ISSN: 2287-688X

Annals of
Plant Sciences

Volume 11, Issue 11 (2022) pp. 5465-5490

Research Article

Seed to Plant to Seed Transmission of Wheat Blast Pathogen *Magnaporthe oryzae* Triticum

Md. Yeasinul Haque Rayhan[1], Md. Humayun Kabir[2], Md. Mahbubur Rahman[3], Md. Ashraful Islam[4], Md. Imtiaz Uddin[5], Md. Ayub Ali[6], and M Bahadur Meah[7]

[1]*Bangladesh Agricultural University, Mymensingh, MS Fellow, Department of Plant Pathology, Mymensingh 2202*
[2]*Department of Agricultural Extension, Upazila Agricultural Officer, Bhuyanpur, Tangail*
[3]*Bangladesh Institute of Nuclear Agriculture, Scientific Officer, Habiganj*
[4]*Bangladesh Institute of Nuclear Agriculture, Research Fellow, Biotechnology Division*
[5]*Bangladesh Institute of Nuclear Agriculture, Chief Scientific Officer, Biotechnology Division, Mymensingh*
[6,7]*Bangladesh Agricultural University, Mymensingh, Professor, Department of Plant Pathology, Mymensingh 2202*

Abstract

That *Magnaporthe oryzae* Triticum is seed-borne, it is confirmed. Random distribution of infected seeds during sowing plays a vital role in epidemic occurrence of wheat blast in the field. Once the spike is infected, local spread of wheat blast occurs by spores from the infected spike. Under Bangladesh field condition, plants with bleached spike seldom shows leaf infection. So, question arises if a bleached spike on a single plant is through systemic infection, however, it is not known. Experiments were conducted to explore the possibility of systemic infection of a wheat spike i.e., when seed is sown the *Magnaporthe oryzae* Triticum traverses from seed to all the way to developing spike of wheat. Seed samples of wheat variety BARI Gom23 were collected from the farmers of three upazila of blast hotspot Meherpur district. Seeds were incubated in moist blotter in room temperature (30 C ± 1). Any growth of *Magnaporthe oryzae pv* Triticum (MoT) seen under stereo-binocular microscope was confirmed through microscopic slide preparation. In seed samples, seed infection of MoT ranged 0-22% were grouped into four treatments (0, 1-10, 11-20 and >20%) which were later sown in pots. Seed germination ranged from 83 to 87% for the four seed categories. Seeds and plant parts were processed for DNA extraction, PCR was run with primer MoT3. For anatomical study, TSs of root, stem and rachis were made. Plants grown out of the four groups of seeds all except healthy seeds carried blast infection showing different levels of bleached spikes. Agarose (1.5%) gel electrophoresis produced monomorphic bands of 361bp in all categories of seeds and plant parts except those from healthy seeds. TSs of plant parts displayed both intra- and inter-cellular mycelium of MoT in the ground tissues and xylem bundles. The results of this study confirmed the seed infection as observed in moist blotter was of *Magnaporthe oryzae* pv Triticum and confirmed the transmission of pathogen from seed to plant to seed through vascular bundle. It may be concluded that MoT is both seed-borne and seed transmitted.

Keywords: *Magnaporthe oryzae Triticum, Anatomy, Seed to plant to seed transmission.*

Introduction

Wheat is grown in more than 70 countries of five continents (Dixon, 2007; Baenziger, *et al.*, 2009).It is the second most important cereal crop, next to rice contributing 7% of the total food production in Bangladesh. It is largely grown as winter crop and covers almost 351213 ha of land (BBS, 2019). Wheat blast caused by *Magnaporthe oryzae* Triticum,

***Corresponding Author:**
M Bahadur Meah;
DOI: http://dx.doi.org/10.21746/aps.2022.11.11.1

appeared for the first time in 2016 as a serious production constraint in Bangladesh. The disease was reported for the first time in Brazil in 1985 (Igarashi, *et al*., 1986) which later spread to Bolivia, Argentina and Paraguay (Duveiller, *et al*., 2011) causing yield losses of up to 100% (Peng, *et al*., 2011). Blast disease of wheat was first spotted in Bangladesh at Kustia, Meherpur, Chuadanga, Jhenaidah, Jashore, Barisal, Bhola and other southern districts in the middle of February of 2016 (Aman, 2016). The affected areas were approximately~15% of Bangladesh's total wheat producing area in 2015-16, presenting a significant threat to the country's aggregate wheat production (Malaker, *et al*., 2016; Islam, *et al*., 2016).

Blast infections at middle and late stages of grain filling led to the entrance of the pathogen into the seeds and the seeds carry the pathogen to the next season. Seeds from infected spikes look small, wrinkled, and deformed (Urashima, *et al*., 2009), these seeds may disperse the pathogen in long-distance, as *Magnaporthe oryzae* is a seed borne pathogen (Maciel, *et al*., 2014).

M. o. Triticum has been isolated in plenty of spores from seeds of freshly harvested infected wheat spikelet (Meah, *et al*, 2016). However, presence of *M. o.* Triticum declined in stored seeds with time. Under this situation, it is not clear how far seed-borne inoculum contributes to the infection of emerging head of wheat plant. The disease is generally spread by infected seeds and airborne spores, and the fungus can survive in infected crop residues and seeds (Yamamoto, *et al*., 2000). Research in many countries gave evidence that MoT is seed-borne. However, findings indicate the probability of triggering wheat blast epidemic from seed-borne inoculum is less than 5% (Urashima, *et al*., 2009, Goulart, *et al*., 2007). It is not clear if the *M o*. Triticum borne by seeds is also transmitted to wheat spike. Also till now it is not known how *M. o*. Triticum in the seeds moves to the spike i.e., is it an internal or external transmission.

These aspects have not been addressed in Bangladesh, but it is very important. Because seeds carrying MoT infection if sown might contribute to head infection. So, seeds selected for sowing must be treated to eliminate MoT. For performing seed treatment properly, it is important to know how MoT is carried by the seed-externally or internally i.e., to which part of the seed MoT is attached. Also, it is important to know if MoT, from germinating seed, passes through the seedling to the mature plant traversing all the way from shoot to spike. No answer to all these questions is available so far neither in Bangladesh nor in other parts of the world. But we need to know the answer to plan the strategy for successful cultivation of wheat. With all these ideas in mind, the present research was designed to determine the mode of transmission of *M. o*. Triticum from seeds to the spike of wheat and thereby to the seeds.

Materials and Methods

Experimental Site

The experiments were conducted in Plant Disease Diagnostic Clinic (PDDC), Bangladesh Agricultural University (BAU) and Biotechnology Lab, and BINA Field, Bangladesh Institute of Nuclear Agriculture (BINA) during the period from November 2018 to March 2021.

Collection of Seed Samples

The seeds of wheat variety Bijoy (BARIGom 23) were collected from the farmers of different regions (Gangni, Meherpursadar, Mujibnogor) of Meherpur (Figure 1).

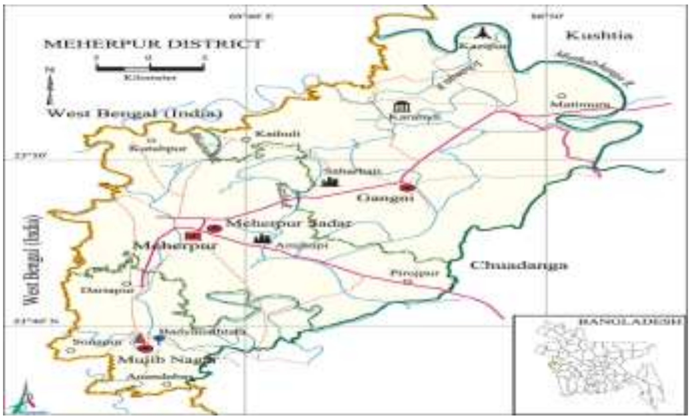


Figure 1: Regions of Meherpur of seed collection

The seed samples were stored in PDDC and IPM lab at Bangladesh Agricultural University, Mymensingh at 4^{0}C.

Analysis of Seed Samples for *Magnaporthe oryzae Triticum (MoT)*

Seeds were analyzed employing blotter technique (ISTA, 2010) and molecular techniques. The seeds in the petri dishes were incubated at 30±1^{0}C under alternating cycles of 12 hours near ultraviolet light (NUV) and darkness for 7 days. Time to time watering was done to keep the blotting paper moist.

Data Collection

Data on i. %Seed germination and ii. %Seed infection by MoT were collected. Data on seed germination were recorded at 7th day after incubation in blotting papers and expressed in percentage. Number of seeds infected by MoT were counted per plate and percent seed infection was calculated. Seed infection by MoT was observed under stereo-binocular microscope for characteristic conidia bearing on the slender conidiophore and confirmed through observation under compound microscope of the slides made from fungal structures.

Grouping of Seeds Based on the Level of Infection by MoT

After inspection, seeds were categorized into four groups based on the level of seed infection by MoT (Table 1).

Table 1: Grouping of seeds based on level of infection by MoT

Level of seed infection by MoT (%)	Group (Treatment)
0%	I
0-10%	II
11-20%	III
> 20%	IV

MoT: *Magnaporthe oryzae* Triticum

Treatments

Each level of seed infection was considered a treatment. Each treatment was replicated thrice.

Sowing of the Seeds in Pots

Plastic pots of 16L size were each filled with soil and cow dung mixture added MoP and TSP. Urea was added after sowing of seeds in the pot as per recommendation of BARC, (2018). In each pot 35 seeds were sown on 29th November 2018.

Data Collection

Appearance of blast symptoms, incidence, and severity of blast disease, percent seed germination and agronomic features of the plants grown in pots out of four categories of seeds (0.0, 1-10, 11-20 and > 20% seed infection) were recorded six times starting from 58 days of sowing up to 73 days age of the crops @ 3 days interval.

Disease Incidence *(%)* =Pi/Pt x 100. Where, Pi = Number of panicles (spikes) infected, and

Pt = Total number of panicles (spikes) observed (*Rajput and Bartaria, 1995*).

