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Research Article

Irradiation of Wheat Seeds for Developing Mutants Resistant to Blast *(Magnaporthe oryzae* Triticum*)*

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Abstract

Seeds of four wheat BARI Gom varieties were irradiated with four different doses of Gamma rays (150Gy, 200Gy, 250Gy, 300Gy). Both M1 and M2 population were exposed to wheat blast under natural field condition. In the present study, M3 seeds harvested off M2 population were grown under both confined inoculated and natural field condition. In BINA campus, M3 plants were inoculated with spore load (CFU 1×107 spores/ml) of *Magnaporthe oryzae* Triticum (MoT) at pre-heading stage (53 days age). Inoculation was repeated thrice to cover pre-heading stage of some late mutants. In Madnadanga Meherpur Sadar, M3 population was exposed to blast infection under natural field condition in the farmer's field. Among the radiation doses, 200Gy produced significant reduction in MoT infection. Inoculated M3 plants showed differential reaction to Magnaporthe infection. Only one entry (BWM 5) was completely free of blast infection. Another one entry (BWM 6) carried only 0.13 % blast severity. Eight entries (BWM 2, 3, 7, 9, 20, 21, 22, 23) had <5% blast severity while other four entries had< 10% blast severity. Here blast severity means percent spike surface area bleached. Molecular analysis in PCR done with MoT3 marker produced typical bands of MoT (361 bp) in all the entries except for the entry BWM 5. In Madnadanga farmer's field, blast disease pressure was very low; most of the entries did not catch infection. Entry BWM 5 and entry BWM 6 and some other 12 entries which showed 0-10% blast severity indicated the inheritance of blast resistance which needs advancing through further checking under inoculated epiphytotic condition.

Keywords: *wheat blast, resistant mutant, seed irradiation*

Introduction

Wheat *(Triticum aestivum* L.) is one of the most important cereal crops in the world. It is the second most important cereal crop contributing to food security of Bangladesh (Mottaleb *et al*., 2019). However, this widespread cultivation of wheat has always attracted a number of biotic and abiotic constraints and resulted in the emergence of diseases, blast *(Magnaporthe oryzae* Triticum) is the most devastating one. 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 eight southern districts of Bangladesh for the first

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time 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)

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 strain and the Brazil outbreak strains were the same phylogenetic lineage (Sadat and Choi, 2017). Wheat blast is considered primarily a disease of the wheat inflorescence (Igarashi, 1990), the pathogen infects all above ground parts of the wheat plant though. On highly susceptible cultivars, peduncle and rachis infections cause entire spikes to become bleached out. Seeds from infected spikes are usually small, wrinkled and deformed (Urashima *et al*., 2009). Infected seeds may disperse the pathogen in long-distance, as *Magnaporthe oryzae* is a seed borne pathogen (Maciel *et al*., 2014). On the leaves, the symptoms of wheat blast are elliptical to elongate lesions with light to dark green centers and yellow borders.

Due to lack of resistant cultivar and ineffectiveness of fungicides at higher disease pressure, wheat blast is widely distributed across the wheat growing areas. Most of the wheat cultivars are susceptible to wheat blast in Bangladesh (Malaker *et al*., 2016). Tolerant cultivars should have to be identified in specific areas, but still yet to develop variety with durable resistance. It is reported that the strains of *Magnaporthe oryzae* Triticum in Bangladesh are more aggressive than those reported earlier (CIMMYT, 2016). The conventional breeding methods of gene isolation and transfer have been tried. One approach the irradiation of seeds to bring a small change in the genetic makeup has not been tried against the blast pathogen.

Currently, mutation breeding has become popular among the breeders and scientists. Mutation breeding has been used since the 1930s (Ahloowalia *et al*., 2004). It's a means of accelerating the process of developing different traits for selection, such as disease resistance, tolerance to harsh growing conditions. Irradiation of seeds with ionizing radiation is aimed to generate mutants with desirable traits. Gamma radiation from radioactive cobalt (^{60}Co) is widely used for this purpose. It has high penetrating potential (Kunzang *et al*., 2017). In 2004, Wonchu mutant variety of Oryza sativa was developed by irradiating seeds at 250 Gy of gamma rays (Raina and Danish, 2018). The mutant variety of durum wheat 092 was developed by irradiation with gamma rays (200Gy) for resistance to stripe and stem rust (IAEA, 2017). Bangladesh Institute of Nuclear Agriculture (BINA) developed some resistant varieties of rice and cereal crops. The rice varieties developed by BINA using irradiation are Binadhan-22, Binadhan-23 and Binadhan-24(BINA, 2019).

