REACTIONS OF SOME WHEAT VARIETIES TO *MAGNAPORTHE ORYZAE* PATHOTYPE *TRITICUM* IN BANGLADESH

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ABSTRACT

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The experiment was carried out to evaluate the reaction of some wheat varieties of Bangladesh against *Magnaporthe oryzae* pathotype *Triticum* (MoT). The experiment was conducted in controlled pot condition. Twelve isolates of MoT were obtained and characterized morphologically based on their colony colour, colony shape and radial mycelial growth. The isolates were inoculated on seedlings of wheat cultivar BARI Gom 25 in pot conditions. Based on their virulence patterns, most aggressive isolate, BMoT 7, was selected for further screening experiment of different wheat varieties against MoT. None of the wheat cultivars was resistant to the isolate of MoT, but the cultivars differed in degree of tolerance. The highest disease incidence (%) and disease severity (%) were found in BARI Gom 25 and BARI Gom 30. On the other hand, lowest disease incidence (%) and disease severity (%) were recorded in BARI Gom 26, BARI Gom 27 and BINA Gom 1. Therefore, BARI Gom 26, BARI Gom 27 and BINA Gom 1 can be cultivated in Bangladesh.

Key words: Wheat, blast, *Magnaporthe oryzae Triticum,* screening.

INTRODCTION

Wheat is cultivated in the world over a large area and under varied climatic conditions ranging from sub tropical to temperate. The widespread cultivation of wheat has always attracted a number of constraints and resulted in the emergence of various biotic and abiotic constraints. Among these, blast is one of the very devastating diseases of wheat. However, in 1985, wheat blast was first identified in Brazil (Igarashi *et al*. 1986). Blast causes enormous loss to wheat production and it is now a serious production constraint in the tropics and sub tropic regions, including Brazil, Argentina, Bolivia and Paraguay causing yield losses of up to 100% (Peng *et al*. 2011). Blast disease of wheat was spotted in Bangladesh at Kustia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, Bhola and other southern districts in the middle of February of 2016 (Aman 2016). This first incidence of wheat blast in Bangladesh was significantly widespread accounting for approximately 15% of Bangladesh’s total wheat area (Malaker *et al.* 2016). Occurrence of this disease caused up to 3.5% reduction of the total wheat fields in Bangladesh *(*Islam *et al*. 2016).

The wheat blast pathogen belongs to the *Magnaporthe oryzae* species complex causing blast disease on multiple hosts in the *Poaceae* family (Choi *et al*. 2013). Phylogenetic analysis revealed that the Bangladesh outbreak strains and the Brazil outbreak strains were the same phylogenetic lineage, suggesting that they might be migrated from Brazil to Bangladesh during the seed import (Sadat and Choi 2017). Development of blast resistant wheat varieties should be a long-term solution and combination of different methods with partial resistant lines may suppress this disease for some time. Wheat blast is considered primarily a disease of the wheat inflorescence (Igarashi 1990). On highly susceptible cultivars, peduncle and rachis infections cause entire spikes to become bleached out. Seeds from infected spikes are usually small, wrinkled, deformed, and have low test-weight (Urashima *et al.* 2009). The highest yield losses occur when infections start during flowering or early grain formation (Urashima *et al*. 2009). Conidia are considered the primary means for spread and infection of the peduncle and rachis. Infected seeds may disperse the pathogen in long-distance, as *Magnaporthe oryzae* is a seed borne pathogen (Maciel *et al*. 2014). Generating information on host-pathogen interactions, developing resistant wheat lines adapted to Bangladesh, the research is aiming to investigate the reaction of some wheat varieties of Bangladesh to *Magnaporthe oryzae* pathotype *Triticum* (MoT).

MATERIALS AND METHODS

The experiment was conducted at Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh from September 2016 to May, 2017. Blast infected samples such as leaves, sheath, panicle and grain were collected and taken to the laboratory. *Magnaporthe oryzae Triticum* (MoT) was cultured in PDA by tissue culture method and pure culture was prepared by transferring fungal block to new PDA plates. The fungus was identified by following Monsur *et al*. (2017). The pure culture of the fungus was maintained by routine sub-culturing after 14 days. All these work were done by maintaining aseptic condition. Morphological characterization of the isolates was performed by observing colony colour, colony shape, down size colour (Back side of petridishes) and radial mycelial growth.

Adequate amount of wheat seeds of ten varieties namely Satabdi, Bijoy, Prodip, BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 29, BARI Gom 30 and BINA Gom 1 were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. The soil of the experiment was collected from farm field, BINA, Mymensingh. The collected soil was sterilized with 5% formalin solution @ 200 ml/ cft soil kept covered with polythene sheets for 2-3 days. Then the soil was uncovered and kept for two days to release the gas of formalin. The prepared soil was poured into plastic pot (16 cm height×16 cm top diameter×11 cm bottom diameter). Apparently good looking healthy seeds of wheat were sown in line in the pot on September 27, 2016 and January 5, 2017. Seeds were placed maintaining adequate distance. After placing the seeds on the pot soil, the upper portion of the pot soil were leveled manually. Continuous supervisions were done to protect the experimental pots from external hazards and staked with bamboo sticks to keep the plants erect.