Disease Severity: Percent surface area of spike infected/bleached was estimated following the figures shown in Figure 2. Spikes per pot were counted and arithmetic means for single plants were calculated.

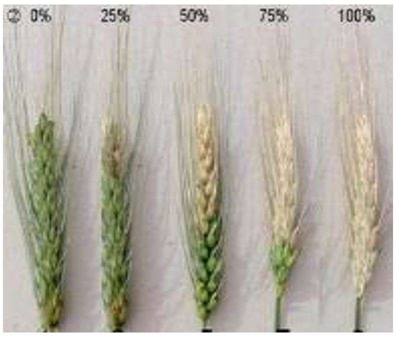


Figure 2: Estimation of spike surface area bleached

Statistical Analysis of Data

The data were statistically analyzed using Minitab 18 computer package program (www.minitab.com: Minitab Statistical Software) and means were compared by DMRT (Duncan's Multiple Range Test).

Collection of Plant Samples from the Pot Experiment

Two plants were collected from each pot (one is apparently healthy, and another is apparently unhealthy). So, there were 24 plants in total. Each of the plants was cut into 3 parts (root, stem, and spike), stem portion was divided into three smaller parts (stem I, stem II and stem III in ascending order from the root) and labeled separately. All the specimens were preserved at -70^{0}C.

Data Collection

Presence of MoT in roots, stem and spike was detected by blotter techniques, free-hand sections, and molecular technique. In blotter (moist), wheat plant parts were planted following the procedure followed for seed plating (ISTA, 2010) (Figure 3).

Root and Spike Stem

Figure 3: Plating of wheat plant parts in moist blotter

Free hand sections of the specimens collected from all treatments were made and each section was examined under compound microscope. Photographs of the sections were taken.

Molecular Techniques by MoT Marker (MoT3)

A modified mini-prep DNA extraction method developed at The International Rice Research Institute (IRRI, 2016), The Philippines, was followed in this experiment.

DNA Extraction

DNA was extracted using the mini preparation CTAB method (Edwards, *et al.*, 1991). The seed and plant samples were crushed in small mortar. Then extracted with 800 µl re-heated extraction buffers by vortexing and inverting. The tubes were placed in a 65°C water bath in a tube holder for 20 minutes (after 10 minutes mixed by inverting and returned to the water bath). Then 800 µl L chloroform mix was added (24:1 mixture of chloroform and isoamyl alcohol). Tubes were closed tightly, placed in tube rack, covered with paper towels, and hold a second tube rack against the top of the tubes and inverted repeatedly for 3 minutes. The tubes were centrifuged for 8 minutes at 11,000 rpm. 500 µl supernatants were removed to a new 1.5 mL tube (already labeled). Later, the chloroform and plant DNA binding solution and 1000 µl of cold 100% ethanol were added into a liquid organic waste container and centrifuged at maximum speed (13,200 rpm) for 12 minutes. When a small pellet was visible then the solution was decanted by pouring the solution into a beaker. 1000 µl cold 70% ethanol was added to all tubes (adding at an angle away from the side of the tube with the DNA pellet) and centrifuged at 13,200 rpm for 3 minutes. The pellet was dried by inverting the tubes on a bench top on top of tissue for 30-45min. The pellet was re-suspended in 100 µl TE buffer and dissolved pellet by warming in a 65°C water bath for up to 1 hr (with frequent mixing or flicking the tube with finger). After the pellet was dissolved, the concentrated DNA was stored at -20°C.

PCR

PCR cocktail (5µl master mix, 3µl of sterilized ddH_2O, 1µl of primer) including 1µl of each template DNA had total volume of 10µl reaction based on a wheat protocol (Pieck, *et al.*, 2013) were placed in the PCR tubes and run in the DNA thermal cycler (Table 2). The cycling parameter were 94°C, 1.5 min to initial denaturation and 94°C, 30 seconds at denaturation; anneal at 62°C, 30 seconds; extend at 72°C, 1 min; final extension at 72°C, 2 min; holding temperature at 4°C until removing from thermocycler. The amplification products were separated on the 1.5% agarose gel for 90 minutes at 90v. About 2µl of each PCR was loaded in each well and 1Kbp DNA ladder was used for size determination. After staining with ethidium bromide (10 mg/ml) the gel was placed on high performance ultraviolet light box (UV-trans-illuminator) of GEL Doc for checking the DNA bands.

Table 2: The Name, sequence and size of the selected specific primer used

Primer Name	Seq[a]	Sequence		Annealing temperature (°C)	Target species
MoT3	WB12	Forward	GTCGTCATCAACGTGAC	62	*Magnaporthe oryzae*Triticum
		Reverse	ACTTGACCCAAGCCTCG		

Results

Seed Germination and %Seed Infection in Blotter Plates

In moist blotter, seeds yielded different levels of infection by MoT(Figure 4, Table 3). Percent seed germination varied and ranged between 81 – 96%, the highest was recorded in the samples which yielded no MoT (Table 3). This means seed infection of MoT didn't affect seed germination because seed germination of wheat at a level of 80% is considered acceptable.

A B C

Figure 4: Wheat seeds incubated in moist blotter displaying germination, seed infection (A, B) and growth of *Magnaporthe oryzae* Triticum (C) as seen under stereo-binocular microscope (40X).

Seed samples with different levels of MoT infection were categorized into four groups. The four groups were designated as L_0, L_1, L_2 and L_3, and named as treatments for the pot experiments (Table 3).

Table 3: Categorizing farmer's seeds based on % seed infection by *Magnaporthe oryzae* Triticum

Seed Sample no.	(%) Seed germination	(%) Seed infection by MoT	Level of infection categorized
1	82 b	22.00 a	L_3
2	84 b	6.00 c	L_1
3	81 b	14.00 b	L_2
4	96 a	0.00 d	L_0
LSD(0.05)	5.73	3.26	

MoT: *Magnaporthe oryzae* Triticum, L_0: no seed infection, L_1: 1-10% seed infection, L_2: 11-20 % seed infection, L_3: >20% seed infection by MoT. Figures with different letters differ significantly at P=0.05.

Growth and Yield Contributing Components of Wheat Plants Grown in Pot Soil Out of Infected Seeds of Variety BARI Gom 23

The seed germination percentage varied from 65.70 - 82.86%. Number of tillers per pot varied from 36-43 and the spike number from 22-26 for the four levels of seed infection. There were not statistically differences for the three growth components among the treatments (Table 4).

Percent spike showed blast symptoms were minimum in L_0 and maximum in L_3. In case of yield, maximum was obtained from L_0 plants and the minimum yield from L_3 plants. Percent spike infection and yields (per pot) varied significantly among the treatments (Table 4).

Table 4: Growth and yield contributing components of wheat plants grown out of farmer's seeds of BARI Gom 23 infected with *Magnaporthe oryzae* Triticum

Treatments	% Germination	Tiller number* Per pot	Spike number* Per pot	Spike infected with MoT (%)	Yield Per pot (g)
L_0	82.86% a	39.00 a	25.00 a	8.500% c	69.10 a
L_1	74.28% a	42.33 a	24.33 a	28.12% b	59.85 b
L_2	65.70% a	43.00 a	26.00 a	35.92% ab	54.77 b
L_3	70.15% a	36.00 a	22.00 a	42.45% a	46.05 c
LSD (0.05)	22.74	7.99	11.83	9.96	7.49

L_0: Seeds with no MoT infection, L_1: Seeds with 1-10% MoT infection, L_2: Seeds with 11-20% infection, L_3: Seeds with > 20% infection. * Tiller number and Spike number is the average of three replication.

Incidence and Severity of Blast in Wheat Plants Grown out of Infected Seeds of Variety BARI Gom 23

Blast Incidence

First record of blast incidence was taken on 55th day after sowing (DAS). Next two readings were taken @ four days interval on 59th and 63rd DAS. Incidence of blast increased with time in all the treatments. On wheat plants grown out of no seed infection (L_0), the spike incidence was the lowest. Blast incidence was higher in the plants grown out of seeds with higher MoT infection. Consequently, plants grown out of seeds with >20% MoT infection had the significantly higher % spike infection (Table 5).

Table 5: Incidence of spike infection in plants of BARI Gom23 grown out of seeds with different levels of *Magnaporthe oryzae* Triticum infection under natural condition

Treatments	% Disease Incidence		
	I*	II*	III*
L_0	3.67 b	6.33 c	9.00 d
L_1	5.33 ab	21.33 b	24.67 c
L_2	6.00 ab	29.00 a	35.67 b
L_3	8.00 a	33.33 a	43.00 a
LSD (0.05)	3.84	6.92	6.15

L_0: Seeds with no MoT infection, L_1: Seeds with 1-10% MoT infection, L_2: Seeds with 11-20%, L_3: Seeds with > 20%MoT infection. *I: 55 days after sowing, II*: 59 days after sowing, III*: 63 days after sowing

Blast Severity

Severity of blast (% spike surface area bleached) in wheat plants were observed at 58, 61, 64, 67, 70 and 73 DAS. First record of blast severity was taken on 58th day after sowing (DAS). Next readings were taken @ 3 days interval.

On 58th DAS, spike bleaching on plants grown out of seeds with all levels of MoT infection did not vary statistically. However, with time the spike infection severity for different levels of seed infection varied significantly. On the 73rd DAS, significantly the highest spike infection was recorded in plants grown out of seeds with >20% MoT infection (Table 6).

Table 6: Severity of spike blast in wheat plants of BARI Gom23 grown out of seeds with different levels of *Magnaporthe oryzae* Triticum infection under natural condition

Treatments	% Disease Severity					
	I	II	III	IV	V	VI
L_0	8.33 a	15.33 a	19.33 b	26.67 b	35.00 b	44.67 b
L_1	16.00 a	24.33 a	32.67 ab	44.00 ab	51.67 ab	59.00 ab
L_2	12.67 a	19.00 a	31.70 ab	41.33 ab	50.00 ab	60.00 ab
L_3	19.67 a	31.67 a	46.67 a	58.33 a	68.00 a	76.00 a
LSD(0.05)	11.56	12.52	19.25	17.93	18.78	19.68

L_0: Seeds with no MoT infection, L_1: Seeds with 1-10% MoT infection, L_2: Seeds with 11-20%, L_3: Seeds with > 20% MoT infection. *I: 58 days after sowing, II*: 61 days after sowing, III*: 64 days after sowing, IV*: 67 days after sowing, V*: 70 days after sowing, VI*: 73 days after sowing

Severity of spike bleaching increased with time in all the four levels of seed infection. Rate of increase of severity was slower in plants from no seed infection and the rate was the highest in plants from seeds with >20% infection (Figure 5).