To minimize the impact of blast disease, environment-friendly and cost- effective technologies are needed. The use of mutation breeding techniques to develop disease resistant varieties is a viable tool in the development of appropriate germplasm and varieties. Based on these points, the possibility of creating wheat mutants resistant to *Magnaporthe oryzae* Triticum was explored through seed irradiation

Materials and Methods

Field experiments

The experiments were carried out in the Bangladesh Institute of Nuclear Agriculture (BINA) farm, Mymensingh (24.7471° N, 90.4203° E) and farmer's fields of

Madnadanga, Meherpur sadar (23.8052° N, 88.6724° E) during 2018-2019 cropping season. Laboratory works were accomplished in the IPM Lab of Bangladesh Agricultural University (BAU), and Biotechnology Lab of BINA. Seeds of BARI Gom 21, BARI Gom 23, BARI Gom 24, BARI Gom 25, BARI Gom 26, BARI Gom 28, BARI Gom 29 and BARI Gom 30 were used in this experiment. Four different doses of gamma rays in Gray (150Gy, 200Gy, 250Gy and 300Gy) were applied on dry seeds of BARI Gom 21, 25, 29 and 30 from 60Co (Gamma Chamber 5000) at BINA Molecular Laboratory, Mymensingh in November 2017.Remaining four varieties used as check were not exposed to gamma radiation.

In BINA farm, M3 plants were artificially inoculated with *Magnaporthe oryzae* Triticum (MoT) whereas, the M3 plants of Madnadanga farmer's field were left to develop infection naturally. Land was prepared following standard procedure of wheat cultivation. Randomized Complete Block design was maintained with unit plot size 2.5m×4m. Continuous line sowing was done with spacing between line to line 20 cm and replication to replication 1m. Three replications were maintained per treatment. The land was fertilized with 220 kg Urea, 125 kg TSP, 120 kg MOP, 65 Gypsum, 6 kg Zinc sulphate, and 6kg Boric acid per hectare (BARC, 2018). Plant thinning was not performed for the crop. Besides, two irrigations were given to the crop at 20 and 58 days after sowing. No pesticides were applied.

Inocula preparation

Spikes of wheat infected with blast were collected from Meherpur, hotspot of wheat blast. Inocula of 2-3 cm size were prepared out of the specimens. 3-4 inocula were placed on the moist blotter in equal distance and incubated at room temperature (30±1℃). Observations were made for the growth of *Magnaporthe oryzae* Triticum (MoT) out of the inocula. The fungus was identified microscopically following keys for *M. oryzae* Triticum (Tembo *et al*. 2020). The conidia along with conidiophore of *Magnaporthe oryzae* Triticum were transferred to Oatmeal Agar (OMA) medium (Oat 50 g, Agar 15 g, Streptopen4-5 spoon, Distilled water 1000 ml) with the help of sterile pointed needle and incubated at 30±1℃ temperature with 55% relative humidity for luxuriant growth. Viewing under stereobinoular microscope, single conidia were transferred to fresh OMA plates and incubated under the same conditions. In 7-10 days, colonies of typical deep ash colour with occasional zoning developed in all the plates. Thus, the pure cultures of the pathogen obtained were multiplied in OMA. The plates were then placed in an incubator with a 12 h/12 h (light/dark) cycle under NUV light for 10 to 15 days at 30±1℃ with 55% relative humidity to promote conidiation (Barksdale and Asai, 1961).

Preparation of *M. oryzae* Triticum spore suspension

Conidia were harvested by rinsing the surface of cultures with sterile distilled water and scraping it using a glass slide. The conidial suspension was filtered through two layers of muslin cloth to remove mycelia and agar. The number of conidia in the suspension was determined using a hemocytometer and a spore concentration of 1×107 conidia/ml was used for inoculation (Akagi *et al*., 2015).

Wheat ear and leaf inoculation

Inoculation was done at pre-heading stage of wheat right at 53 days after sowing in BINA farm. This time varied as some entries flowered late. Before inoculation, the plot was covered with polythene shed to maintain high temperature (30 ± 10C) and high relative humidity

and to keep it in darkness. Inoculations were repeated thrice through spraying conidial suspension onto the leaves and ear. Inoculated plants were watered daily for maintaining high humidity (> 80%). Symptoms of blast appeared on spike 12 days (65 days after sowing) after MoT inoculation.

Data collection and analysis

Data were collected on days to symptom expression, disease incidence and disease severity.

The percentage of disease incidence was calculated by following formula:

$$\text{Percent disease incidence} = \frac{\text{Number of diseased spikes}}{\text{Total Number of spikes counted}} \times 100$$

Percent surface area of spike infected/bleached was estimated as disease severity. All spikes in a 1 x 1 m^2 area per plot were tagged. Percent spike surface area bleached was determined through eye estimation and average of all readings was calculated for one plot (replication).

Data were analyzed statistically by using Minitab 17 software (www.minitab.com). The mean of all the treatments were compared by critical difference value at 5 % level of significance.

Infected samples of wheat spikes were collected and analyzed for the presence of *Magnaporthe oryzae* Triticum (MoT) following both conventional pathological techniques (moist chamber technique) and molecular technique (PCR) using specific primer.

