SURVEY ON WHEAT BLAST AND MORPHOLOGICAL VARIABILITY OF *MAGNAPORTHE ORYZAE TRITICUM* IN TWO SOUTH-WESTERN DISTRICTS OF BANGLADESH

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ABSTRACT

Tanjina, A., Aminuzzaman, F. M., Islam, M. R., Joty, A. A., Laila, L. and Rayhanul, M. I. 2019. Survey on wheat blast and morphological variability of *Magnaporthe oryzae triticum* in two south-western districts of Bangladesh. Bangladesh J. Plant Pathol. 35(1&2):39-46

A survey on wheat spike blast caused by *Magnaporthe oryzae* pathotype *triticum* (MoT) was conducted in eight village sites of four upazilas under two districts of south-western wheat growing regions of Bangladesh during November 2017 to March 2018. Incidence and severity of the disease varied among the survey sites. The highest blast incidence (43%) was recorded in Chithlia of Mirpur and the lowest (15%) in Bollobpur of Mujibnagar. The highest blast severity (20%) was observed in Dhanakkhula of Gangni, while the lowest severity (10%) was recorded in Chithilia and Amla of Mirpur, and Bollobpur and Doriapur of Mujibnagar. Significant losses in grain yield due to wheat blast were observed in all the survey sites of the two districts. A total of 470 symptomatic spikes, and infected stem and leaf samples were collected, dried up in the field, kept in brown paper envelopes and preserved at 4^0C for further study. MoT was isolated on Potato Dextrose Agar (PDA) and Oat Meal Agar (OMA) and identified based on 2-septate pyriform conidia, cultural and microscopic characteristics and pathogenicity study. Eight MoT isolates from eight village sites varied in their cultural characteristics observed on PDA and OMA. Mycelial growth rate/day of the isolates ranged 2.17-3.58 mm on PDA and 1.70-5.99 mm on OMA. Sporulation of the isolates varied from poor to excellent with a range of 13.3 × 2.5 µm to 20.0 × 6.0 µm spore size. Sporulation of MoT isolates was rarely found on PDA.

Key Words: Wheat blast, *Magnaporthe oryzae triticum*, variation, incidence, severity, Bangladesh

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most important cereal crop after rice in Bangladesh. It has a significant impact on the national economy and global trade. It provides 20% of the total food calories and is staple food for nearly 40% of the world population. It produces 93% of total cereal production in Bangladesh (BBS 2014). However, since the last decade, wheat consumption has been increasing rapidly due to increase in population and change in peoples' dietary habit. Outbreak of various fungal diseases has become a great threat to world's food security (Fisher *et al.* 2012). For example, blast disease of rice, wheat and other cereals can destroy enough food supply (Fisher *et al.*, 2012; Pennisi, 2010; Liu *et al.*, 2014).

Wheat blast is caused by the ascomycetous fungus *Magnaporthe oryzae* (synonym *Pyricularia oryzae*) pathotype *triticum* (Urashima *et al.* 1993, Kato *et al.* 2000, Tosa *et al.* 2006). However, the wheat infecting isolates of *Magnaporthe oryzae* are genetically different from rice infecting isolates and generally do not infect each other (Prabhu *et al.* 1992 Urashima *et al.* 1993, Urashima and Kato 1994, Farman 2002, Faivre Rampant *et al.* 2008). Blast disease of wheat was first reported in the Parana state of Brazil in 1985 (Igarashi *et al.* 1986) and spread to Bolivia in 1996 (Barea and Toledo 1996), Paraguay in 2002 and Argentina in 2007. The disease caused about 70–80% wheat production loss (Alberione *et al.* 2008, Viedma and Morel 2002). There are still some regions in South America where wheat is not cultivated because of the potential threat of this disease (Callaway 2016). However, no case was reported outside of South America except one on a single spike in the experimental field at Kentucky University, USA in 2011 (Callaway 2016).