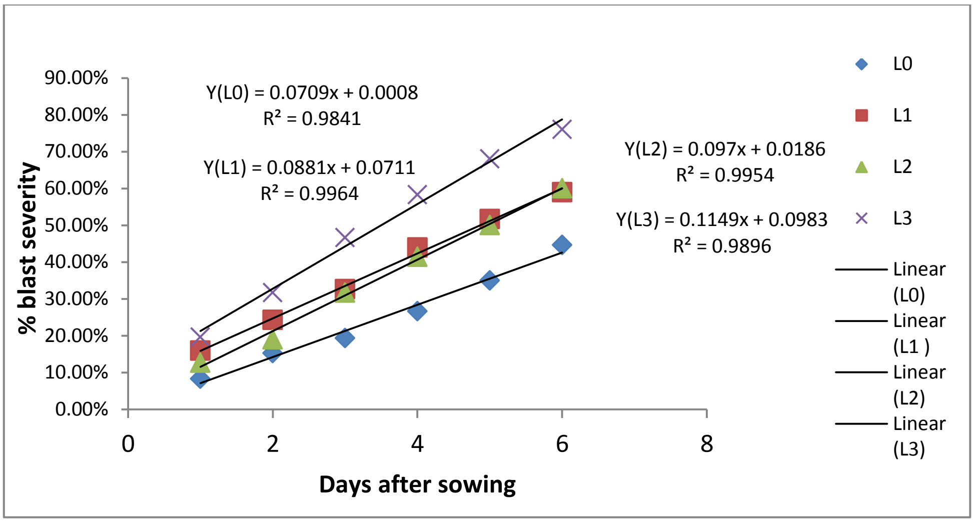
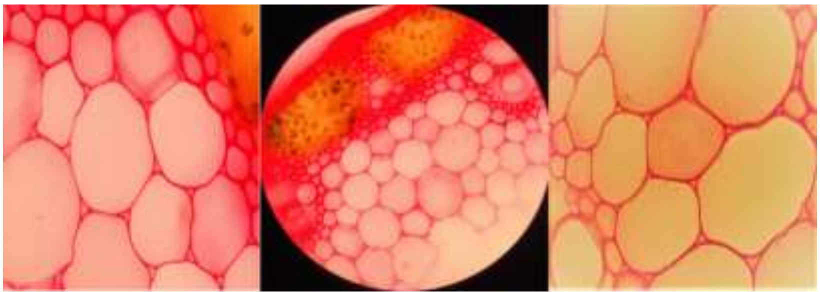


Figure 5: Increase in severity of spike bleaching by *Magnaporthe oryzae* Triticum infection over 15 days in wheat plants grown out of four levels of seed infection under natural condition. L_0: Seeds with no MoT infection, L_1: Seeds with 1-10% MoT infection, L_2: Seeds with 11-20%, L_3: Seeds with > 20% MoT infection

Anatomy of the Wheat Plants Infected with *Magnaporthe oryzae Triticum*

TSs of healthy wheat plant, collected from control pot of the experiment did not carry any MoT mycelium in any of the parts and no DNA band was detected (Figure 6).



Root Stem Rachis

Figure 6: TS of root, stem and rachis of a healthy wheat plant with no sign of mycelium of *Magnaporthe oryzae* Triticum

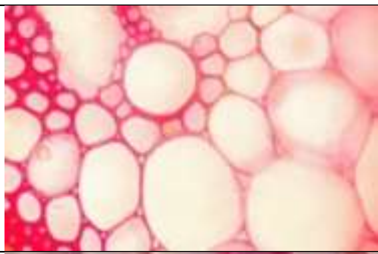
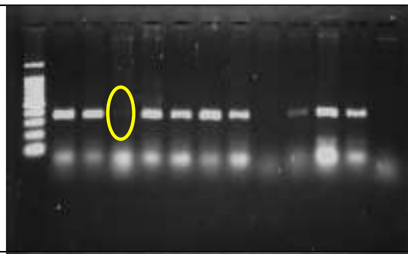
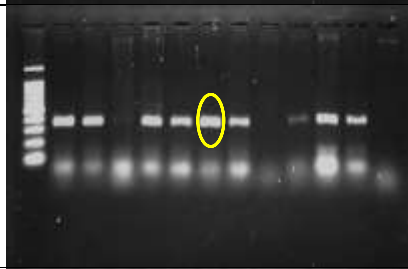
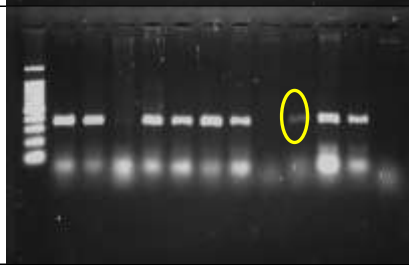
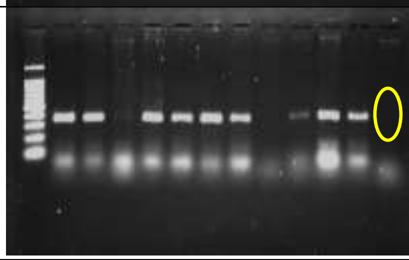
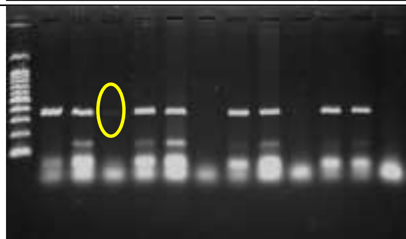
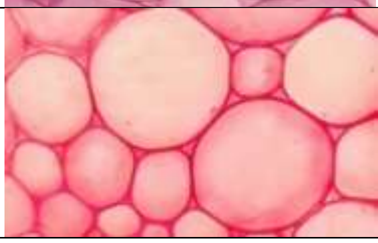
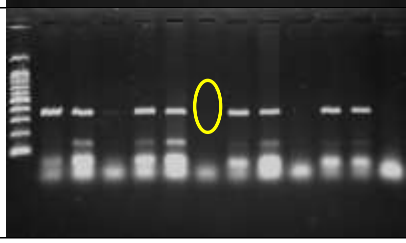
Presence of fungal structures in different parts of the plant and DNA of the fungal structures in the corresponding parts were determined (Table 7).

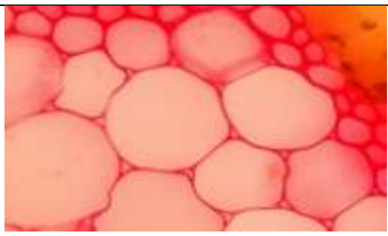
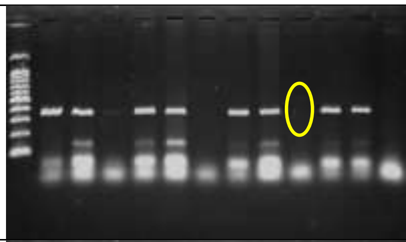
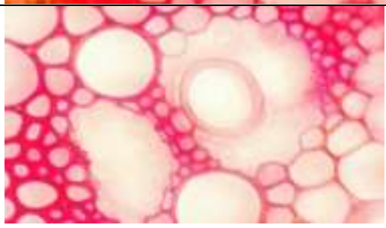
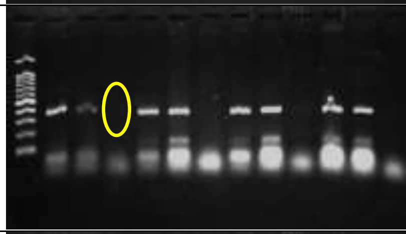
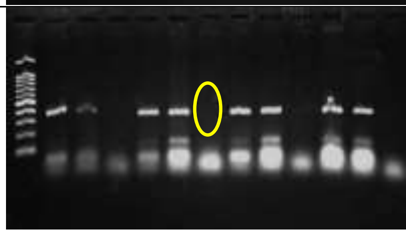
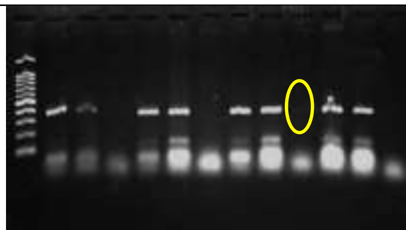
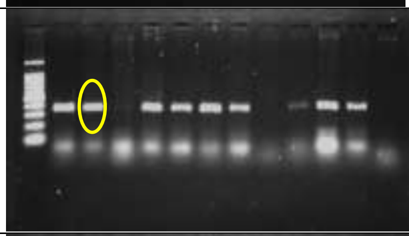
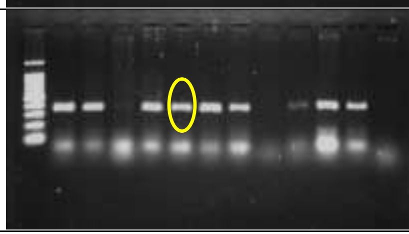
In the sections of root of wheat, presence of mycelium of MoT was scanty or not detected at all, with one exception, in all categories of seed infection (L0, L1, L2 and L3). This is supported by the non-scoring of DNA band in the corresponding sections from root (Table 7).