PCR Analysis

DNA extraction

DNA was extracted using the mini preparation CTAB method (Edwards *et al*. 1991) in the Biotechnology Laboratory, BINA, Mymensingh. The spike samples were cut into 2-3 cm pieces and crushed in small mortar. Then extraction was done with 800µl 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 800µl 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 1.5ml tube (already labeled). Later, the chloroform and plant DNA binding solution and1000 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. 1000µl 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 100µl TE buffer and dissolved pellet by warming in a 65°C water bath for up to 1h (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) (Table 1) 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. The cycling parameters were 94°C, 1.5min to initial denaturation and 94°C, 30 seconds at denaturation;

anneal at 62°C, 30 seconds; extend at 72°C,1min; final extension at 72°C, 2 min; holding temperature at 10°C until removing from thermo cycler. 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 mgml-1) the gel was placed on high performance ultraviolet light box (UV-trans-illuminator) of GEL Doc System for checking the DNA bands.

Table 1. The name, sequence and size of the selected specific primer used for M3 survey

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

Results & Discussion

Symptom expression of blast on M_3 plants

Spike infection

Infection appeared on the emerging heads at pre-heading stage of wheat plants (both in BINA, Mymensingh and in the farmer's fields of Madnadanga, Meherpur) as whitening of the spike most commonly from the top. Infection also occurred randomly on the emerging head in different points i.e., in the middle or near the tip or at the base. Common thing was observed that irrespective of the point of infection, bleaching occurred from the top to downward. It means if infection occurred at the base, the whole of the spike bleached, an infection near the top caused bleaching of the spike from the top to down the point of infection and an infection in the middle-caused bleaching of the half of the spike from the top. However, with time the whole of the spike in all cases turned silvery white i. e. completely bleached (Figure 1a, 1b, 1c).

Leaf infection

Initially small brown dots appeared on leaf and rapidly expanded in inoculated wheat plants observed in BINA, Mymensingh. Older lesions on the leaves were elliptical with pointed ends (elongated diamond-shaped spot or eye shaped spot). The centers of the spot were usually whitish or gray with brown to reddish –brown necrotic border (Figure 1d, 1e).

Blast infection on the leaves of M3 plants were never observed under natural field condition in Madnadanga.

Stem infection

Symptoms appeared on stem had similarities with the symptoms observed on the leaves. Lesion on stems showed pale tan centers with brown margins (Figure 1f).

Blast infection i.e. bleaching of spike progressed rapidly and wheat population showed a burning appearance in the confined experimental field.

Figure 1. a. typical eye-shaped spot on the spikelet, **b**. whitening of spike from top, **c**. bleached wheat ears with straw color, **d**. initial brown spotting on the leaf, e. typical eye-shaped blast lesion on the wheat leaf, **f.** lesion on stems showing pale tan centers with brown margins.

Time to expression of blast symptoms on M_3 plants

The symptoms of blast first appeared as blackening of awns on the inoculated M_3 plants in BINA at 65 days after sowing, approximately 12 days after inoculation and spike infection succeeded the awn infection approximately 7 days after. In Madnadanga, infection appeared on the emerging heads generally at 85-88 days age of wheat plants**.**

Incidence and severity of wheat blast on M_3 population

Wheat blast on inoculated M_3 plants in BINA Campus

On Spike

Incidence of wheat blast on M_3 plants of BARI Gom varieties did not differ significantly for different irradiation doses. M_3 plants of BARI Gom25 and radiation dose of 200Gy had comparatively lower blast severity (Table 2).

Table 2: Main effect of wheat varieties and radiation dozes on the incidence and severity of blast on th spike of M_3 population inoculated at pre-heading stage with spores of *Magnaporthe oryzae* Triticum.

Entries	Disease incidence (%) at irradiation					Disease severity (%) at irradiation				
	00 Gy	150 Gy	200 Gy	250 Gy	300 Gy	00 Gy	150 Gy	200 Gy	250 Gy	300 Gy
BARI Gom 21	100a	100a	97.95a	100a	100a	100a	92.67 b	93.00 b	83.0 b	95.33 a
BARI Gom 25	96.67a	94.76 a	87.7 a	90.37 a	95.21a	91.93 b	78.33 c	76.33c	81.66b	80.33 b
BARI Gom 29	100a	100a	100a	100a	100a	100a	99.67a	100a	100a	100a
BARI Gom 30	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
CV (%)	2.88	4.53	10.82	8.33	4.15	3.44	2.06	1.53	2.25	2.63
LSD (%)	5.43	8.54	20.37	15.69	7.82	6.47	3.88	2.88	4.24	4.95

In each column values with same letters indicate statistically similar.