Pathotypes of MoT were transferred from pure culture and grown in PDA media for 15 days at room temperature (about 25 ºC). The conidia were removed from the plate with the aid of a brush and distilled water. Then the suspension was filtered through cheese cloth. Leaf inoculation on young plants was performed at the four leaves stage and at the adult stage at beginning of the anthesis. Wheat plants were sprayed with the inoculums suspension using hand sprayer at seedling stage on October 20, 2016 and February 10, 2017 and panicle initiation stage on March 15, 2017 as separate experiment. Just after inoculation; the pots were covered with polythene bags for four days. After removing the polythene bags the plants were exposed but watering was continued for maintaining moisture. The experimental pots were observed regularly to observe the infection. Data were taken at seedling stage and from panicle initiation to maturity. Percent disease incidence was calculated by the following formula of Ansari (1995).

To record disease severity, percentage of leaf area with necrotic spots and proportion of chlorosis were assessed separately for all unfolded leaves and arithmetic means for single plants were calculated. Percent disease severity was calculated following the method described by Chaube and Singh (1991). Data were analyzed with the help of a Computer Package Program MSTAT-C and the mean differences among the varieties were compared by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Morphological study of different isolates of MoT isolated from wheat blast grown in PDA medium is presented in Table 1. Pure culture of BMoT 1 (Isolate 1) showed regular shape surrounding with blackish colour on upper side, light black down side and radial mycelial growth 4.4 mm/day. BMoT 2 was regular in shape surrounding with whitish colour on upper side, light Black in down side and radial mycelial growth 5.0 mm/day. BMoT 3 showed regular shape surrounding with ash colour on upper side, black in down side and radial mycelial growth 4.7 mm/day. BMoT 4 showed regular shape surrounding with whitish upper side, centre – Black, surrounding cream black down side and radial mycelial growth 5.3 mm/day. BMoT 5 showed regular shape, centre black surrounding white upper side, light black down side and radial mycelial growth 5.1 mm/day. BMoT 6 showed regular shape, centre black surrounding white upper side and black down side and radial mycelial growth 5.0 mm/day. BMoT 7 showed regular shape surrounding with whitish upper side and centre – black, surrounding cream black down side and radial mycelial growth 4.7 mm/day. BMoT 8 showed regular shape surrounding with ash colour on upper side, black in down side and radial mycelial growth 5.0 mm/day. I BMoT 9 showed regular shape surrounding with ash colour on upper side, centre – black, surrounding cream black down side and radial mycelial growth 4.9 mm/day. BMoT 10 showed regular shapes surrounding with dark ash colour on upper side, black in down side and radial mycelial growth 5.1 mm/day. BMoT 11 showed regular shape, centre black surrounding with black ash colour on upper side , black in down side and radial mycelial growth 5.0 mm/day. BMoT 12 showed regular shape surrounding with whitish upper side, centre – light Black, surrounding dark black down side and radial mycelial growth 4.9 mm/day. The findings are consistent with data obtained from previous studies of

Gashaw *et al*. (2014) where they reported morphological variability of different isolates of *Pyricularia grisea* (*Magnaporthe grisea)*, which were characterized based on cultural, morphological (growth pattern, spore shape and septation), physiological character. Castroagudín *et al*. (2016) observed the cultural and morphological characteristics for *Pyricularia graminis-tritici* and *Pyricularia oryzae* pathotypes *Triticum* and *P. oryzae* pathotype *Oryza but, n*o morphological differences were observed among these species, but a distinctive pathogenicity spectrum was observed. Morphobiometrical characteristics of the typical pyriform (pear-shaped) and 2-septate hyaline conidia were in agreement with the identification of the fungus as MoT (Subramanian 1968). Molecular analysis with MoT-specific markers and comparative genome analysis of isolates confirmed that the wheat blast observed in Bangladesh is caused by MoT pathotype and has strong genetic identity to a strain from South America (Malaker *et al*. 2016). The characteristic feature of blast disease is the pyriform conidia that give rise to the disease. Conidium has three cells, with each having single nucleus per cell (Saharan *et al*. 2016)

