gh	0.16 a	0.54 b
Bari Gom 27	23.90 ab	34.78 a	0.19 a	0.86 a
Bari Gom 28	22.40 ac	10.63 gi	0.17 a	0.16 df
Bari Gom 29	18.00 eg	17.07 ce	0.18 a	0.17 de
Bari Gom 30	21.47 ad	12.02 fh	0.17 a	0.36 c
Level of significance	**	**	**	**
CV %	7.18	10.01	21.59	16.74

Same letter(s) within the same column do not differ significantly at 5% levelof significance.
** Highly significance (p≤ 1%)

3.5 Association of seed borne fungi in different wheat genotypes

3.5.1 *Fusarium sp*. (%)

The present study revealed that, there was a significant variation of the association of Fusariumsp.among the varieties (Table 4.1). The highest incidence of *Fusarium* sp. (24.25%) was recorded in the variety of Sonara-64 which was statistically similar the variety of Bijoy (24.25%), whereas, the lowest infection (3.250%) was found in the varieties of BARI Gom 27 and BARI Gom 26. Parry et al. (1995) had listed *F. grammearumas* one of the several *Fusarium* sp. associated with the head blight of wheat. *F. moniliforme* was found to be associated with pink/purple seeds. Grain contaminated with *Fusarium* species which produce mycotoxins may be completely unusable for feed, food and malting purposes. Several studies have shown that, up to 17 species of *Fusarium* can be readily isolated from grain cereals. Similar observation was reported by Neergaard (1979). Saari and Prescott (1986) reported that at least three different fungi possibly causing black point of wheat seed. *D. sorokiniana* causes some reduction in seed germination and also causes discoloration of embryo end of seed, while *Fusarium sp*. causes discoloration of such grains.

3.5.2 *Bipolaris sp*. (%)

Bipolaris sp. was found as the most prevalent associated fungus among all the varieties used (Table 4.1). The most (16.50%) of Bipolarissp. was observed in variety of Bijoy followed by Gourab (16.00%) and Shatabdi (15.50%) although the lowest *Bipolaris sp*. was recorded by BARI Gom 28 (1.750%). Higher level of Bipolaris infected seed causes higher level of disease in adult plant (Bazlur Rashid, 1996; Malaker, 2003). Leaf spot/Leaf blight disease is consequence of seed to plant and again to seed transmission of *B. sorokiniana*. In the present study, increased number of black pointed seed/spike was found with the treatment of increased percentage of black pointed seed. This result was also reported by other workers (Nema and Joshi, 1974; Hossain, 2000).

3.5.3 *Alternaria sp*. (%)

From Table 4.1, we see that maximum association of Alternariasp. was observed in Kanchan (11.50%) while the lowest was recorded in BARI Gom 29 (2.00%), which was statistically similar to the varieties of BARI Gom 25 (2.25%), BARI Gom 27 (2.50%), BARI Gom 26 (2.50%), BARI Gom 28 (2.75%), and BARI Gom 30 (3.00%). A considerable number of seed-borne fungal pathogens belonging to the genera *Bipolaris, Alternaria, Curvularia, Fusarium, Penicillium* and *Aspergillus* have been detected in wheat seeds as reported by many researchers (Ashrafuzzaman and Hossain, 1992; Suratuzzamanet al., 1994; Hossain and Schlosser, 1993; Khan and Kumar, 1992; Cappelliet al., 1993).

3.5.4 *Curvolaria sp*. (%)

The maximum percentage of *Cuvularia sp.* was observed (3.75%) in the variety of Sonara-64 (Table 4.1), however, statistically similar result was found by Khri (3.50%), Kahyonso (3.50%) Aghrani (3.50%), Bijoy (3.50%) and Shatabdi (3.25%) whereas, the minimum percentage was observed in the variety of BARI Gom 26 (1.00%), which was statistically similarto the BARI Gom 27 (1.00%), BARI Gom 29 (1.00%), BARI Gom 28 (1.25%), BARI Gom 30 (1.25%) BARI Gom 25 (1.50%). A considerable number of seed-borne fungal pathogens belonging to the genera *Bipolaris, Alternaria, Curvularia, Fusarium, Penicillium* and *Aspergillus* have been detected in wheat seeds as reported by many researchers (Ashrafuzzaman and Hossain, 1992, Suratuzzamanet al.,1994, Rahman, 1998, Hossain and Schlosser, 1993, and Khan and Kumar, 1992).