Incidence and severity of spike blast on BARI Gom 26 were 100%, and in three other BARI Gom varieties were 100 and 93-97% respectively (Table 3). Entry BWM 5 carried no blast (Figure 2). In the remaining 23 entries, blast disease incidence varied from 2 – 93% while severity ranged between 0.13 – 94%. Among them entry BWM 6 carried the lowest blast infection. Three entries BWM 3, BWM 20 and BWM 21 had blast incidence <20% while blast severity was between 1.5 – 2.2% (Table 3). Five entries (BWM 2, BWM 7, BWM 9, BWM 22 and BWM 23) had blast severity <5% though the blast incidence was in the range of 30-40%. Again, three entries (BWM 1, BWM 15 and BWM 19) carried blast severity <10% (Table 3).

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Figure 2. Entry BWM 5 did not catch *Magnaporthe oryzae* Triticum infection

Table 3: Incidence and severity of blast in wheat spike of 27 entries inoculated with spores of *Magnaporthe oryzae* Triticum at pre-heading stage under confined nethouse condition in BINA.

Entries	Disease Incidence (%)	Disease Severity (%)
BARI Gom 23	100.00 a	95.47abcde
BARI Gom 24	100.00 a	97.67 abc
BARI Gom 26	100.00 a	100.00 ab
BARI Gom 28	100.00 a	93.13 abcdef
BWM 1	52.983 cdefgh	7.1333 jk
BWM 2	40.980 fghi	4.8667 jk
BWM 3	17.953 hijk	1.5333 k
BWM 4	77.223 abcde	94.333 abcde
BWM 5	0.0000 k	0.0000 k
BWM 6	2.1800 jk	0.1333 k
BWM 7	27.717 ghijk	4.8667 jk
BWM 8	93.343 ab	86.333 abcdefgh
BWM 9	40.980 fghi	4.8667 jk
BWM 10	71.617 abcdef	69.667 h
BWM 11	90.050 ab	75.333 gh
BWM 12	57.627 ef	44.667 i
BWM 13	53.623 cdefgh	49.667 i
BWM 14	83.747 abcd	14.333 jk
BWM 15	52.983 cdefgh	7.1333 jk
BWM 16	40.920 fghi	11.667 jk
BWM 17	41.893 efghi	6.000 jk
BWM 18	51.177 defghi	20.867 j
BWM 19	37.193 fghij	6.067 jk
BWM 20	20.073 hijk	2.2667 k
BWM 21	15.643 ijk	1.9333 k
BWM 22	29.400 ghijk	4.8667 jk

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BWM 23	30.190 ghijk	4.6667 jk
CV (%)	12.99	6.47
LSD (%)	21.22	10.56

In each column values with same letters indicate statistically similar. BARI Gom 23, 24, 26 and 28 are cultivated blast susceptible wheat varieties used as check. BWM: BINA Wheat Mutant.

On leaves

Incidence and severity of blast infection on leaves of M_3 population did not vary for varieties or radiation doses. The blast incidence ranged from 69.85 – 91.38%. Severity of blast ranged from 6.60 –12.33% (Table 4).

Table 4: Main effect of wheat varieties and radiation dozes on the incidence and severity of blast on leaves of M3 population inoculated at pre-heading stage with spores of *Magnaporthe oryzae* Triticum in BINA nethouse.

Entries	Disease incidence (%) at irradiation					Disease severity (%) at irradiation				
	00 Gy	150 Gy	200 Gy	250 Gy	300 Gy	00 Gy	150 Gy	200 Gy	250 Gy	300 Gy
BARI Gom 21	83.85 a	75.75 a	79.01 a	83.01 a	85.87 a	9.60 a	8.07 a	7.27 a	8.27 a	6.80 b
BARI Gom 25	69.85a	71.867 a	86.51 a	80.443 a	74.62 a	7.73 a	6.60 a	9.33 a	7.86 a	7.60 b
BARI Gom 29	85.24a	80.0a	89.0a	86.67a	85.55a	10.33 a	9.07 a	8.93 a	9.00 a	7.87 b
BARI Gom 30	76.30a	91.38a	74.5a	80.43a	73.92a	9.53 a	9.53 a	12.33 a	10.60 a	11.46 a
CV (%)	14.76	15.30	10.33	6.79	9.46	1.66	2.39	2.82	1.59	1.82
LSD (%)	27.79	28.8	19.38	12.80	17.77	3.13	4.49	5.31	2.99	3.43

In each column values with same letters indicate statistically similar.

Blast infection observed on the leaves of 27 entries varied significantly. Blast incidence varied from 29.57 – 88.70% while severity ranged from 1.67 – 12.00%. Severity of blast infection on the leaves of all entries was low. Entry BWM 5 carried the lowest blast incidence and severity on leaves (Table 5).

Table 5: Incidence and severity of blast in wheat leaf of 27 entries inoculated with spores of *Magnaporthe oryzae* Triticum at pre-heading stage under confined nethouse condition in BINA.