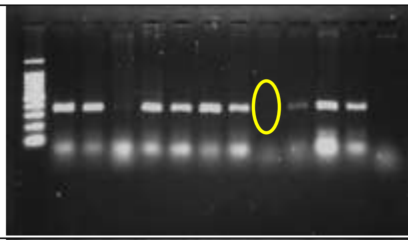
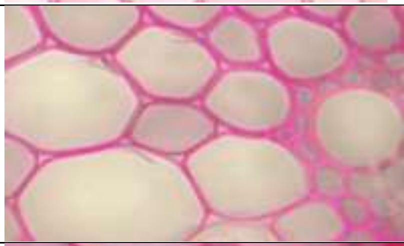
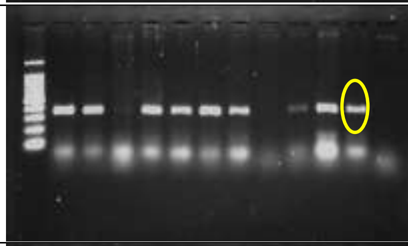
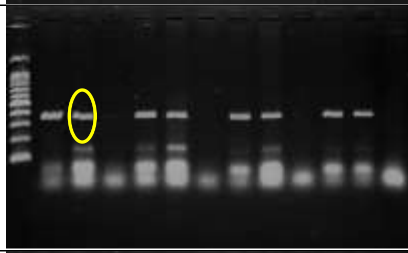
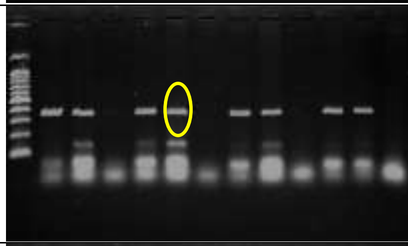
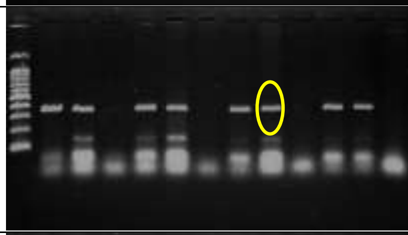
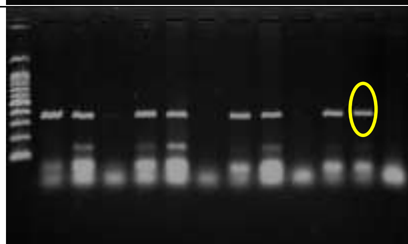
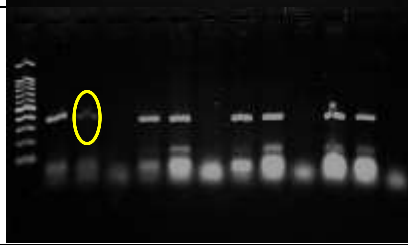
Stem Section: In the three stem parts (I, II, III from lower to upper), MoT mycelium was detected in all the sections from all four categories of seed infection and DNA bands were scored in the corresponding sections. However, in a few cases of L0 sections, no mycelium was detected, and no DNA band was scored (Table 7).

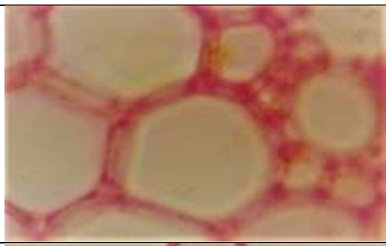
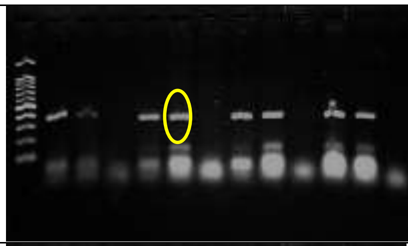
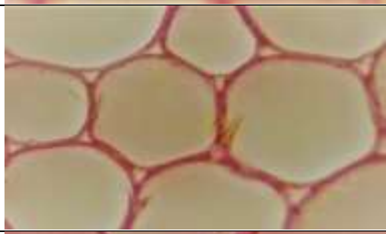
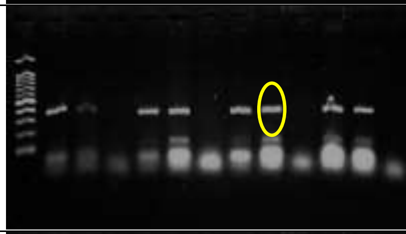
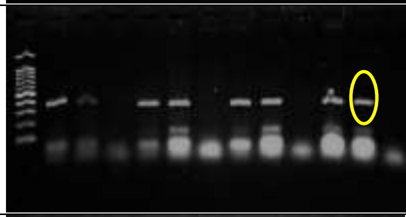
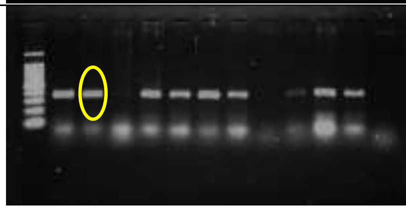
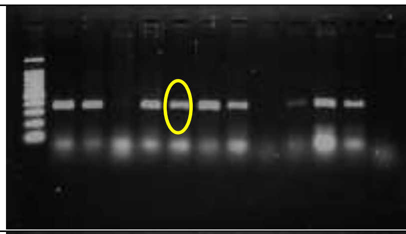
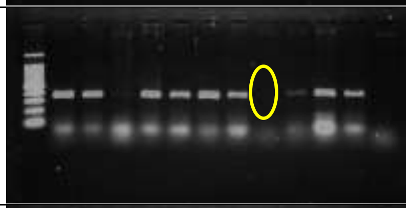
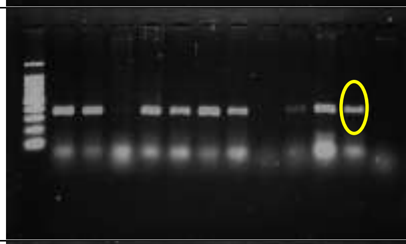
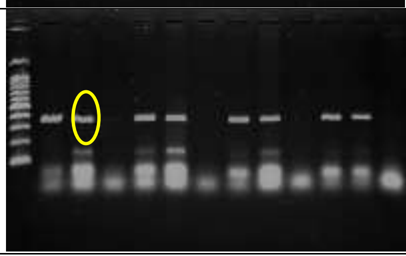
Spike Sections: In all sections of spike, MoT mycelium was detected in all four categories of seed infection and DNA bands were scored in all the corresponding sections (Table 7).

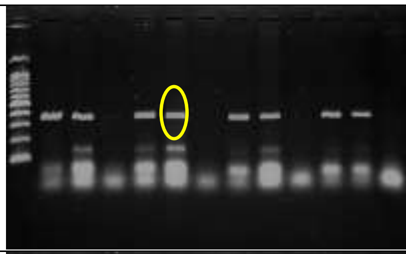
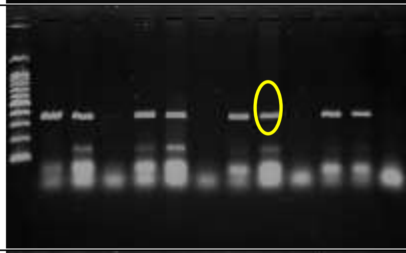
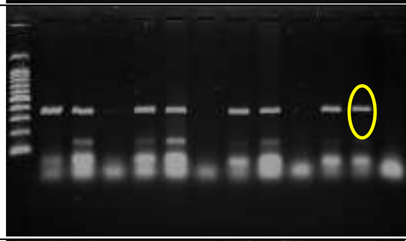
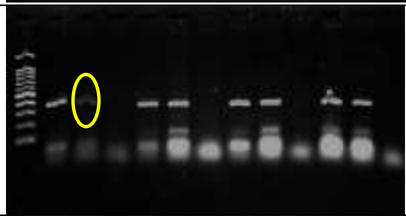
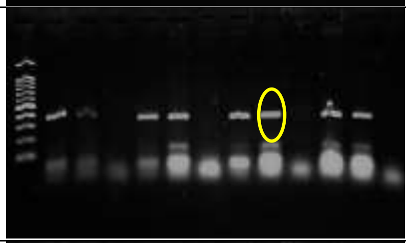
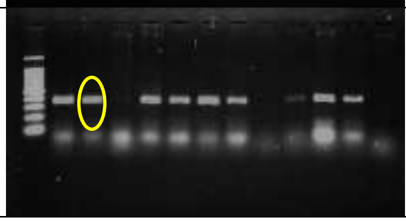
Table 7: Anatomical detection of *Magnaporthe oryzae*Triticum in different parts of wheat plants grown out of infected seeds of variety BARI Gom 23

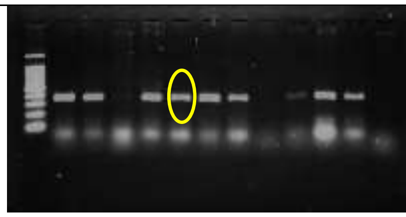
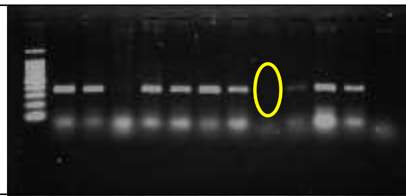
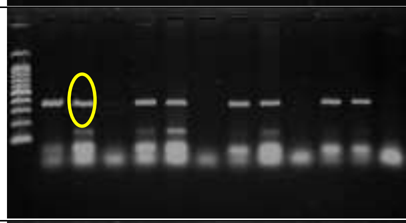
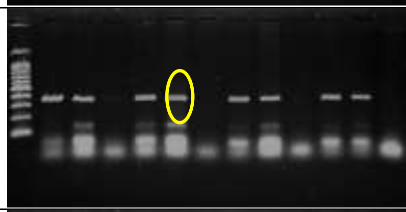
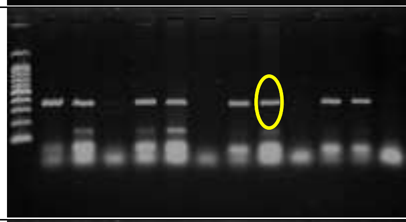
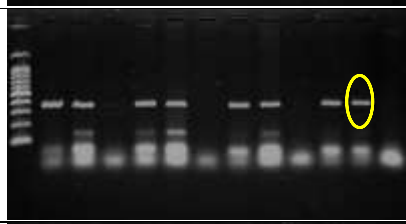
Plant Part(s)	Slide	Mycelium of MoT	Photo	DNA Band
Root	L_0R_1	-		
	L_0R_2	+		
	L_0R_3	+		
	L_1R_1	-		
	L_1R_2	+		
	L_1R_3	-		