But unfortunately, wheat blast was spotted in Bangladesh in 2016 and this was the first outbreak of the disease in Asia (Callaway 2016, Islam *et. al*. 2016, Malaker *et. al*. 2016). This outbreak proved the predictions of International Maize and Wheat Improvement Center (CIMMYT) experts that wheat blast could be spread to Asia and Africa from disease existing countries because of similar climatic conditions in these regions (CIMMYT 2016). Scientists also warned that this disease has the chance to spread to India, Pakistan, and China due to favourable environment for disease development. In Bangladesh, wheat blast appeared first in the middle of February in Chuadanga, Meherpur and Kushtia districts and rapidly spread to adjacent four districts within fifteen days (http://en.prothom-alo.com/bangladesh/news/102091/). Reports also indicated the risk of wheat production in other parts of Bangladesh and in neighbour countries. Because, wheat blast was also found in Barisal and Bhola districts which are quite far from the first spotted areas (Islam *et al.* 2016).

Continuous monitoring of disease pressure and determining possible variation among the pathogen population are of significant importance to find out sustainable disease management solutions. The present research work was therefore conducted to assess wheat blast incidence and severity in some selected sites of the south-western wheat growing region of Bangladesh and to study the variability in morphological and cultural characteristics of *Magnaporthe oryzae triticum* isolates collected from different survey sites.

MATERIALS AND METHODS

Survey area and survey period

Survey was conducted in two south-western wheat growing districts *viz.*Kushtia at $23^0$55′Nlatitude and $89^0$13′Elongitude, and Meherpur at $23^0$45′N latitude and $88^0$42′E longitude.Survey was made in the selected sites during November 2017 to March 2018.

Collection of wheat blast samples

Wheat blast samples were collected from eight village sites of four upazilas, two from Kushtia (Doulatpur and Mirpur) and two from Meherpur (Gangni and Mujibnagar). The spikes, stems and leaves were collected wherever the symptoms were seen. In each field, three random spots of $2\times2m^2$ were selected for survey and sampling (Meena 2005). The samples were kept in paper envelopes with proper labelling, air-dried overnight and brought to the plant pathology laboratory of Sher-e-Bangla Agricultural University (SAU), Dhaka and preserved at 4°C in a refrigerator before pathogen isolation.

Determination of disease incidence, severity, yield loss and economic loss

Incidence of wheat blast was assessed using the following formula of Waller *et al*. (2002):

$$\% \text{ Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plant assessed}} \times 100$$

A common formula as follows was used to calculate disease severity.

$$\% \text{ Disease severity} = \frac{\text{Spike area diseased}}{\text{Total spike area}} \times 100$$

Yield was estimated by randomly placing a quadrate (2×2m) in the field of each variety maintaining three replicates and harvesting all the plants within the quadrate. Data were converted to yield per hectare (kg ha^{-1}). Yield and economic losses were calculated by comparing the grain yield and existing market price of wheat obtained from diseased and disease free plots.

Monoconidial isolation of MoT

The symptomatic wheat spikes, infected leaves and stems were cut into 1 cm pieces, surface sterilized with mercuric chloride ($HgCl_2$) and then washed serially with sterile double distilled water and allowed for sporulation on sterilized glass slides by incubating in a moist chamber at 26ºC for 48 h. Well sporulated pieces of spikes, leaves and stems were placed in test tubes containing sterile distilled water and vortexed for 1 min. About 1 ml of spore suspension was poured onto sterilized plates containing 2% agar and dispersed. Single spores were located, picked up microscopically and eventually transferred to potato dextrose agar and oat meal agar media in test tube slants. The slants were incubated at 26ºC for 2 days.

The composition and procedures followed for preparation of the media used in this experiment were as described by Tuite (1969). For oat meal agar, 30 g oat flakes were boiled with 500 ml distilled water for 30 min. and filtered through muslin cloth. Agar 20 g was melted in 500 ml water separately. Both the solutions were mixed thoroughly with distilled water added to make the volume up to 1000 ml. For potato dextrose agar, 200 g small pieces of peeled potato were boiled with 500 ml distilled water and filtered through muslin cloth. Agar 20 g was melted in 500 ml water separately and 20 g dextrose was dissolved. The solutions were mixed with distilled water added to make 1000 ml. The media were sterilized by autoclaving at 121ºC (15 psi pressure) for 15 min.

