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Comparative efficacy of fungicide and gamma radiation for controlling blast of wheat

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ABSTRACT

This research is conducted for comparison of an eco-friendly gamma radiation method and chemical fungicidal spray in reducing wheat blast incidence and severity in BINA under both artificial and natural conditions during 2019-2022. Wheat blast infection occurred on the leaves, spikes and newly emerged heads contained typical eye shaped blast symptoms. *Magnaporthe oryzae Triticum* (MoT), a causal agent of wheat blast was isolated by tissue panting method, cultured on OAT medium at 30 $\pm 1^0$C, confirmed by PCR amplified with universal MoT_3 primer pairs that resulted 361 bp. The MoT inoculated (@ $5x10^6$ CFU/mL) wheat plants successfully developed blast symptoms after 16 days in four tested wheat varieties/germplasm. An *in vitro* evaluation of five chemical fungicides was done against MoT where Nativo and Filia completely inhibited (100%) its radial mycelial growth. A total three spray, combination of half doses of Nativo + Filia [(0.3g + 1mL)/L] at 10 days intervals starting from one week of pre-heading or critical stage significantly reduced wheat blast incidence and severity in both artificially inoculated and natural conditions. Gamma radiated M3 population of wheat vars. also showed better performance in reduction of blast severity. Among the four doses of gamma radiation, 300 Gy significantly showed the better performance in reducing blast disease incidence and severity on wheat var. BG 26. Although combined fungicidal spray treatment (TF) significantly reduced 93.1% DI and 94.3 % DS in var. BG26 over control, but the highest percent reduction of wheat blast incidence (97.7%) and severity (98.4%) was observed on gamma irradiated (300 Gy) M_3 line (BG26M3L4) under natural condition indicating gamma radiation brought a change in the genetic level positive for developing source of resistance against wheat blast. Moreover, gamma irradiated M_3 line BG26M3L4 increased higher vegetative traits / yield than the combined fungicidal spray indicating gamma radiation might be a good replacement of chemical fungicides for management of blast of wheat.

Keywords: Wheat blast, fungicide, gamma irradiation, comparison, control.

INTRODUCTION: Wheat (*Triticum aestivum* L.) is one of the world's most significant cereal crops, and also Bangladesh's second most important cereal crop. Wheat is the core of global food security, supplying 20% of the protein and calories consumed by the majority of people (Singh, 2017). The extensive wheat farming has always attracted a variety of constraints, resulting in the emergence of biotic and abiotic limits (Mushtaq *et al.*, 2019; Nazir, 2021). Among them, the blast is one of the deadliest wheat diseases caused by *Magnaporthe oryzae Triticum (MoT)*. It is currently a major production barrier in the tropical and subtropical regions, causing yield losses of up to 100% in Brazil, Argentina, Bolivia, and Paraguay (Peng *et al.*, 2011). In the middle of February 2016, blast disease of wheat was reported in Bangladesh in Kustia, Meherpur, Chuadanga, Jhenaidah, Jashore, Barisal, Bhola, and other southern districts. In 2015-16, the afflicted regions accounted for about 15% of Bangladesh's entire wheat producing area, posing a significant threat to the country's aggregate wheat production (Chowdhury *et al.*, 2017). The pathogen infects all portions of the wheat plant that are above ground. It causes elliptical to elongated lesions on the leaves with light to dark green centers and yellow margins. Infections of the peduncle and inflorescence cause whole spikes to be bleached out in highly susceptible cultivars). If the blast fungus continues to show the same migratory ability, it may quickly move to other hot and humid wheat-growing areas in South Asia and beyond. The detection of a blast in Bangladesh in early 2016. Callaway (2016) confirmed the fear. *Magnaporthe oryzae* is a seed-borne disease, therefore infected seeds may spread the infection across long distances (Maciel *et al.*, 2013) . The wheat blast can be devastating under favorable conditions, leading to a 100% yield loss (Kohli *et al.*, 2011).