The highest disease incidence (100%) was found in wheat isolate BMoT 7 and the lowest incidence (18.87%) was found in BMoT 6 (wheat isolate 6) which was statistically similar with BMoT 3 (19.30%), BMoT 2 (19.53%) and BMoT 1 (19.57%) as shown in Table 2. The highest disease severity on wheat seedling was found in BMoT 7 (89.20 %), which was statistically similar with isolate twelve, BMoT 12 (89.93%) and the lowest severity (10.53%) was found in BMoT 3. Based on these results, most aggressive isolate, BMoT 7 was selected for screening experiment of different wheat varieties against MoT under inoculated pot condition at different stages of growth. Peng *et al*. (2011) reported that isolates of blast pathogen from different species displayed differential infection abilities and host parasite specificity between wheat cultivars and pathogen isolates was observed. It can be mentioned that *Magnaporthe oryzae* pathotype *Triticum* is host specific. Prabhu *et al*. (1992) reported that all of the *Pyricularia grisea* isolates from rice, wheat and grass weeds were pathogenic to wheat and barley cultivars but none of isolates from wheat and from grass could infect rice.

The highest incidence (98.70%) was found in Bijoy variety which was statistically similar with Satabdi (95.00), Prodip (91.87%), BARI Gom 25 (95.13%), BARI Gom 28 (92.73%), BARI Gom 29 (92.17%) and BARI Gom 30 (95.53%) as shown in table 3. .The lowest incidence (45.37%) was found in BARI Gom 26. The highest severity (97.53 %) was found in BARI Gom 28 which was statistically similar with BARI Gom 30 (97.50%) followed by Satabdi (95.17%), BARI Gom 25 (95.17%), Bijoy (92.27%), Prodip (88.27%), BARI Gom 29 (75.17%)and the lowest severity (22.53%) was found in BARI Gom 26 followed by BARI Gom 27 (48.60%). Maciel *et al*. (2013) reported the correct quantification of blast caused by the fungus *Magnaporthe oryzae* on wheat (*Triticum aestivum*) spikes is an important component to understand the development of this disease aimed at its control. The durable blast resistance sources against wheat blast are still not found (Bockus *et al*. 2013).

Incidence (%) and severity (%) of blast in wheat plants inoculated by MoT were evaluated at 90 DAS, 100 DAS and 110 DAS (Table 4). Significant differences of percent blast incidence were observed among the different varieties at different days after sowing. At 90 DAS, the highest blast incidence (18.87%) was found in BARI Gom 25 followed by Bijoy (13.80%) and the lowest disease incidence (6.90%) was found in BARI Gom 26 followed by Satabdi (8.10%), Prodip (8.16%), BARI Gom 29 (8.23%), BARI Gom 30 (8.50%), BARI Gom 27(8.89%), BARI Gom 28 (9.33%). At 100 DAS the highest blast incidence (39.17 %) was found in BARI Gom 30 followed by Prodip (34.50%), BARI Gom 25 (33.73%), BARI Gom 29 (29.10%), BARI Gom 27(27.00%), Satabdi (26.10 %), Bijoy (22.77%) and the lowest disease incidence (14.20%) was found in BINA Gom 1 which was statistically similar with BARI Gom 26 (14.90%) followed by BARI Gom 28 (19.50%). At 110 DAS, the highest disease incidence (86.93%) was found in BARI Gom 30 followed by Prodip (66.37%), BARI Gom 25 (61.35%), Bijoy (55.17%), BARI Gom-28(53.00%) and the lowest blast incidence (26.90%) was found in BARI Gom 26 followed by Satabdi (35.00%), BARI Gom 27 (37.50%), BINA Gom 1 (43.90%).

In case of percent blast severity of wheat plants, significant differences were observed among the different varieties at different days after sowing. At 90 DAS the highest blast severity (15.90 %) was found in Bijoy followed by BARI Gom 25 (9.16 %), BARI Gom 30 (8.53 %) and the lowest blast severity (4.56 %) was found in BARI Gom 27 followed by Prodip (5.60%), BARI Gom 26 (6.46%), BARI Gom 29 (6.70%), BINA Gom 1(7.70%), Satabdi (7.77%). At 100 DAS the highest blast severity (25.60%) was found in BARI Gom 25 followed by BARI Gom 29 (23.33%), BARI Gom 30 (22.10%), Satabdi (21.43%) and the lowest blast severity (14.37%) was found in BARI Gom 26 which was statistically similar with BINA Gom 1 (14.67%) followed by BARI Gom 28 (17.47%), Prodip (18.57%), BARI Gom 27 (18.75%), Bijoy (19.37%) At 110 DAS the highest blast severity

(75.40%) was found in BARI Gom 30 followed by Bijoy (68.20%), BARI Gom 25 (67.67%), Prodip (67.20%), BARI Gom 29 (57.60%), BARI Gom 28 (54.57%) and the lowest blast severity (31.80%) was found in BARI Gom 26 which was statistically similar with BINA Gom 1 (34.57%), Satabdi (41.57%), BARI Gom 27 (45.67%). Arruda *et al.* (2005) examined the relationship between incidence of blast in wheat spikes and infection of harvested seeds using inoculation tests with three fungal isolates on five wheat cultivars. The varieties showing susceptible reaction at seedling stage were also susceptible to spike infection. Arruda *et al.* (2005) also found that the BH1146 was the sole variety resistant at seedling stage, which subsequently produced a significantly lower incidence and severity of blast. The percentage of infected seeds was less in varieties exhibiting a resistant reaction to spike infection than in susceptible varieties.