	L_2R_1	-		
	L_2R_2	-		
	L_2R_3	-		
	L_3R_1	-		
	L_3R_2	-		
	L_3R_3	-		
Stem-1	L_0R_1	+		
	L_0R_2	+		

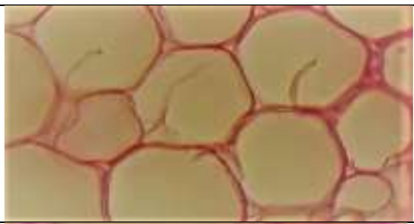
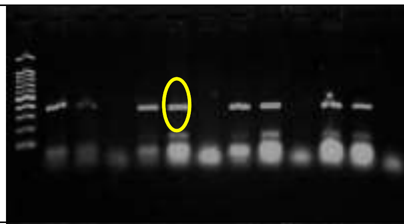
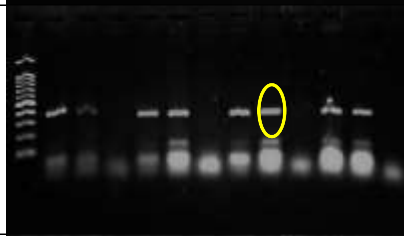
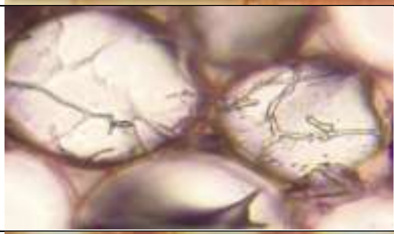
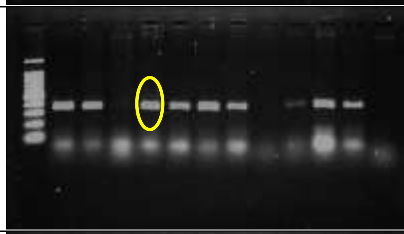
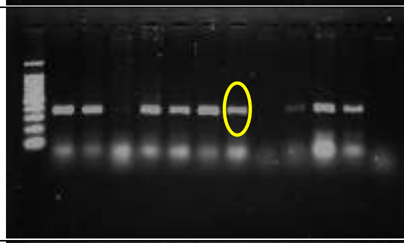
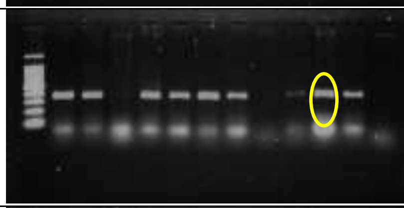
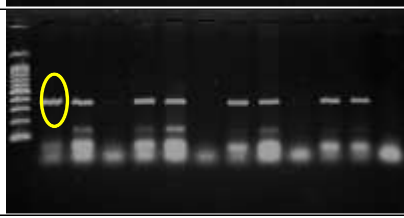
	L_0R_3	-		
	L_1R_1	+		
	L_1R_2	+		
	L_1R_3	+		
	L_2R_1	+		
	L_2R_2	+		
	L_2R_3	+		

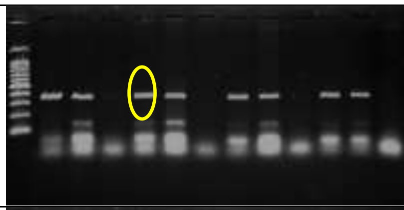
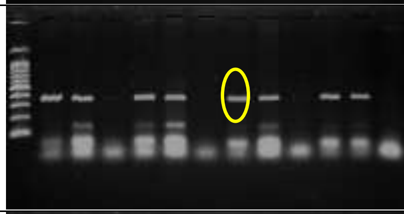
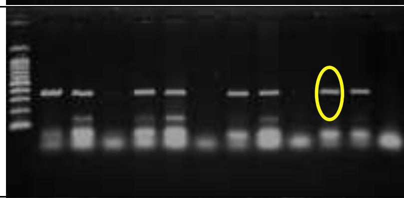
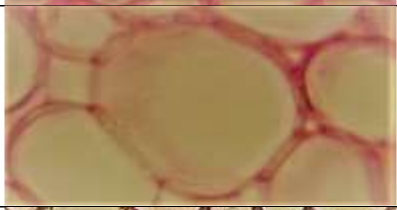
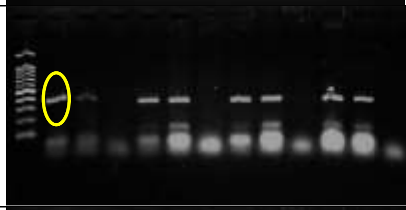
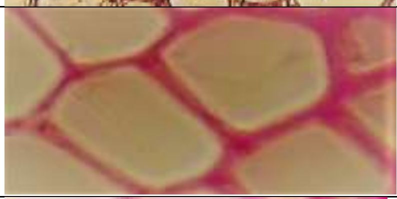
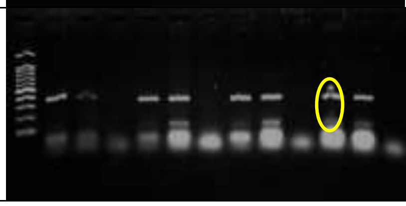
	L_3R_1	+		
	L_3R_2	+		
	L_3R_3	+		
Stem-II	L_0R_1	+		
	L_0R_2	+		
	L_0R_3	-		
	L_1R_1	+		
	L_1R_2	+		

	L_1R_3	+		
	L_2R_1	+		
	L_2R_2	+		
	L_2R_3	+		
	L_3R_1	+		
	L_3R_2	+		
	L_3R_3	+		
	L_0R_1	+		

Stem-III	L_0R_2	+		
	L_0R_3	-		
	L_1R_1	+		
	L_1R_2	+		
	L_1R_3	+		
	L_2R_1	+		
	L_2R_2	+		
	L_2R_3	+		

 Volume 11, Issue 11 (2022) pp.5465-5490

	L_3R_1	+		
	L_3R_2	+		
	L_3R_3	+		
Spike	L_0R_1	+		
	L_0R_2	+		
	L_0R_3	+		
	L_1R_1	+		
	L_1R_2	+		

	L_1R_3	+		
	L_2R_1	+		
	L_2R_2	+		
	L_2R_3	+		
	L_3R_1	+		
	L_3R_2	+		
	L_3R_3	+		

Lo: Seeds with no MoT infection, L_1: Seeds with 1-10% MoT infection, L_2: Seeds with 11-20%, L_3: Seeds with > 20%. R_1, R_2, R_3: Replication. +: Presence and -: absence of MoT in seeds incubated in the moist blotter

Molecular Study of the Cut Plant Parts

Gel electrophoresis of the wheat plant parts revealed the presence of DNA bands of 361bp size as depicted against the primer MoT3. The DNA bands were scored for almost all the samples of roots, stem and rachis with a few exceptions. Presence of DNA bands mostly corresponded to the presence of MoT mycelium in the corresponding TS of the plant parts (Table 7).

In roots, presence of DNA bands was scarce irrespective of sample types L0 - L3. DNA bands were detected in the root part corresponding to the presence of MoT mycelium. Here, DNA bands were not scored in the parts where MoT mycelia were not seen (Table 7).

In case of stem, all the three parts revealed DNA bands where all TSs carried mycelium of MoT except two exceptions. Here, stem part from the plant grown out of no seed infection had no MoT mycelium and DNA bands (Table 7).

In case of spike, all sections irrespective of their origin i.e., infected seeds or no, had both MoT mycelium and DNA bands (Table 7).

In general, presence of MoT mycelium and corresponding DNA bands did not depend on the seed infection by MoT. The exception to this observation may be attributed to random error in the experimental procedure. Mostly non-detection of MoT mycelium in the roots may be explained that the seed-borne MoT possesses mode of moving upward along with the growth habit of wheat plant. This is entirely a personal explanation of the researcher, yet to be supported by any available literature or further research.

Analysis of Plants Grown out of MoT Infected and Healthy Seeds

Analysis of Seedlings Selected from Seed lots by Blotter Method

MoT infected seeds developed both infected and apparently healthy seedlings in the moist blotter. Seedlings from moist blotter when planted in pot soil some proceeded to death, and some survived to grow into apparently healthy plants. These wheat plants produced apparently healthy spikes which finally all got bleached (Figure 7).

Figure 7: Seedlings and wheat plants developed out of infected seeds from moist blotter. A: Seedlings from the moist blotter planted in pot soil, B: One seedling survived, C: Plants developed from the lone seedling, D: Apparently healthy plants, E: All the plants carried bleached spike.

Seedlings in the pot soil showed typical eye-shaped blast symptoms on the leaves and stem that girdled the stem (Figure 8).

Figure 8: Wheat seedlings from infected seeds (in moist blotter) with typical eye-shaped blast symptoms when transplanted to pot soil.

Analysis of Infected Seedlings

25 MoT infected seeds of wheat variety BARI Gom23 were sown in one pot soil. There were such four pots served as replication (Table 8). In pot-1, % seeds germinated 56, number of dead seeds 11, seedlings infected 14%, apparently healthy seedlings 42%, number of infected seedlings planted-3, % seedlings showed symptom 100%, seedlings killed 2, number of seedlings grew up 1, Number of adult plants sustained 16, %adult plants carried spike 100 and % number of spikes

showed blast symptom 100. Similar trend was recorded in case of other pots. The observations confirm that *Magnaporthe oryzae* Triticum is seed borne and the blast of wheat is a seed transmitted disease (Figures 9).