Wheat blast affects the majority of wheat varieties in Bangladesh (Chowdhury *et al.*, 2017). Tolerant cultivars should be found in particular regions, although varieties with long-term resistance are still unavailable. BWMRI (Bangladesh Wheat and Maize Research Institute) produced "BARI Gom 33" a blast resistant wheat variety, using a breeding line from the International Maize and Wheat Improvement Center. But recently this variety also showed susceptible reaction to MoT. Chemical controls are expensive and possibly harmful to our health, so cultivating wheat with built-in resistance is a better option. Therefore, mutation breeding is becoming more popular among breeders and scientists. The use of gamma radiation from radioactive cobalt (60Co) is common; plant varieties with pathogen and disease resistance have been created using induced mutagenesis (Ikram *et al.*, 2010; Katiyar *et al.*, 2022). Bhuiyan *et al.* (2022) observed high values of heritability and genetic advance with high genotypic coefficient of variance (GCV) for plant height, branch number, pod number and yield in irradiated (250 Gy) soybean plants of M4 population. Sarker *et al.* (2014) found that 200 Gy irradiation improve morpho-physiological characters of BARI soybean-5 and produced the highest seed yield than non-treated plants. Malek *et al.* (2012) developed two high yielding mutant varieties of mustard (*Brassica juncea* L.) through using gamma rays (700Gy). Wheat seeds have never been irradiated here previously to create a mutant resistant to the wheat blast disease. On the other hand, farmers are using huge amount of fungicide to control this disease that makes environment hazardous including socio-economic losses.

OBJECTIVES: The objectives of this study were as follows: (1) to evaluate the efficacy of fungicides against wheat blast pathogen (2) to develop blast resistant wheat variety through screening of gamma radiated wheat mutants and (3) to compare the gamma irradiated mutant variety with chemical fungicidal spray for ecofriendly management of wheat blast.

MATERIALS AND METHODS: The experiments (2019-2022) were conducted at Plant Disease Clinic, Department of Plant Pathology, Bangladesh Agricultural University (BAU) and Bangladesh Institute for Nuclear in Agriculture (BINA) Farm, Mymensingh, Bangladesh.

Isolation and detection of MoT: Wheat blast infected spikes were collected from farmers' field and isolated by tissue planting method (Harun-Or-Rashid *et al.*, 2019). Symptomatic seed and spike tissues were surface disinfected in 1% clorox (NaOCl) solution for 30 sec. and rinsed twice with sterile distilled water. The samples were incubated at 30 ± 1°C on moistened Whatman blotter paper (figure 1f) with 80% relative humidity. Single conidia were transferred onto petri dishes containing oat meal agar (OMA) (Oat 50g, agar 18g amended with Streptopen 0.65g/L) and incubated for 8 days in a laminar air flow with continuous fluorescent light at 30±1°C. Colony characteristics and conidia were observed under microscope. The MoT culture was multiplied on PDA (200 g sliced potato, 15 g agar powder, and 12 g dextrose in 1 L distilled water) media. The plates were put in a growth chamber with a 12h/12h (light/dark) cycle under NUV light for 10-12 days at 25 ± 1°C and pure culture was preserved for DNA extraction and inocula preparation (Harun-Or-Rashid *et al.*, 2019).

Molecular identification: The genomic DNA was extracted with CTAB method (Edwards *et al.*, 1991) and MoT3 forward (5' GTC GTC ATC AAC GTG ACC AG 3') and reverse (5' ACT TGA CCC AAG CCT CGA AT 3') primers were used for amplification and PCR was performed with 10 µL/reaction mixture (3.5 µL of *Taq polymerase*, 0.5 µL of dNTPs, 0.25 µL of DNA, 1µL forward primer and 1µL reverse primer and 3.75 µL of ddH_2O) maintaining the thermal profile with initial denaturation at 94°C for 90 sec; followed by 30 cycles of 94°C for 30 sec, 62°C for 30 sec, and 72°C for 60 sec; and a final extension at 72°C for 120 sec. (Pieck *et al.*, 2017). Amplified PCR products were analyzed on ethidium bromide stained agarose gels (1.8%) with 1 Kb DNA ladder.

***In vitro* evaluation of fungicides:** The experiment was conducted in both laboratory and pot at BINA following CRD with four replications. The laboratory experiment was performed for the analysis of radial mycelia growth of MoT on PDA through poison food technique (Debnath *et al.*, 2019) with five fungicides (table 1). For control treatment, only sterile water was used instead of fungicides. The plates were then placed at 30±1°C for 10 days.

Fungicides	Chemical	Dose (L)
Nativo	Tebuconazole 50%+ Trifloxystrobin 25%	0.6 g
Filia	Tricyclazole (40%) and Propiconazole (12.5%)	2 mL
Two-in-one	Hexaconazole 3% + Tricyclazole 22% 25 SC	1 mL
Opponent	Tebuconazole 50% + Trifloxystrobin 25% 75 WG	0.6 g
Azonil	Azoxystrobin 6% + Chlorothalonil 50% 56 SC	1 mL

Table 1: Fungicide's concentration used to mangae MoT.