Table 4.1: Fungi associated with wheat seed of twenty varieties

Variety	*Fusarium sp.*	*Bipolaris sp.*	*Alternaria sp.*	*Culvolaria sp.*
Kheri	23.00 bd	12.75 c	8.25 bc	3.50 ab
Kalyansona	23.75 ac	9.750 e	9.00 b	3.50 ab
Sonora 64	24.25 a	12.75 c	9.25 b	3.75 a
Sonalika	22.75 cd	14.25 b	6.50 de	1.75 eg
Pavon 76	22.00 d	13.75 bc	5.50 ef	2.50 cd
Kanchan	18.75 e	14.25 b	11.5 a	2.25 de
Aghrani	18.75 e	14.25 b	3.75 gh	3.50 ab
Protiva	15.00 f	13.75 bc	4.25 g	1.75 eg
Sourav	22.75 cd	11.00 d	8.50 b	3.00 bc
Gourab	23.50 ac	16.00 a	7.25 cd	2.00 df
Shatabdi	24.00 ab	15.50 a	9.00 b	3.25 ab
Sufi	19.50 e	7.50 f	4.50 fg	2.25 de
Bijoy	24.25 a	16.5 a	4.75 fg	3.50 ab
Prodip	23.00 bd	13.25 bc	6.50 de	3.00 bc
Bari Gom 25	14.25 f	8.50 f	2.25 i	1.50 fh
Bari Gom 26	3.25 i	8.50 f	2.50 i	1.00 h
Bari Gom 27	3.25 i	3.50 g	2.50 i	1.00 h
Bari Gom 28	4.75 h	1.75 h	2.75 hi	1.25 gh
Bari Gom 29	3.25 i	7.50 f	2.00 i	1.00 h
Bari Gom 30	8.75 g	8.50 f	3.00 hi	1.25 gh
Level of significance	**	**	**	**
CV %	4.63	6.88	14.74	20.78

Same letter(s) within the same column do not differ significantly at 5% levelof significance.
** Highly significance (p≤ 1%)

3.5.5 Epicoccumsp. (%)

It is observed in Table 4.2 that in Sonalika, Epicoccumsp. was the most predominant (19.75%) while the least association was recorded in BARI Gom 25 (1.00%), which was statistically similar with variety Sourav (1.00%), Gourab (1.00%), Khri (1.25%), Protiva (1.25%), BARI Gom 26 (1.50%), BARI Gom 29(1.50%), Prodip (1.50%), Shatabdi (1.50%) and BARI Gom 27 (1.75%).Monsura (2011) reported that the newly released 22 wheat varieties collected from WRC were categorized into large healthy, small healthy, black pointed, and shriveled and tested by blotter method. Prevalence of different fungi associated with the seed was recorded as *A. tenius, Aspergillus sp., B. sorokiniana, Curvularia sp., Epicoccum sp.* and *Fusarium sp*. The highest prevalence of *B. Sorokiniana* (20%) on Pavon. Similar reports were reported in different times by (BARI 2006, 2007 and 2009).

3.5.6 *Nigrospora* sp. (%)

Table 4.2 summarizes that *Nigrospora* sp. incidence was recorded higher in the variety of Shatabdi (6.25%), whereas the lowest incidence was found in thevariety of Bari Gom 25 (1.00%), which was statistical similar to BARI Gom 28 (1.00%), BARI Gom 26 (1.25%), BARI Gom 27 (1.25%) and BARI Gom 30 (1.25%). The present findings are consistent with the findings ofthe wheat varieties Protiva, BARI Gom 27 and BARI Gom 28 were free from the infection of *Nigrospora* sp. The highest 5.25% infection of *Nigrospora* sp.was found in the variety BARI Gom 25. The lowest 3.50% infection of *Nigrospora* sp was recorded in Sourav which was similar with BARI Gom 26 having 3.75% infection of *Nigrospora* sp. (BARI, 2006; Islam, 2015).