Pathogenicity test of MoT isolates

Inoculation of MoT isolates was individually performed at 3-leaf stage (3 weeks old) of wheat seedlings. After harvesting conidia from fungal cultures on oat meal agar using sterile distilled water, the concentration was adjusted to 1×10^5 conidia/ml. Three seedlings were sprayed with 20 ml of conidial suspension using a hand sprayer, dried for a while and covered by plastic bags to maintain high relative humidity. The inoculated seedlings were kept in a growth chamber at 25ºC with 16 hr/8 hr light and darkness and 80 % relative humidity. Lesion types reflecting disease severity were assessed 10 days after inoculation.

Maintenance of isolates of *Magnaporthe oryzae triticum*

The MoT isolates were sub-cultured on oat meal agar medium and kept at 26±1ºC for 15 days. Subsequent sub-culturing of isolates was done at an interval of 20 days. Such isolates were stored in a refrigerator at 5ºC and revived monthly.

Cultural characterisation

The MoT isolates were grown on PDA and OMA. Radial mycelial growth and cultural characteristics such as colony color and colony shape were recorded at 15 days after inoculation of media. Experiment was laid out in Completely Randomized Design (CRD) with three replications.

Morphological characterisation of conidia

A microscopic slide was prepared by a fine needle from the infected host tissue placed in a moist chamber and also from the OMA culture and mounted in lactophenol. Spores were mixed with lactophenol, a uniform spread was obtained and then a cover slip was placed over it. Number of spores per microscopic field was counted. Photographs were taken to show the typical spore morphology of the MoT isolates. Sporulation was determined by a rating system described by Meena (2005) as follows:

Sporulation Type	Number of spores/ microscopic field	Index
Excellent	>30	4
Good	20-30	3
Fair	10-20	2
Poor	<10	1
Nil	0	0

Statistical analysis of data

The data were analyzed by using the "R" Software. The mean values were compared according to LSD test at 5% level of significance.

RESULTS AND DISCUSSION

Disease survey and collection of infected samples

Incidence and severity of wheat blast and disease symptoms were recorded in different varieties grown in eight survey sites of four upazilas under Kushtia and Meherpur districts (Table 1). Samples of infected spikes, stems and leaves were collected wherever the disease symptom was observed. The survey was conducted in eight village sites of four upazilas belonging to the two districts. During the survey three wheat varieties *viz*. BARI Gom 24 (Prodip), BARI Gom 26 and BARI Gom 28 were found to be infected by wheat blast. A total of 470 samples were collected for isolation of the pathogen. Typical eye-shaped lesions with light grey centre, and dark brown margin were observed on blast infected wheat leaf. Blast infected spikes had typical bleached head symptom above the point of infection on the rachis. Most of the spikes had become completely bleached with grey colour blast infection at the neck of the spike (Figs. 1 & 2).

Table 1. Wheat blast survey sites with land area surveyed and number of infected samples collected from different varieties during 2017-2018 cropping season

District	Upazila	Village	Land (Deci.)	Plant parts infected	Variety	No. of samples
Kushtia	Doulatpur	Kalidaspur	40	Spike, Stem, Leaf	BARI Gom 24 (Pradip)	50
	Mirpur	Chithlia	80	Spike, Leaf, Stem	BARI Gom 24 (Pradip)	70
		Amla	120	Spike, Leaf, Stem	BARI Gom 28	75
Meherpur	Gangni	Saharbati	80	Spike, Leaf, Stem	BARI Gom 26	60
		Dhanakkhula	80	Spike, leaf, stem	BARI Gom 28	50
		Tentulbaria	40	Spike, Leaf, Stem	BARI Gom 28	50
	Mujibnagar	Bollobpur	120	Spike, Leaf, Stem	BARI Gom 28	55
		Doriapur	80	Spike, Stem	BARI Gom 28	60
Total	4	8	640		3	470

Fig. 1. Wheat blast symptoms; Infected field (A) and bleached spikes (B, C and D).

Fig. 2. Typical eye shaped blast lesions on stem (A), Infected rachis with point of infection (B), Infected glumes (C) and infected seeds (D).

Blast incidence among the survey sites ranged from 15 to 43% where the highest blast incidence was recorded in Chithlia (Mirpur, Kushtia), followed by Kalidaspur (35%) and Dhanakkhula (32%) and the lowest in Bollobpur (Mujibnagar, Meherpur) (Fig. 3). The spike blast severity (20%) was found highest in Dhanakkhula (Gangni, Meherpur), while the lowest disease severity (10%) was observed in Chithlia and Amla of Mirpur, Kushtia and Bollobpur and Doriapur of Mujibnagar, Meherpur (Fig. 3).