Table 8: Analysis of infected wheat seeds and seedlings transferred from moist blotter to pot soil for sign and symptoms of wheat blast

Pot Number	Number of seeds plated	% seeds germinated	Number of seedlings Infected	Number of infected seedlings planted in pot soil	% seedlings showed symptoms	Number of seedlings killed	Number of seedlings grew up	Number of Adult plants sustained	% spike showed blast symptom
Pot-1	25	56 a	14 ab	3	100	2	1	16 b	100
Pot-2		52 a	18 a	4	100	2	2	36 a	100
Pot-3		56 a	16 ab	4	100	2	2	32 ab	100
Pot-4		52 a	12 b	3	100	1	2	36 a	100
CV (%)	-	14.5	18.6	11.65	-	-	11.65	24.79	-
LSD (0.05)	-	12.32	4.35	0.88	-	-	0.62	13.31	-

**Each pot: 4 Replication

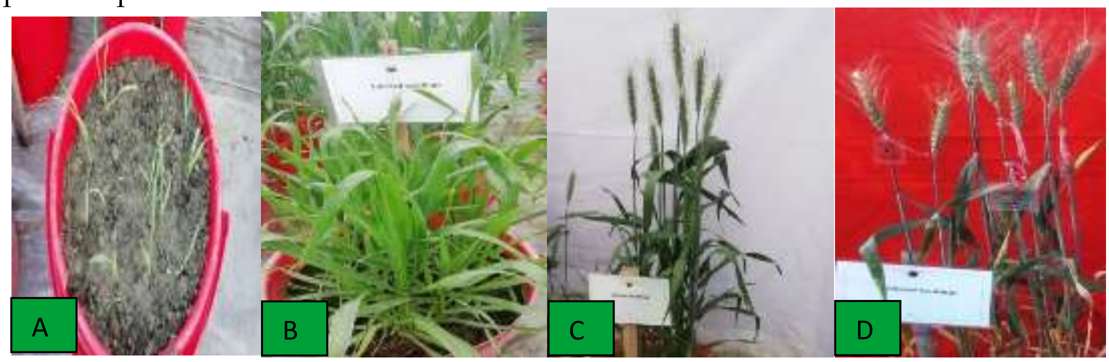


Figure 9: Infected seedlings produced infected spikes(Without inoculation). A: Infected seedlings planted in the pot soil, B: Apparently healthy plants developed from the infected seedlings, C: Apparently healthy spikes developed, D: Spikes were bleached.

Analysis of Seedlings Grown up From Apparently Healthy Seeds

Two pots were maintained. 25 seeds were sown in each pot. The seeds were randomly taken from seed lot, appeared healthy; samples were planted in moist chamber yielded infected seedlings. In pot-1, observation revealed % seed germinated 76, number of dead seeds 6, seedlings infected 12%, apparently healthy seedlings 64%, number of infected seedlings planted-3, number of seedlings showed symptom 1, seedlings killed 1, number of seedlings grew up 2, number of adult plants sustained 28, number of adult plants carried spike 28 and % spike showed blast symptom 100. Similar trend in case of other pots was observed. This analysis confirms that *Magnaporthe oryzae* Triticum is seed borne and blast is a seed transmitted disease. The results also revealed that apparently healthy seedlings are not healthy (Table 9, Figure 10).

Table 9: Analysis of apparently healthy wheat seeds planted in moist chamber and seedlings produced afterwards transferred to pot soil for sign and symptoms of wheat blast

Pot number	Number of seeds plated	%Number of seeds germinated	%Number of seedlings		Number of dead seeds	Number of infected seedlings planted	Number of seedlings showed symptoms	Number of seedlings killed	Number of seedlings grew up	Number of adult plants sustained	Number of adult plants carried spike	%Number of spikes showed blast symptom
			Infected	Apparently healthy								
Pot-01	25	76 a	12 a	64 a	6 a	3 a	1	1	2 a	28a	28 a	100
Pot-02	25	72 a	9 b	64 a	7 a	2 b	1	1	1 b	16 b	26 b	100
CV(%)	0	8.23	9.52	10.12	23.43	0	0	0	0	14.86	14.83	-
LSD(0.05)	0	10.57	2.44	11.3	2.64	0	0	0	0	5.65	1.41	-

**Each pot had four replications

A B C

Figure 10: Apparently healthy seeds produced healthy seedlings which grew up to carry blast infected

Spikes A: Apparently healthy seedlings produced from apparently healthy seeds, B: Apparently healthy adult plants, C: All the spikes carried blast symptoms

Analysis of Seedlings Grown up from Healthy Seeds

The seeds drawn randomly from seed lot did not yield any MoT infection in moist chamber. Those seeds were planted in pots. In case of pot I, observation revealed % number of seed germinated 88, seedlings infected 0%, apparently healthy seedlings 88%, number of dead seeds 3, number of healthy seedlings planted-3, % seedlings showed symptom 0%, number of seedlings killed 0, number of seedlings grew up 3, Number of adult plants sustained 44, number of adult plants carried spikes 44 and % spike showed blast symptom 0. Similar trend in case of other pots was recorded. From this observation, it is confirmed that healthy seeds did not carry *Magnaporthe oryzae* Triticum and that's why none of the plants showed any blast symptoms (Table 10, Figure 11).

Table 10: Analysis of healthy wheat seeds for blast sign and symptoms planted in moist blotter and seedlings produced afterwards transferred to pot soil.

Treatments	Number of seeds plated	%Number of seeds germinated	%Number of seedlings		Number of dead seeds	Number of healthy seedlings planted	%Number of seedlings showed symptoms	%Number of seedlings killed	Number of seedlings grow up	%Number of adult plants sustained	Number of adult plants carried spike	%Number of spike showed blast symptom
			Infected	Apparently healthy								
Pot-01	25	88 a	0	88 a	3 a	3 a	0	0	3 a	44 a	44 a	0
Pot-02	25	92 a	0	92 a	2 a	2 b	0	0	2 b	32 b	32 b	0
CV(%)	0	3.63	0	3.63	32.64	0	0	0	0	11.75	11.75	0
LSD(0.05)	0	5.65	0	5.65	1.41	0	0	0	0	7.99	7.99	0

*Each pot 4 replication

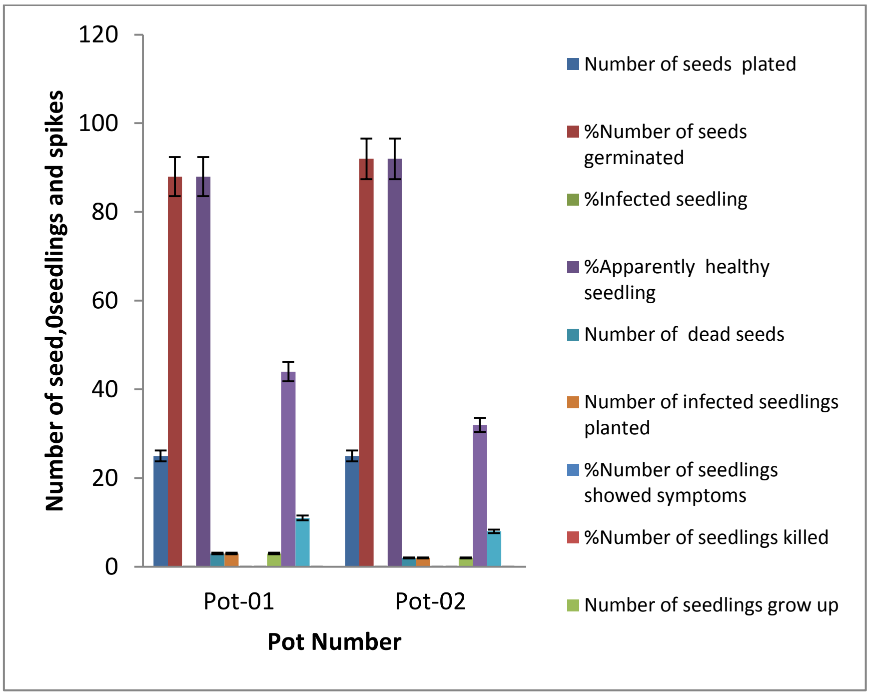


Figure 11: Analysis of healthy wheat seeds for blast sign and symptoms planted in moist blotter and seedlings produced afterwards transferred to pot soil.

Healthy seeds yielded healthy seedlings which all carried healthy spikes. None of the spikes got blast (Figure 12).

Figure 12: Healthy seeds produced healthy seedlings grew up to carry no blast symptoms

Molecular Detection of *M. oryzae pv. Triticum* in Wheat Plants and Spikes

PCR run with primer MoT3 produced band of 361 bp in 12 out of 12 infected and apparently healthy wheat plant samples tested (Fig. 13).

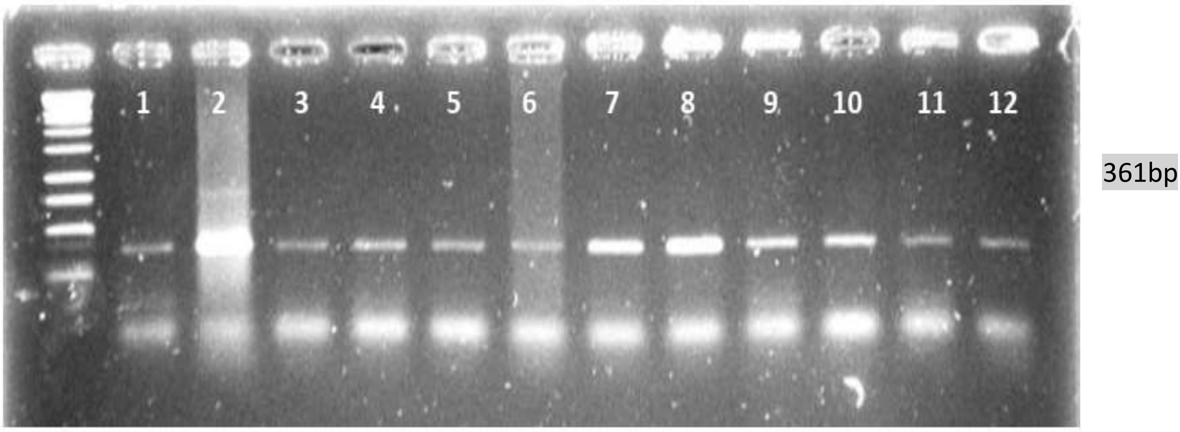


Figure 13: Agarose (1.5%) gel electrophoresis of DNA of 46 days aged wheat seedlings. The DNA was amplified with primer MoT3. Lane M- 1000bp DNA ladder; Lane (1-12) contains PCR products of 12 plant samples. Lane 1-6: infected seedlings, Lane 7-12: Apparently healthy seedlings

An amplicon size of 361 bp was found in all wheat samples tested from both infected and apparently healthy spikes (Fig. 14).