Table 1. Morphological Characteristics of different isolates of *Magnaporthe oryzae Triticum* in PDA

Isolates	Colony color	Colony shape	Down side color	Radial mycelial growth (mm/day)
BMoT 1	Blackish	Regular	Light Black	4.4
BMoT 2	Whitish	Regular	Light Black	5.0
BMoT 3	Ash	Regular	Black	4.7
BMoT 4	Whitish	Regular	Centre-Black, Surrounding-Cream Black	5.3
BMoT 5	Center Black, Surrounding White	Regular	Light Black	5.1
BMoT 6	Center Black, Surrounding White	Regular	Black	5.0
BMoT 7	Whitish	Regular	Center Black, Surrounding Cream Black	4.7
BMoT 8	Ash	Regular	Black	5.0
BMoT 9	Ash	Regular	Center Black, Surrounding Cream Black	4.9
BMoT 10	Dark Ash	Regular	Black	5.1
BMoT 11	Center Black, Surrounding Black Ash	Regular	Black	5.0
BMoT 12	Whitish	Regular	Center Light Black, Surrounding Dark Black	4.9

BMoT=Bangladesh *Magnaporthe oryzae Triticum* isolate

Table 2. Pathogenicity of different isolates of MoT causing seedling blast of wheat

Name of the isolates	Disease incidence (%)	Disease severity (%)
BMoT 1	19.57 e	19.67 f
BMoT 2	19.53 e	19.43 f
BMoT 3	19.30 e	10.53 g
BMoT 4	48.70 c	68.87 b
BMoT 5	16.83 f	19.57 f
BMoT 6	18.87 e	19.33 f
BMoT 7	100.0 a	89.20 a
BMoT 8	48.83 c	41.00 d
BMoT 9	28.03 d	29.87 e
BMoT 10	48.90 c	49.40 c
BMoT 11	18.67ef	19.50 f
BMoT 12	78.93 b	88.93 a
Label of significance	**	**
CV (%)	2.86	3.67

BMoT=Bangladesh *Magnaporthe oryzae Triticum* isolate

****** Significant at 1% level of significance

Table 3. Reaction of wheat varieties to MoT (Isolate, BMoT 7) **under inoculated pot condition at seedling stage**

Variety	**Disease incidence (%)**	**Disease severity (%)**
Satabdi	95.00 a	95.17 ab
Bijoy	98.70 a	92.27 ab
Prodip	91.87 a	88.27 b
BARI Gom 25	95.13 a	95.17 ab
BARI Gom 26	45.37 c	22.53 e
BARI Gom 27	78.77 b	48.60 d
BARI Gom 28	92.73 a	97.53 a
BARI Gom 29	92.17 a	75.17 c
BARI Gom 30	95.53 a	97.50 a
Level of significance	**	**

****** Significant at 1% level of significance

Table 4. Incidence (%) and Severity (%) of blast of wheat inoculated by MoT at 90 DAS, 100 DAS and 110 DAS

Varieties	**Disease incidence (%)**			**Disease severity (%)**		
	90 DAS	**100 DAS**	**110 DAS**	**90 DAS**	**100 DAS**	**110 DAS**
Satabdi	8.10 def	26.10 bcd	35.00 f	7.77 bcd	21.43 bcd	41.57 e
Bijoy	13.80 b	22.77 cde	55.17 d	15.90 a	19.37 cde	68.20 b
Prodip	8.16 cde	34.50 ab	66.37 b	5.60 cd	18.57 de	67.20 b
BARI Gom 25	18.87 a	33.73 ab	61.35 c	9.16 b	25.60 a	67.67 b
BARI Gom 26	6.90 f	14.90 e	26.90 g	6.46 bcd	14.37 f	31.80 f
BARI Gom 27	8.89 cd	27.00 bcd	37.50 f	4.56 d	18.75 de	45.67 d
BARI Gom 28	9.33 c	19.50 de	53.00 d	8.80 bc	17.47 ef	54.57 c
BARI Gom 29	8.23 cde	29.10 bc	61.07 c	6.70 bcd	23.33 ab	57.60 c
BARI Gom 30	8.50 cde	39.17 a	86.93 a	8.53 bc	22.10 bc	75.40 a
BINA Gom 1	7.60 ef	14.20 e	43.90 e	7.70 bcd	14.67 f	34.57 f
Level of significance	**	**	**	**	**	**

DAS= Day after sowing, ****** Significant at 1% level of significance

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