Yield losses due to wheat blast varied among the survey sites where the highest yield loss (67%) was recorded in Dhanakkhula (Gangni, Meherpur) and the lowest (31%) in Bollobpur (Mujibnagar, Meherpur) (Fig. 4). The highest economic loss (64%) was also estimated in Dhanakkhula (Gangni, Meherpur) and the lowest (26%) in Bollobpur (Mujibnagar, Meherpur) (Fig. 4).

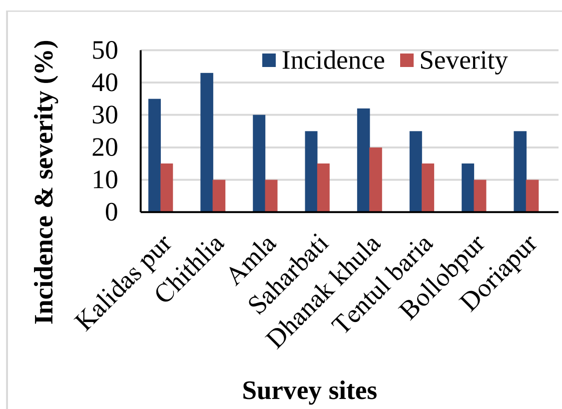

Fig. 3. Incidence and severity of wheat blast observed in eight village sites of two districts.

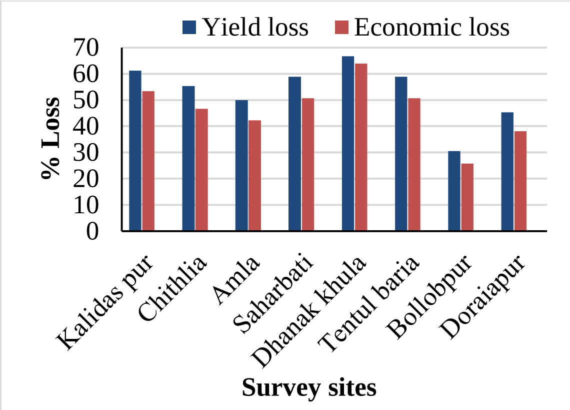

Fig. 4. Yield loss and economic loss of wheat due to spike blast observed in eight village sites of two districts.

Cultural characteristics of *Magnaporthe oryzae triticum* isolates

Magnaporthe oryzae triticum was cultured on potato dextrose agar (PDA) and oat meal agar (OMA). Mycelial growth and colony characters are presented in Table 2. The isolates showed variation in mycelial growth and colony characters on both the media used.

Mycelial growth on oat meal agar

Mycelial growth on OMA ranged from 15.2 mm to 25.1 mm at 7 days after inoculation (DAI) where the highest mycelial growth was recorded in MoT 2 isolate. The lowest mycelial growth was recorded in MoT 4 isolate. Colony colour of all the isolates was usually ash with good growth, whereas MoT 1 and MoT 8 showed greyish black colour with irregular whitish margin (Plate1).

Mycelial growth on potato dextrose agar

Mycelial growth on PDA ranged from 25.6 mm to 89.90 mm at 15 DAI where the highest mycelial growth was recorded in MoT 5 isolate. The lowest mycelial growth was recorded in MoT 8 isolate. Colonies were greyish black with irregular margin. The isolates MoT 1, MoT 3, MoT 5, MoT 7 and MoT 8 showed irregular colony margin, while the other isolates had smooth colony margin (Plate 2).

Morphological characteristics of conidia of MoT isolates

The shape of conidia was pyriform in all the isolates with rounded base and narrowed apex. The conidia were almost hyaline to pale olive and 2-septate (3-celled) with middle cell broader than others (Table 3 and Plate 3). The average conidial size of different isolates varied from 13.3 × 2.5 µm to 20.0 × 6.0 µm. Sporulating ability of the 8 isolates of *Magnaporthe oryzae triticum* was shown in Table 4. The isolates MoT 1, MoT 3 and MoT 5showed poor sporulation, whereas excellent sporulation was observed in isolate MoT 8 with 35 spores per microscopic field. However, no sporulation was found in the isolate MoT 6.