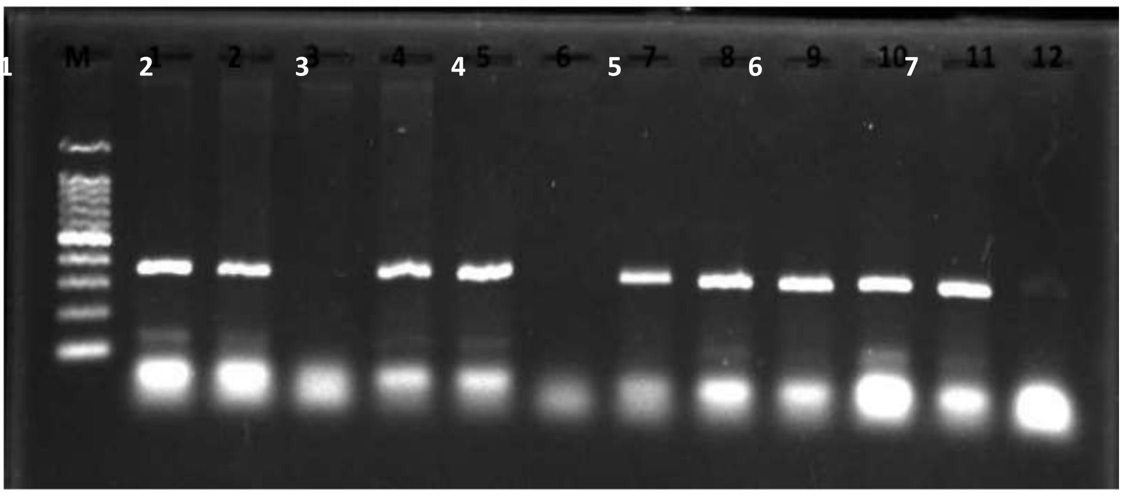


Figure 14: Agarose (1.5%) gel electrophoresis of DNA of wheat spikes. The DNA was amplified with primer MoT3. Lane M- 1000bp DNA ladder; Lane 1-6: Infected spikes, Lane 7-12: apparently healthy spikes.

There were no bands found in the samples of healthy spikes (Fig. 15).

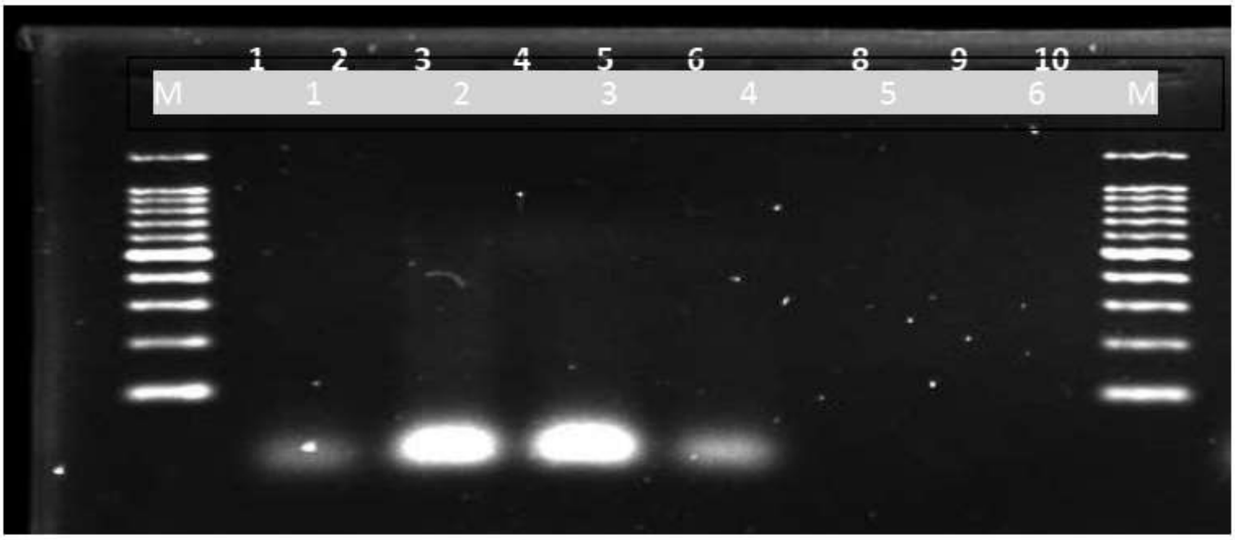


Figure 15: Agarose (1.5%) gel electrophoresis of DNA of six healthy wheat spikes. The DNA was amplified with primer MoT3. Lane M- 1000bp DNA ladder; Lane (1-6) contains PCR products of 6 spikes samples.

Discussion

Wheat blast is caused by the Ascomycetes fungus *Magnaporthe oryzae* pv Triticum. In 2016, it appeared first time in eight different districts of south-western region in Bangladesh, which resulted in burning the total fields in most of the cases (Islam*, et al.*, 2016). So, management of wheat blast is very important for sustainable production of wheat.

As seeds have been suspected to be the carrier of *M. o.* Triticum by many researchers (Urashima*, et al*., 2009, Urashima*, et al*., 1999, Goulart*, et al*., 1995), seed treatment prior to sowing should give a good protection of the wheat crops from blast pathogen. However, it is important that seed lots intended for sowing need to be checked for the possibility of the presence of *M. o.* Triticum in these seeds. Seeds in the samples collected from different areas of Meherpur district had some abnormal appearance which concede the similar opinion of Goulart*, et al*., (2007) who reported that grains from blast-infected spikes from highly susceptible cultivars are often small, shriveled and deformed. Presence of *M. o.* Triticum in seeds as detected in the present study indicates seeds as the primary source of inoculum which agrees with the similar opinion of Greer and Webster, (2001). This finding confirms the idea of Goulart, *et al.*, (1995) that infected rachis of wheat did pass on pathogen to harvested seeds. Presence of fungal contamination of wheat seeds leads the seed transmission to blast occurrence in new wheat-growing areas. This finding confirms the idea of Urashima*, et al.,* (1999) that seeds lead the transmission of blast occurrence in new wheat-growing areas.

One of the seed samples (L_0 treatment) used for PCR analysis and anatomical study was healthy looking but produced a band of typical 361bp size and showed mycelium in transverse section, supporting the opinion of Urashima*, et al.,* (2009) that seeds collected from diseased and healthy- looking spikes of certain cultivars may have similar degree of infection. Seed lots having ~20% seed infection indicated these seeds were harvested from fields with blast incidence and that the seed –borne inoculum of *M. o.* Triticum infected the wheat seeds developed on the mother plant. Therefore, these seeds cannot be recommended for sowing (Comes*, et al.*, 2017).

The results of the present investigation indicate that seeds looking healthy or diseased might carry wheat blast pathogen. Also, this phenomenon does not depend on if the seeds were collected from blast affected field or not because contaminated seeds were considered to play an important role in MoT long distance dispersion (Goulart and Paiva, 1990). As seed is the primary source of inoculum to new areas (Greer and Webster, 2001) fungal contamination of wheat seeds leads the seed transmission to blast occurrence in new wheat-growing areas (Urashima, *et al.*, 1999, Duveiller, *et al*., 2007).

TSs of plant parts displayed both intra- and inter-cellular mycelium of MoT in the xylem tissues of all the parts (root, stem, and rachis) of plants. Agarose (1.5%) gel electrophoresis produced monomorphic bands of 361bp. Most root samples, all the 11 stem samples and 12 spike samples yielded clearly amplified bands of 361bp by MoT3 primer. This confirmed the seed infection as observed in moist blotter was of *Magnaporthe oryzae* pv Triticum and confirmed the process of transmission of pathogen from seed to plant through vascular bundle.

Based on our research, we have summarized how *M o* Triticum reaches spike through the wheat plant body.

Movement of *Magnaporthe oryzae Triticum* from Seed to Seed-an Analysis

With the germination of seeds, fungi also germinate in seeds. Then fungi directly penetrate (active penetration) into young shoot of the wheat plant (sometime in both radical and plumule). With the growth of the wheat plant, fungi also grow inside of the plant without showing any symptom on plant. Fungi enter their haustorium into plant cells and take nourishment from the cells for their growth and development. We have found so many haustoria in almost all the ground tissues of the plant (Figure 8). For this work, we divided the whole wheat plant into 5 parts (Root, stem-I, stem-II, stem-III and rachis). We made several transverse sections for all the five portions and found the presence of haustorium and mycelium in nearly all portions of the plant (in case of root it wasn't regular). Sometimes we found that, fungal mycelium passed through the plasmodesma of the cells. We also found fungal mycelium in some xylem tissues, that's why we think that it also may be found in phloem tissue (the cells of the phloem tissue are too small to identify any fungal body in it). We also did molecular work on it to make sure about the presence of *Magnaporthe oryzae* Triticum in different plant parts. DNA bands of 361bp were scored in electrophoresis gel run against MoT3 marker. This indicates the presence of *Magnaporthe oryzae* Triticum in all the three portions of plant (in case of root it wasn't regular) (Figure 16).

The findings clearly demonstrate that *Magnaporthe oryzae* Triticum moves through the plant cells internally (both intra- and inter-cellularly) and reach to rachis. At the base of the rachis fungal mycelium colonized in high amount and blocked the phloem tissues. As a result, translocation of food materials is hindered and ultimately hinders grain filling and thereby grains become chaffy.