3.5.7 *Rhizopus* sp. (%)

Table 4.2 indicated that maximum percentage of *Rhizopus* sp. found in Sourav (7.00%)and the minimum percentage in Paron-76 (1.00%), which was statistically similar to Aghrani (1.00%), Shatabdi (1.50%), Gourab (1.00%), Bijoy (1.00%), BARI Gom 25 (1.00%), BARI Gom 26 (1.00%), BARI Gom 27 (1.00%), BARI Gom 28 (1.00%), BARI Gom 29, (1.00%) and BARI Gom 30 (1.00%). Kolawole et al. (2013) also isolated eight different fungi from wheat seed including *A. niger, A. flavus, A. fumigates, Penicillum sp., F. solani, Rhizopus sp., Alternaria sp., Tricoderma sp*. from wheat in Lagos state, Nigeria.

Table 4.2: Fungi associated with wheat seed of twenty varieties

Variety	*Epicocum sp.*	*Nigrospora sp.*	*Rhizopus sp.*	*Phoma sp.*	*Aspergillus sp.*
Kheri	1.25 ef	5.50 b	2.50 bc	2.75 c	1.00 d
Kalyansona	2.00 ce	3.75de	1.25 d	3.50 b	4.25 a
Sonora 64	13.75 b	4.75 c	1.25 d	3.50 b	1.75 bc
Sonalika	19.75 a	4.25 cd	1.25 d	2.00 d	4.50 a
Pavon 76	1.00 f	3.00 f	1.00 d	4.75 a	1.25 cd
Kanchan	2.25 cd	2.75 fg	3.00 b	2.00 d	1.00 d
Aghrani	1.50 df	2.75 fg	1.00 d	3.50 b	1.25 cd
Protiva	1.25 ef	3.25 ef	2.50 bc	1.75 de	1.25 cd
Sourav	1.00 f	3.00 f	7.00 a	1.25 ef	1.50 bd
Gourab	1.00 f	2.25 gh	1.00 d	1.50 df	1.50 bd
Shatabdi	1.50 df	6.25 a	1.50 d	1.25 ef	1.25 cd
Sufi	2.00 ce	2.00 hi	2.25 c	1.25 ef	1.50 bd
Bijoy	2.25 cd	4.25 cd	1.00 d	1.25 ef	2.00 b
Prodip	1.50 df	5.50 b	1.00 d	1.25 ef	1.75 bc
Bari Gom 25	1.00 f	1.00 j	1.00 d	1.00 f	1.00 d
Bari Gom 26	1.50 df	1.25 j	1.00 d	1.00 f	1.00 d
Bari Gom 27	1.75 df	1.25 j	1.00 d	1.00 f	1.00 d
Bari Gom 28	2.75 c	1.00 j	1.00 d	1.00 f	1.00 d
Bari Gom 29	1.50 df	1.50 ij	1.00 d	1.00 f	1.00 d
Bari Gom 30	2.00 ce	1.25 j	1.00 d	1.00 f	1.00 d
Level of significance	**	**	**	**	**
CV %	18.93	17.07	21.80	21.77	24.73

Same letter(s) within the same column do not differ significantly at 5% levelof significance.
** Highly significance (p≤ 1%)

3.5.8 *Phoma* sp. (%)

Table 4.2 also shows that the most incidence of Phomasp.was found in Paron-76 (4.75%) and the lowest in Bari Gom 25 (1.00%), which was statistically similar to BARI Gom 26 (1.00%), BARI Gom 27 (1.00%), BARI Gom 28 (1.00%), BARI Gom 29 (1.00%) and BARI Gom 30 (1.00%). In previous studies, it has been reported that *Alternaria*, *Aspergillus, Chaetomium, Fusarium, Helminthosporium, Myrothecium, Nigrospora, Penicillium, Phoma, Rhizopus,* and *Stemphylium* species have usually been isolated from black pointed wheat seeds (Martin and Gilman, 1976; Agarwal et al., 1983; Rees et al., 1984; Conner and Kuzyk, 1998; Conner and Whelan, 1989; Sisterna and Sarandon, 2005).