Entries	Disease Incidence (%)	Disease Severity (%)
BARI Gom 23	77.44abcde	11.13 abcd
BARI Gom 24	85.14abcd	12.00 ab
BARI Gom 26	88.70a	11.73a
BARI Gom 28	80.10abcde	8.47 abcd
BWM 1	50.78bdefg	5.47 abcd
BWM 2	85.81abcd	8.133 abcd
BWM 3	46.50efg	5.33 bcd
BWM 4	77.19abcde	6.40 abcd
BWM 5	29.57g	3.53 cd
BWM 6	31.53fg	3.27 d
BWM 7	49.82cdefg	5.73 abcd
BWM 8	70.65 abcde	7.47 abcd
BWM 9	66.0 abcdefg	5.60 abcd
BWM 10	57.12 abcdefg	6.67 abcd

BWM 11	81.39abcde	8.20 abcd
BWM 12	73.53 abcde	11.67 abcd
BWM 13	73.9abcde	9.00 abcd
BWM 14	67.72 abcdef	8.93 abcd
BWM 15	71.76 abcde	10.0 abcd
BWM 16	76.25abcde	10.33 abcd
BWM 17	74.44abcde	1..67 abcd
BWM 18	70.16 abcde	11.0abcd
BWM 19	69.36abcde	7.53 abcd
BWM 20	81.75abcde	9.93 abcd
BWM 21	78.24abcde	6.00 abcd
BWM 22	67.62 abcdef	6.00 abcd
BWM 23	70.42 abcde	6.00 abcd
CV (%)	10.43	2.62
LSD (%)	17.03	4.28

In each column values with same letters indicate statistically similar. BARI Gom 23, 24, 26 and 28 are cultivated blast susceptible wheat varieties used as check. BWM: BINA Wheat Mutant.

Incidence and severity of wheat blast on M_3 population in farmer's fields of Madnadanga

Blast disease incidence and severity was low on the spikes of M_3 population sown in the farmer's fields of Madnadanga. The blast incidence ranged from 6.57- 44.10% and severity varied from 1-58.33% for different varieties and radiation dozes (Table 6).

Table 6: Incidence and severity of blast on wheat spike of M_3 population in the farmer's field of Madnadanga

Entries	**Disease incidence (%) at irradiation (Gy)**					**Disease severity (%) at irradiation(Gy)**				
	00	**150**	**200**	**250**	**300**	**00**	**150**	**200**	**250**	**300**
BARI Gom 21	31.52b	44.17 a	17.87 c	27.50 a	44.10 a	28.33 b	3.67 a	11.67 b	33.33 a	30.0 a
BARI Gom 25	32.77b	12.33 d	13.57 d	13.20 c	6.57 c	4.33 c	2.33 b	3.67 c	2.33 b	1.0 d
BARI Gom 29	40.20 a	20.70 c	23.40 b	11.37 d	12.33 b	58.33 a	4.67 a	4.0 c	1.67 b	1.67 b
BARI Gom 30	18.20 c	28.83 b	33.37 a	17.47 b	8.2 c	1.67 c	1.33 b	50.0 a	33.33 a	1.33 c
CV (%)	1.65	1.21	1.09	1.59	1.37	2.14	0.76	1.63	2.14	0.41
LSD (%)	3.10	2.29	2.07	2.99	2.59	4.03	1.44	3.08	4.03	0.24

In each column values with same letters indicate statistically similar.

In Madnadanga, blast infection did not develop on the spikes of 16 entries. Blast infection was low on the remaining 11 entries. Incidence and severity ranged between 0-57.83% and 0-61.67% respectively (Table 7). Inoculum pressure was very low under natural field conditions.

Table 7. Incidence and severity of blast in wheat spike of 27 entries under field conditions in Madnadanga

Entries	Disease Incidence (%)	Disease Severity (%)

BARI Gom 23, BARI Gom 24, BARI Gom 26, BARI Gom 28	12.73 -57.83	2.33 – 61.67
BWM 2, BWM 3, BWM 5, BWM 6, BWM 7, BWM 9, BWM 12, BWM 13, BWM 15, BWM 16, BWM 17, BWM 18, BWM 19, BWM 20, BWM 21, BWM 22	0.00	0.00
BWM 1, BWM 4, BWM 8, BWM 10, BWM 11, BWM 14, BWM 23	5.73 – 11.80	1.00 – 1.33

BARI Gom 23, 24, 26 and 28 are cultivated blast susceptible wheat varieties used as check. BWM: BINA Wheat Mutant.

Progress in spike bleaching due to blast infection

Progress in spike bleaching in M_3 population was followed. Spike bleaching (blast severity) was recorded for 6 times @ 2 day's intervals beginning from 1st sight of blast symptoms. In variety BARI Gom 25, spike bleaching progressed more slowly in 250Gy plants than in plants radiated with other doses (Figure 3).