Table 2. Radial mycelial growth and cultural characteristics of eight isolates of *Magnaporthe oryzae triticum* on potato dextrose agar and oat meal agar media

MoT Isolates	Radial mycelial growth (mm)				Cultural characteristics on	
	OMA 7 DAI	Growth rate /day	PDA 15 DAI	Growth rate/day	OMA	PDA
MoT 1	22.5ab	3.21ab	85.1b	5.67a	Ash colour, smooth margin	Greyish Black colour, with concentric ring, irregular whitish margin
MoT 2	25.1a	3.58a	89.6a	5.97a	Ash colour, irregular margin	Greyish black colour, irregular margin, velvety
MoT 3	19.6bc	2.80bc	74.9c	4.90b	Greyish Black colour, Irregular margin	Greyish black colour, irregular margin, velvety
MoT 4	15.2d	2.17d	89.3ab	5.36a	Ash colour, smooth margin, raised mycelial growth	Greyish black colour, irregular margin, velvety
MoT 5	16.6cd	2.37cd	89.9a	5.99a	Ash colour, smooth margin	Greyish black colour, irregular margin, velvety
MoT 6	15.5d	2.21d	89.8a	5.98a	Yellowish colour, smooth margin	Greyish black colour, irregular margin, velvety
MoT 7	18.4cd	2.62cd	85.2b	5.68a	Greyish colour, irregular margin	Greyish black colour irregular margin, velvety
MoT 8	16.3d	2.32d	25.6d	1.70c	Ash colour, irregular margin	Greyish black colour, irregular whitish margin, velvety

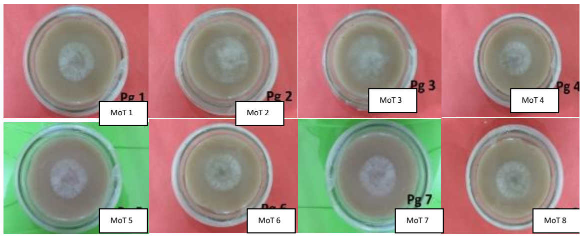

Plate 1. Pure culture of eight *M. oryzae triticum* isolates on oat meal agar at 7 days after inoculation

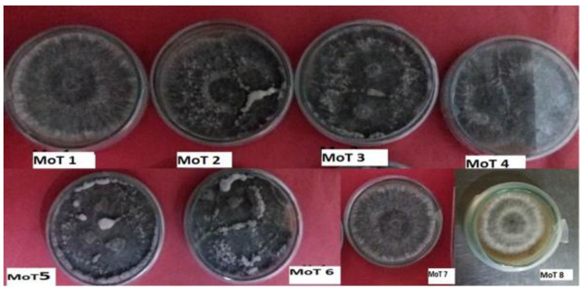

Plate 2. Pure culture of eight *M. oryzae triticum* isolates on PDA at 15 days after inoculation.

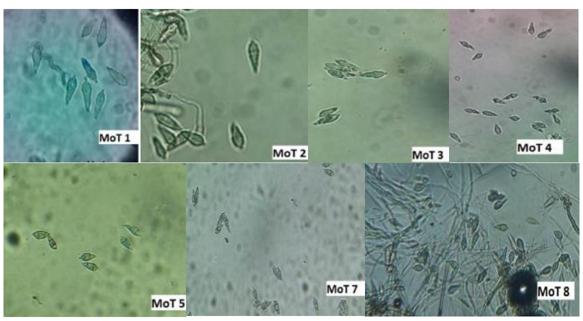

Plate 3. Spore morphology of seven MoT isolates (× 400) on OMA

In the present study it has been found that wheat blast incidence and severity varied among the survey sites. This variation might be due to difference in temperature, relative humidity, varieties grown and crop stage during disease development. A strong influence of climate on the appearance of blast epidemics in rice was reported by Suzuki (1975). Atkins (1966) also demonstrated that the weather was an important factor in the variability of disease development. When there were no fluctuations in relative humidity and temperature, there appeared no change in the number of diseases and disease severity. Wei (2015) found severe ear bleaching at temperatures above 26°C compared to lower temperatures and spike wetness significantly contributed to disease severity.