3.5.9 *Aspergillus sp*. (%)

It is observed in Table 4.2 that maximum percentage of *Aspergillus* sp. was associated with Kahyonso (4.25%), which was statistically similar to Sonalika (4.25%) and the minimum percentage in BARI Gom 25 (1.00%), which was statistically similar to the BARI Gom 26 (1.00%), BARI Gom 27 (1.00%), BARI Gom 28 (1.00%), BARI Gom 29, (1.00%) and BARI Gom 30 (1.00%). A considerable number of seed-borne fungal pathogens belonging to the genera *Bipolaris, Alternaria, Curvularia, Fusarium, Penicillium* and *Aspergillus* have been detected in wheat seeds as reported by many researchers (Ashrafuzzaman and Hossain, 1992; Suratuzzamanet al.,1994; Rahman, 1998; Hossain and Schlosser, 199; Khan and Kumar, 1992).

3.6 Percent of black point incidence of wheat seed under different grades and PDI of twenty varieties

3.6.1 Disease Grade '1'

The highest black point disease of grade one was recorded in the variety of Aghrani (42.00%) where the lowest was in BARI Gom 17 (2.00%), which is statistically similar to the variety of BARI Gom 19 (2.00%), BARI Gom 25 (3.00%) and BARI Gom 30 (3.00%) (Table 5). Rahman and Islam (1998) studied the effect of black point seeds of wheat on its qualitative characters such as weight of 1000 grains, total crude protein, total crude fibre, total ash dry matter and seed shoot vigour in respect of germination and root growth into five different grades (Grade-0, 1, 2, 3, and 4) on the basis of level of black point infection. All the qualitative

parameters except total crude proteins decreased significantly with the increase of black point infection. The decrease was more pronounced in grade 3 and grade 4 infected seeds. Germination percentage decreased sharply with the increased severity of infection level.

3.6.2 Disease Grade '2'

The highest prevalence of black point disease was recorded (Table 5) in Bijoy (44.00%) while the least prevalence of the pathogen was recorded in the variety of BARI Gom 29 (2.00%), which was statisticallysimilar to the variety BARI Gom 17 (3.00%) and BARI Gom 30 (3.00%). Different grades of black point infection were observed higher in the wheat varieties, significantly yield losses were reported in the wheat when terminal heat stress aggravates the disease. However, the degree of disease severity and yield losses were found to depends on variety and growing condition (BARI 2013).

3.6.3Disease Grade '3'

The highest percentage of grade three was recorded (Table 5) in Gourab (11.00%) where the lowest was in BARI Gom 17 (2.00%) which was statistically similar to the variety of BARI Gom 25 (2.00%) and BARI Gom 29 (3.00%). Wheat Research Center of Bangladesh (WRC), 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. The present result is in line with the result of Afroz (2012) who reported the highest black point grade 3 in the variety of Gourab.

3.6.4 Disease Grade '4'

Table 5 represents that the maximum black point disease of grade four was recorded in the variety of Protiva (7.00%) which was statistically similar to the variety of Shatabdi (7.00%) where the lowest disease incidence was recored in BARI Gom 27 (1.00%), BARI Gom 25 (1.00%), Sufi (1.00%) and Paron-76 (1.00%). Highest percentage of black pointed seed under different grades and PDI in the Variety Kanchan was also reported by different authores (Kabiret al., 2009; Malaker, 2003).

3.6.5 Disease Grade '5'

Table 5 indicates that in the grade five, most prevalence was observed insourav (11.00%) whereas, the lowest was BARI Gom 30 (1.00%) same as Bijoy (1.00%) and Prodip (1.00%). It was alsoreported that 95.0% to 0.00% germination in seeds of grade-0 (free from infection, the best seeds, apparently healthy) and grade-5 (grains are shrivelled, almost completely discolored or more than ½ of grains discolored, respectfully). Seeds of grade-5 and grade-0 resulted the highest (92.50) and the lowest (40.50) incidence of B.sorokiniana, respectively,Hossain (2000).