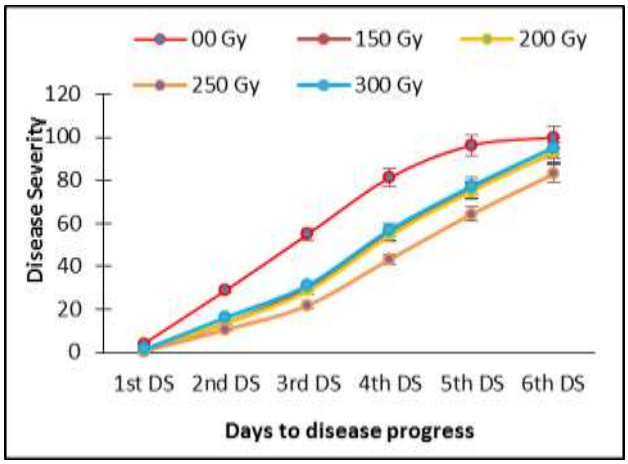

Figure 3. Progress in spike bleaching (blast severity) in radiated wheat plants of BARI Gom 25 over a period of 12 days

In other four varieties, effect of dose of radiation was not significantly different. Non-radiated seeded plants of five wheat varieties showed higher rate of progress in spike bleaching. Among them, wheat variety BARI Gom 25 had slower rate of progress (Figure 4).

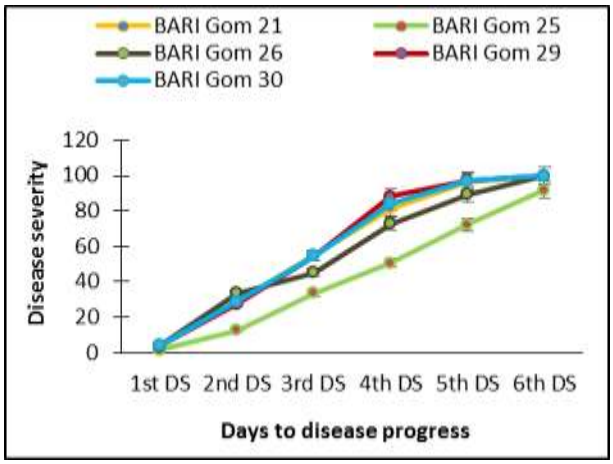

Figure 4. Progress in spike bleaching (blast severity) in non-radiated wheat plants used as control over a period of 12 days

Gamma radiated seeded plants of four wheat varieties showed differential rate of progress in spike bleaching. Among them, wheat variety BARI Gom 25 had slower rate of progress in spike bleaching at 250 Gy (Figure 5).

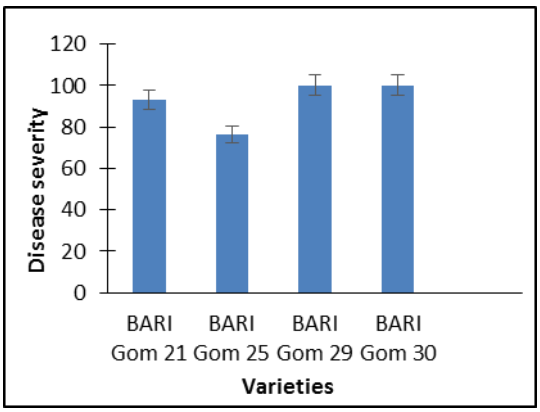

Figure 5. Progress in spike bleaching of different wheat varieties radiated at 250Gy over a period of 12 days

PCR based detection of Magnaporthe oryzae* Triticum *using specific primer

Genomic DNA was extracted from 25 infected M_3 wheat spike samples, representative of a total of 48 entries and DNA concentrations were quantified by Nano Drop™ spectrophotometer. The DNA concentration ranged between 463.13 ngµl^{-1} (BWM 10) to 2227.46 ngµl^{-1} (BWM 13). A working DNA solution 100 ngµl^{-1}was prepared for each sample. Molecular analysis in PCR done with MoT3 marker produced typical bands of MoT (361 bp) in all the entries except for the entry BWM 5 (Figure 6). The size of the band

confirms the infection of M_3 population by *Magnaporthe oryzae* Triticum (MoT). This confirms the field reaction of the inoculated M_3 entries.