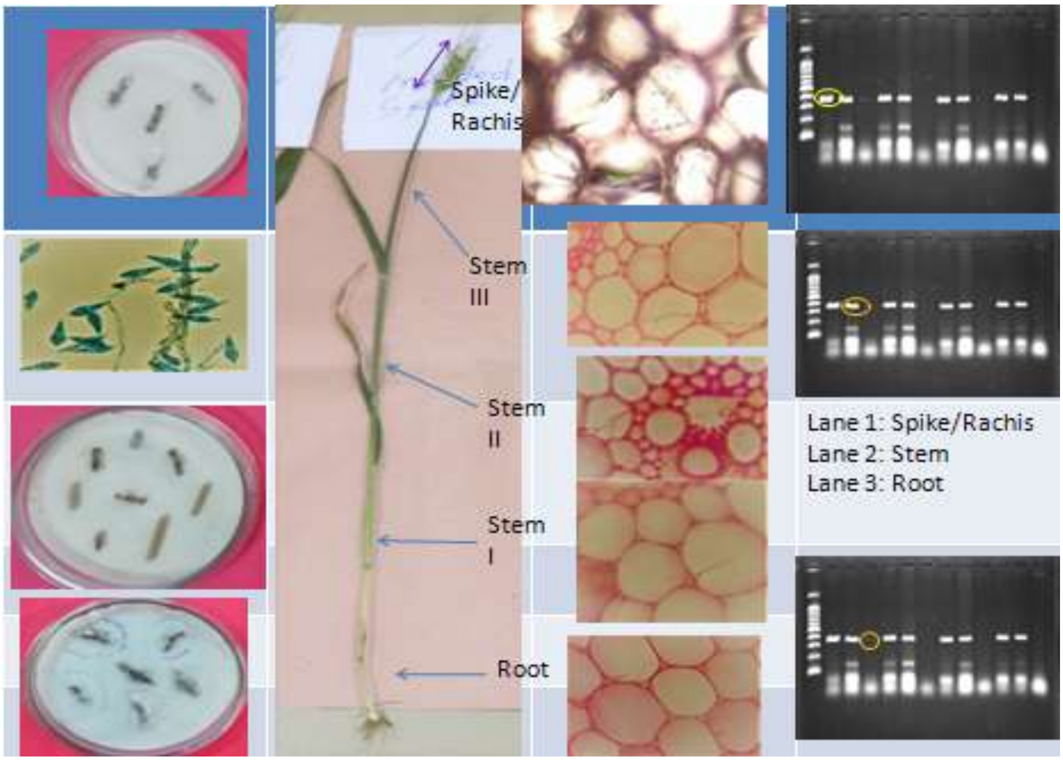


Figure 16: Movement of *Magnaportheoryzae* Triticum from seed to seed

This is a new finding confirming the seed to plant to seed transmission of *Magnaporthe oryzae* Triticum. The finding supports the results of the recent investigation revealing the presence of *M. o*. Triticum at all growth stages of wheat. The investigation was done in the wheat fields of Meherpur, hot spot of blast, during the cropping seasons of 2018-19 and 2019-20 (Meah, 2020).

Conclusion

The results of the present investigation confirmed the process of transmission of wheat blast pathogen, *Magnaporthe oryzae* pv Triticum from seed to plant through vascular bundle.

Acknowledgement

Krishi Gobeshona Foundation (KGF) financed the research (Project no. CN/FRPP: CF-50-C/17).

References

1. Aman, A. "Wheat blast threatens yield—farmers in 6 districts complain of infection." *Daily Star* (2016). *http://www.thedailystar.net/backpage /wheat-blast-threatens- yield 784372* Accessed 6 Jun 2017
2. Baenziger, P., Robert, G., D. Van Sanford. and W. Berzonsky. "Winter and specialty wheat." *Cereals*. Springer (2009): 251-265.
3. Bangladesh Bureau of Statistics. "Statistical Yearbook of Bangladesh." *Bureau of Statistics Division, Ministry of Planning, Government of the People's Republic of Bangladesh; Dhaka, Bangladesh* (2019).
4. Gomes, D.P., Valterley, S.R., Olinto, L.P. and Moacil, A.S. "Damage of wheat blast on the productivity and quality of seeds as a function of the initial inoculum in the field." *Journal of Seed Science* 27 (2017): 33-40.
5. Dixon J. The economics of wheat; Research challenges to field to fork." *Wheat Production in Stressed Environments, The Netherlands, Springer, Dordrecht* (2007): 9-22.
6. Duveiller, E., D. Hodson., K. Sonder. and A. von Tiedemann. "An international perspective on wheat blast." Special Symposium, APS-IPPC Joint Meeting, August 6-10, Honolulu, Hawaii. *Phytopathology* 101(2011): S220.
7. Duveiller, E., Ravi, P. S. and Julie, M.N. "The challenges of maintaining wheat productivity: pests, diseases, and potential

epidemics." *Euphytica* 157.3 (2007): 417–430.

8. Edwards, K., C. Johnstone, and C. Thompson. "A simple and rapid method for the preparation of plant genomic DNA for PCR analysis." *Nuclic Acid Research* 19.6 (1991):1349.
9. Goulart, A.C.P. and Paiva, F.D.A. "Transmission of *Pyricularia oryzae* by wheat (*Triticum aestivum*) seeds." *Brazilian Phytopathology* 15 (1990): 359–362.
10. Goulart, A.C.P., Paiva, F.D.A. and Andrade, P.J.M. "Relationship between incidence of blast in wheat heads and the presence of *Pyricularia grisea* in the harvested seeds." *Brazalian Phytopathology* 20 (1995): 184-189.
11. Goulart, A.C.P., Paulo, G.S. and Alfredo, S.U. "Danosemtrigo causad ospela infecção de *Pyricularia grisea*." *Summa Phytopathological* 33(2007): 358-363. http://dx.doi.org/10.1590/S0100-54052007000400007
12. Greer, C.A. and Webster, R.K. "Occurrence, distribution, epidemiology, cultivar reaction, and management of rice blast disease in California." *Plant Disease* 85.10 (2001): 1096-1102.
13. Igarashi, S., C. Utimada., L. Igarashi., A. Kazuma. And R. Lopes. "*Pyricularia* sp. emtrigo. I. Ocorrencia de *Pyricularia*sp. no Estado do Parana." *FitopatologiaBrasileira* 11(1986): 351–352.
14. Islam, M.T., D. Croll., P. Gladieux., Soanes, D.M., A. Persoons., P. Bhattacharjee., Hossain, M.S., Gupta, D.R., Rahman, M.M., Mahboob, M.G., N. Cook., Salam, M.U., Surovy, M.Z., Sancho, V.B., Maciel, J.L.N., A. NhaniJúnior., Castroagudín, V.L., de Assis Reges, J.T., Ceresini, P.C., S. Ravel., R. Kellner., E. Fournier., D. Tharreau., Lebrun, M.H., McDonald, B.A., T. Stitt., D. Swan., Talbot, N.J., Saunders, D.G.O., J. Win. and S. Kamoun. "Emergence of wheat blast in Bangladesh was caused by a South American lineage of *Magnaporthe oryzae*." *BMC Biol*. 14(2016):84.
15. Maciel, J.L.N., Paulo, C.C., Vanina, L. C., Marcelo, Z., Gerrit, H.K. and Bruce, A. M.D. "Population structure and pathotype diversity of the wheat blast pathogen *Magnaporthe oryzae* 25 years after its emergence in Brazil." *Phytopathology* 104.1 (2014):95-107.
16. Malaker, P.K., Barma, N.C., Tiwary, T.P., Collis, W.J., Duveiller, E.P., Singh, K., Joshi, A.K., Singh, R.P., Braun, H.J., Peterson, G.L., Pedley, K.F., Ferma, M.L. and B. Valent. "First report of wheat blast caused by *Magnoporthe oryzae* pathotype Triticum in Bangladesh." *Plant Disease* 2016. http://dx.doi.org/10.1094/PDIS-05-16-0666-PDN
17. Meah, M.B., Salam, M.U., A. Khan. and Kabir, W. "Wheat Blast in Southwestern Region of Bangladesh." *Report submitted to FAO (BD) on the survey of wheat blast incidence in March 2016* (2016): 7.
18. Meah, M.B. Management of wheat blast: a holistic approach with emphasis on early-stage detection for forecasting. Annual Progress Report submitted to Krishi Gobeshona Foundation (KGF). BARC Campus, Farm Gate Dhaka. 2020. 62pp.
19. Toledo, J. "Tratamiento de Semillas." *Manual de recomendaciones técnicas – Cultivo de trigo. Santa Cruz, Bolivia. Asociación de Productores de Oleaginosas y Trigo* (ANAPO) (2015): 140 .
20. Urashima, A., Grosso, C. and Stabili, A. "Effect of *Magnaporthe grisea* on seed germination, yield and quality of wheat." *Advances in Genetic, Genomics and Control of Rice Blast Disease. New York, NY, USA: Springer Science and Business Media* (2009): 267–77.
21. Urashima, A., Yoko, H., Le, D. D., Motoaki, K., Yukio, T., Hitoshi, N. And Shigeyuki, M. "Molecular analysis of the wheat blast population in Brazil with a homolog of retrotransposon MGR583." *Annual Phytopathology Society* 65.4 (1999): 429-436.
22. Yamamoto, M., Hajime, K., Tomona, Y.O., Hiroyuki, K., Yutaka, I., Hitoshi, N., Yukio, T., Shigeyuki, M. and Naoki, M. "Pathogenicity, mating ability and DNA restriction fragment length polymorphisms of Pyricularia populations isolated from Gramineae, Bambusideae and Zingiberaceae plants." *Journal of General Plant*

Pathology 66.1 (2000): 30-47.

Source of support: Nil;
Conflict of interest: The authors declare no conflict of interests.

Cite this article as:

Rayhan, Y.H., Humayun, K., Mahbubur, R., Ashraful, I., Imtiaz, U., Ayub, A. and Meah, M.B. "Seed to Plant to Seed Transmission of Wheat Blast Pathogen *Magnaporthe oryzae* Triticum." *Annals of Plant Sciences*.11.11 (2022): pp. 5465-5490.

DOI: http://dx.doi.org/10.21746/aps.2022.11.11.1