Table 5: The percent of black point incidence of wheat seed under different grades and PDI of twenty varieties

	Disease grade %						PDI
Variety	**1**	**2**	**3**	**4**	**5**	**Under size**	
Kheri	9.00 i	6.00 g	4.00 ef	3.00 e	2.00 h	4.00 d	22.25 a
Kalyansona	17.00 fg	22.0 b	7.00 c	2.00 f	4.00 f	2.00 f	16.75 b
Sonora 64	16.00 gh	4.00 hi	3.00 fg	4.00 d	9.00 b	1.00 g	16.50 b
Sonalika	18.00 ef	9.00 f	9.00 b	3.00 e	4.00 f	7.00 b	16.25 b
Pavon 76	6.00 j	4.00 hi	3.00 fg	1.00 g	7.00 d	5.00 c	15.25 b
Kanchan	4.00 kl	5.00gh	7.00 c	3.00 e	2.00 h	1.00 g	12.50 c
Aghrani	42.00 a	8.00 f	5.00 de	2.00 f	4.00 f	2.00 f	12.25 c
Protiva	16.00 gh	14.00 c	6.00 cd	7.00 a	4.00 f	1.00 g	12.00 c
Sourav	15.00 h	14.00 c	7.00 c	3.00 e	11.00 a	11.00 a	11.00 c
Gourab	29.00 c	11.00 e	11.00 a	6.00 b	9.33 b	1.00 g	9.25 d
Shatabdi	19.00 e	13.00cd	5.00 de	7.00 a	3.00 g	2.00 f	9.25 d
Sufi	22.00 d	6.00 g	5.00 de	1.00 g	2.00 h	1.00 g	7.00 e
Bijoy	33.00 b	44.00 a	4.00 ef	5.00 c	1.00 i	2.00 f	6.50ef
Prodip	30.00 c	12.00de	6.00 cd	4.00 d	1.00 i	2.00 f	6.00ef
Bari Gom 25	3.00 lm	6.00 g	2.00 g	1.00 g	8.00 c	2.00 f	6.00ef
Bari Gom 26	5.00 jk	4.00 hi	6.00 cd	2.00 f	4.00 f	5.00 c	5.17 fg
Bari Gom 27	2.00 m	3.00 ij	2.00 g	1.00 g	4.00 f	3.00 e	5.00fg
Bari Gom 28	5.00 jk	6.00 g	4.00 ef	5.00 c	3.00 g	2.00 f	5.00fg
Bari Gom 29	2.00 m	2.00 j	3.00 fg	2.00 f	5.00 e	1.00 g	4.25 g
Bari Gom 30	3.00 lm	3.00 ij	5.00 de	3.00 e	1.00 i	2.00 f	3.75 g
Level of significance	**	**	**	**	**	**	**
CV %	6.23	7.90	12.16	13.76	7.73	13.59	9.19

Same letter(s) within the same column do not differ significantly at 5% levelof significance.
** Highly significance (p≤ 1%)

3.6.6 Undersize seed

The upper most frequency of undersized seed was recored (Table 5) inthe variety of sourav (11.00%) while the lowest was in (1.00%) BARI Gom 29 (Islam, 2015). Conducted among the ten wheat variety of time and late sowing Protiva consist the height amount of undersized seed and it was 22.42% 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, BARI gam 25 both time and late sowing variety of Gourab and time sowing variety of Sotabdi having 3.08%, 4.92%, 4.42%, 3.58% and 4.83% undersized seed respectively.

3.6.7 Black point incidence

Table 5 points out that maximum percentage of black point incidence (22.25%) was recorded in Khri where the lowest was observed in BARI Gom 30 (3.75%), which was statistically similar to the variety of BARI Gom 19 (4.25%). The findings of the present study were in agreement with the findingsofmany researchers (BARI, 2009; BARI, 2004; Cappelli et al., 1993, Dey et al., 1992).

IV. Conclusion

Wheat is an important and promising cereal crop having good potential in Bangladesh. However, the yield of wheat is very low in compare to other wheat growing countries because of poor health and low germination of wheat seeds. The results of this study will give valuable informatons to the wheat growers.

Acknowledgement

We very grateful to the authority of Wheat Research Centre of Dinajpur, Bangladesh for supplying the wheat seeds. Special thanks to Md. Mostafa Kamal, MS student, Department of Food Processing and Preservation, HSTU, Dinajpur for his helps in data analysis and writing this manuscript.

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Abubakar Ali Jama et al. "Seed Health Status of Selected Wheat Cultivars Available in Bangladesh. "IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 11.1 (2018): 80-92.