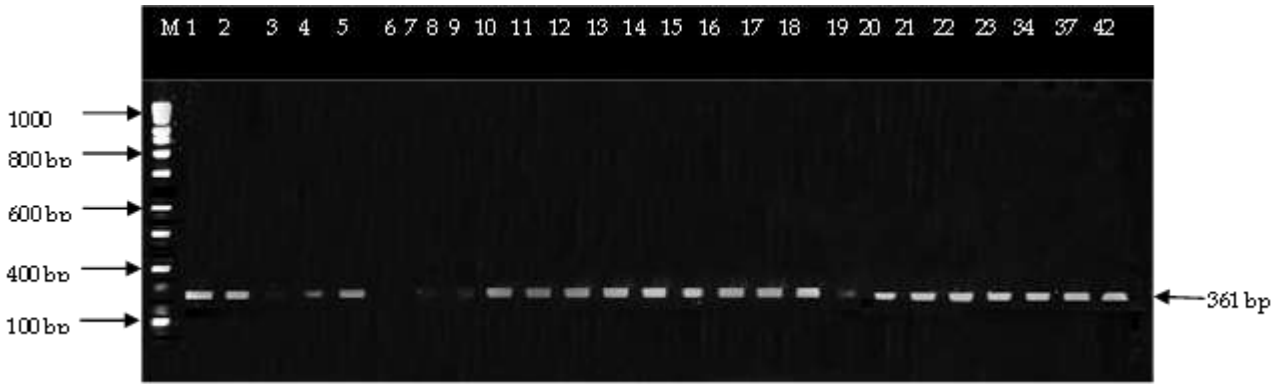

Figure 6 Agarose gel electrophoresis of DNA of wheat blast infected spikes of M_3 population amplified with primer MoT3. Lane M-1000bp DNA ladder. Lane 1: BARI Gom 26, Lane 2: BWM 1, Lane 3: BWM 2, Lane 4: BWM 3, Lane 5: BWM 4, Lane 6: BWM 5, Lane 7: BWM 6, Lane 8: BWM 9, Lane 9 to Lane 22: BWM 9- BWM 23, Lane 23: BWM 34, Lane 24: BWM 37, Lane 25: BWM 42. An amplicon size of 361 bp was found in all the entries except BWM 5.

Disease incidence and severity on the M_3 plants were reflected on the presence or absence of banding on agarose gel except in few cases. The MoT infection was recorded on all the M_3 families except the entry BWM 5 and molecular detection of *Magnaporthe oryzae* Triticum (MoT) infection by MoT3 primer was confirmed on 24 M_3 families. Entry BWM 6 which showed very low blast infection in the inoculated field also produced typical MoT3 band. The primer detected the expression of the disease as an amplification of 361 bp semi-quantitatively which varied in different M_3 families of wheat. For quantitative expression of *Magnaporthe oryzae* Triticum, Real Time PCR study is recommended.

The temperature in the inoculation chamber was maintained approx. 28-30^{0}C. This range of temperature was appropriate for heavy infection by *Magnaporthe*. This action is supported by the report of Cardoso *et al*. (2008) who obtained highest blast intensity at temperature 30^{0}C under controlled condition. However, Kohli *et al*. (2011) had different opinion that continuous rain along with moderate temperature 18-25 ℃ followed by sunny days at flowering stage is the most favourable condition for wheat blast outbreak.

Prominent blast symptoms developed on the inoculated M_3 plants with high severity which was possible because the mutant plants were inoculated with very high load of blast spores. Relative humidity was maintained more than 80%, and temperature-maintained 28-30 ℃, continued water sprays were done to maintain sufficient moisture and field wetness. Conversely, the blast infection was observed very low in the farmer's field of Madnadanga. The plants were not inoculated there, nor were the levels of field moisture taken care of. This might explain the necessity of the presence of sufficient inocula and favorable environment especially wetness period. It is gathered the weather in Meherpur was relatively dry in wheat cropping season of 2018-2019. This opinion of the author has the support of Suzuki (1975) and Asai *et al*. (1967) who opined that spike wetness is the key factor for wheat blast epidemic occurrence.

Inoculated M_3 plants in BINA campus developed typical blast symptoms 12 days after MoT (*Magnaporthe oryzae* Triticum) inoculation at pre-heading stage which coincide with the observation of Cruz *et al*. (2017) who set up a spore trap system for quantification of airborne inoculum of the *Lolium* pathotype in turf field and demonstrated detection of as few as 10 conidia up to 12 days before symptoms developed in inoculated turf grass plots which closely related to *Magnaporthe oryzae* Triticum isolates. In

inoculated wheat field, disease spread rapidly after infection occurred in the field and spikes were totally bleached approximately within 14-16 days. Singh (2017) also gave similar opinion that under field condition, *Magnaporthe* infection of susceptible wheat cultivars occurred quite fast (2-3 weeks).

New observation was noted in the present experiment. In inoculated plants, awn infection as blackening preceded the spike infection /bleaching. This observation got support of Igarashi *et al*. (1986) who also reported the infection of awn by *Magnaporthe oryzae* Triticum. Infected awns showed brown to whitish discoloration while infected rachis, depending on the point of infection presented partial loss or complete death of the head. Blackening of the rachis, lower nodes, has also been observed. Infection in the peduncle kills the upper part of the spike. The data presented by Malaker *et al*. (2016) and Cruz and Valent (2017) supported the observation.

The research findings of the present study have similarities to the findings of Cruz *et al*. (2015) who reported that the *Magnaporthe oryzae* Triticum isolates can infect all above ground parts of the plant, but head infection is the most prevalent symptom in the field. In experimental field of BINA campus, we also observed that under confined inoculated condition *Magnaporthe oryzae* Triticum can also infect leaf and stem.