In the present study, the highest yield loss (66.66%) and highest economic loss (63.85%) were observed in Dhanakkhula (Gangni, Meherpur) and the lowest yield loss (30.55%) and the lowest economic loss (25.75%) were observed in Bollobpur (Mujibnagar, Meherpur). Economic loss, as calculated from estimated yield loss and crop values for each cultivar, varied considerably across survey sites. The losses were positively associated with disease severity recorded in different upazilas. Infection of the rachis blocks the translocation of photosynthates to the part of the spike above the point of necrosis, resulting partial or total sterility with shrivelled kernels and reduced grain yield. Prabhu *et al*. (1992) also reported the typical symptom of wheat blast was the head infection showing partial or total sterility of the spikelets.

Cultural and morphological characteristics of *Magnaporthe oryzae triticum* grown on PDA and OMA showed variation among different isolates in respect to mycelial growth, colony colour, colony margin, sporulation etc. Colour varied from ash to greyish black with smooth to irregular margin and medium to good growth. The highest mycelial growth in PDA was found in MoT 5 (89.90 mm) collected from Dhanakkhula (Gangni, Meherpur) and the lowest in MoT 3 (74.90 mm) from Amla (Mirpur, Kushtia). While working with four different solid media *viz*. Host extract + 2% sucrose agar, OMA, PDA and Richard's agar, Meena (2005) found 51.50 to 90.00 mm growth on OMA and 50.30 to 89.90 mm growth on PDA. However, no variation with respect to conidial shape was found in the present study, where conidia were pyriform, almost hyaline to pale olive and 2-septate. These characters are in agreement with Kumar and Singh (1995).

The isolates varied in their ability to sporulate on OMA but spore production was rare on PDA medium. Among 8 isolates, MoT 8 showed excellent, MoT 4 good, MoT 2 and MoT 7 fair and MoT 1, MoT 3 and MoT 5 poor sporulation, while no sporulation was found in isolate MoT 6. Meena (2005) reported that variation in cultural characters was important from the view point of pathogen biology and physiological specialization.

Table 3. Characteristics of conidia of seven *Magnaporthe oryzae triticum* isolates on oat meal agar

MoT Isolates	Shape of conidia	Number of septation	Number of cell	Colour of conidia	Size of conidia	
					Range (µm)	Average (µm)
MoT 1	Pyriform	2	3	Hyaline	12.2 - 18.1× 2.0 - 3.0	15.15× 2.5
MoT 2	Pyriform	2	3	Hyaline	11.4 - 15.2× 2.5 - 3.0	13.3 ×2.75
MoT 3	Pyriform	2	3	Pale olive	11.1 - 15.5×2.0- 3.0	13.3 ×2.5
MoT 4	Pyriform	2	3	Pale olive	14.5 - 19.7× 4.0 - 6.0	17.1× 5.0
MoT 5	Pyriform	2	3	Hyaline	15.2 - 21.5× 4.2 - 6.0	18.35× 5.1
MoT 7	Pyriform	2	3	Hyaline	15.0 - 22.0 ×4.0 - 7.0	18.5 ×5.5
MoT 8	Pyriform	2	3	Hyaline	17.6 -22.5× 5.0- 7.0	20.0× 6.0

Table 4. Sporulating ability of eight MoT isolates of *Magnaporthe oryzae triticum*

Isolates	No of spores/ microscopic field	Index	Sporulation
MoT 1	8	1	Poor
MoT 2	15	2	Fair
MoT 3	9	1	Poor
MoT 4	25	3	Good
MoT 5	6	1	Poor
MoT 6	0	0	Nil
MoT 7	15	2	Fair
MoT 8	35	4	Excellent

The study determined incidence and severity of wheat spike blast with yield and economic losse in eight village sites of two south-western districts and morphological variation in different isolates of blast pathogen collected from those sites. In order to investigate the population diversity and presence of physiologic races of *Magnaporthe oryzae triticum* more isolates should be collected from other wheat growing regions of Bangladesh. The results from this research would provide useful information for the development of strategies for wheat blast management under field conditions.

ACKNOWLEDGEMENTS

This research work was supported by Sher-e-Bangla Agricultural University Research System (SAURES), Dhaka.

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