Inoculated plants developed symptoms also on leaf. Wheat blast symptoms on leaves are seldom seen in the farmers' field of Bangladesh. Rios *et al*. (2013) also gave similar description of leaf infection as we found in the wheat plants inoculated with *Magnaporthe oryzae* Triticum. On leaves, initial macroscopic lesions are water soaked to gray green. Blast lesions have grey centers during sporulation and white to tan centers after sporulation. Symptoms appeared on the inoculated M_3 plants coincided with the symptoms observed in Madnadanga. Symptoms showed resemblance as infected spikes were collected from the farmer's field in Meherpur sadar.

MoT (*Magnaporthe oryzae* Triticum) isolates were confirmed depending on the spore size, shape and color. The findings are supported by many authors who also gave similar description of MoT (*Magnaporthe oryzae* Triticum). According to Perello *et al*. (2016) *Magnaporthe oryzae* is characterized by simple, short, delicate, conidiophores that carry clusters of conidia at their tips. Conidia are typically obpyriform, hyaline, truncated with a short tooth at the base, 2-septate, usually with a pointed acute apex. The conidia in the present study were measured to the size of 28 µm ×7µm which is comparable with the conidial size (20-24 µm×9-12µm) as reported by Perello *et al*. (2016).

Gel electrophoresis run with MoT3 marker produced bands of typical 361 bp. This is the standard DNA band size of *Magnaporhe oryzae* Triticum as reported by several authors. Pieck *et al*. (2017) support the specificity of MoT3 assay. Conventional PCR primers were designed to amplify a 361-bp product. They used MoT3 marker to identify the Triticum pathotype of *Magnaporthe oryzae*. MoT3 marker was selected for the specificity using DNA from 284 *M. oryzae* isolates collected from a diverse array of host species. They confirmed that the assay reliably distinguished between wheat and rice isolates from Bangladesh. The results confirm the isolates obtained from samples collected from Meherpur and used for inoculation of M_3 population and re-isolated from infected M_3 plants are same and single form of *Magnaporthe oryzae* Triticum.

In M_2 population, 15 entries were tested for the presence of *Magnaporthe oryzae* Triticum. Molecular analysis was done with MoT1 (350 bp) which produced diagnostic amplicon in nine entries (25, 27, 28, 31, 33, 34 and 36-38). The other seven entries did not show amplification by MoT1 (data not shown). In M_3 population, 25 representative mutants were taken, and molecular analysis was done with MoT3 marker produced typical bands of MoT (361 bp). It successfully produced diagnostic amplicon in 24 (BARI Gom 26, BWM 1-4, BWM-6, BWM 9 -23, BWM 34, BWM 37 and BWM 42) entries except BWM 5. Entries BWM-34 and 37 showed amplicons in both M_2 and M_3 generation.

The research findings of the present investigation have some similarities to the findings of Vishwakarma *et al*. (2017) who mutagenized by gamma rays a high yielding variety, DBW-88 (released in 2014) at different doses (viz. 200, 250, 300 and 350 Gy) raised M_2 generation in Kernel, Indian Institute of Wheat and Barley Research (IIWBR), a hot spot for yellow rust. Variable resistance compared to susceptible parent was observed. Continued selection until M_5 generation yielded an immune mutant wheat against the susceptible parent variety (60-80S).

Similarly in 2004, Wonchu variety of *Oryza sativa* was developed by irradiating seeds with 250 Gy of gamma rays. This variety has been shown to have higher yield and better disease resistance (Raina and Danish, 2018). A new rice mutant 'Mwangaza' was developed by irradiation with 170, 201, and 240 Gray (Gy) gamma rays at the International Atomic Energy Agency (IAEA) Seibersdorf Laboratories in May 1994 which is resistant to rice yellow mottle virus (Kihupi *et al*., 2008).

The M_2 population of BARI Gom 25, BARI Gom 29 and BARI Gom 30 were grown under field condition during 2017-18 cropping season. M_2 population of BARI Gom 25 showed lower disease incidence and severity at 200 Gy (Rashid *et al*., 2019). This result resembles with the M_3 population inoculated with *Magnaporthe oryzae* Triticum under controlled condition. In M_3 population, BARI Gom 25 also showed lower incidence and severity of blast disease at 200 and 250 Gy. Thus, both dose of 200 and 250 Gy could be considered as an effective level of seed irradiation for obtaining variability in blast resistance. The doses 150 and 300Gy did not produce significant result as recorded in both M_2 (Rashid *et al*., 2019) and M_3 population.

Conclusion

M_3 generation was screened under inoculated condition. In farmer's field radiation dose 250Gy showed good performance in suppressing wheat blast incidence which also showed similar performance in confined inoculated condition. Some lines were selected those did not produce symptoms under inoculated condition. These mutant lines need to be advanced to M_5 generation for blast resistance. Variability obtained in blast resistance through seed irradiation might be an asset in the attempt of developing mutant wheat resistant to blast.

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