Percentage growth inhibition: The linear growth (cm) of mycelium of MoT was recorded at 2days interval until the control plates were filled in. Efficacy of the treatments in inhibiting radial mycelial growth of MoT *in-vitro* was calculated (Wedajo, 2015).

$$L = \frac{C - T}{C} X\,100$$

Where, L is percentage of inhibition; C is radial growth of the MoT; T is radial growth of MoT in the presence of the fungicides. In both cases, CRD design was followed with thrice replication. Each pot was filled with 5 kg of sterile field soil mixing with Triple Super Phospahte (TSP), Muriate of Potash (MoP), Gypsum, Boric acid and Zinc Sulphate (@ 1.2, 0.34, 0.82, 0.16 and 0.34 g respectively). MoT susceptible 10 wheat seeds of var. BG 26 were sown at mid-November in each pot with thrice replication in CRD design.

The inoculam preparation: *Magnaporthe oryzae* Triticum (MoT) inocula was prepared as described by Harun-Or-Rashid *et al.* (2019). A 5 mm mycelial block of preserved culture of MoT was transferred to PDA and incubated under fluorescent light. Conidia were harvested by rinsing cultures with sterile distilled water and scraping the surface with a glass slide. To remove mycelia and agar, the conidial suspension was filtered through two layers of muslin fabric. The germination ability of the spores was checked through continued microscopic observation of the slide prepared out of spore suspension prepared. A haemocytometer was used to number the conidia in the suspension, and a spore concentration of @$5x10^6$ conidia/mL was used for inoculation.

Detection of critical growth stage: The inoculam ($5x10^6$ CFU/mL) of MoT was sprayed at tillering stage (25 DAS), pre-booting stage (35 DAS), booting stage (45 DAS), pre-heading stage (52 DAS) and heading stage (60 DAS) (Zadoks *et al.*, 1974). Blast symptoms appeared after 16 days of and perecentage disease incidence and severity were recorded up to one week for each stage of inoculation.

Artificial inoculation under confined condition: Pot soil was prepared, seeds were sown as described above and inoclum was sprayed on wheat plants at 42 DAS. The plants were covered for 48 h to maintain relaive high humidity (>80%RH) and temperature (30 ± 1^0C).

Fungicides application: Wheat seedlings were sprayed thrice times at 45, 55 and 65 DAS with Nativo, Filia, and combination of Nativo and Filia as T_0= Control, T_1 = Nativo @ 0.6g/L, T_2= Filia @ 2 ml/L and T_3= Nativo + Filia [(0.3g + 1 mL)/ L)]. After visualization of blast symptoms, % disease incidence severity (0 = No lesions; 1 = 25% or less; 2 = 26-50%; 3 = 51-75%; and 4=

76-100% infected area) were recorded (Maciel *et al.*, 2013).

Percent disease incidence (%DI)

$$= \frac{\text{Number of spikes infected}}{\text{Total number of spikes counted}} \text{ X } 100$$

Percent disease severitey (%DS)

$$= \frac{\text{Sum of all diseases}}{\text{Total number of disease rating}} \text{ X } 100$$

Experiments for gamma radiated: Seeds of BARI Gom 24 (BG 24), BARI Gom 26 (BG 26), BINA Germplasm G2 (G2) and BINA Germplasm G8 (G8) were irradiated from a Cobalt source @ 150Gy, 200Gy, 250Gy and 300Gy. Central dose rate (CDR) was 3.81 KGy/hr, exposure time: 150 Gy for 2 min. 22 sec, 200Gy for 3 min. 9 sec, 250 Gy for 3 min. 57 sec and 300 Gy for 4 min. 44 sec. Then M_3 plants of all four verities were artificially inoculated with *Magnaporthe oryzae* Triticum (MoT) @ $5x10^6$ CFU/ml at 42 DAS. The pot was covered with a polythene shed before MoT inoculation at BINA farm to maintain >90% relative humidity. Non-radiated seeds of all tested varieties were sown as control. After 16 days of inoculation, data were recorded as mentioned above. From 10 entries of M3 BG26, one mutant line BG26M3L4 was also evaluated against MoT under artificially inoculated and natural conditions.

Comparison of fungicidal and M3 mutant under artificial and natural condition: The land was prepared according to conventional procedures fertilization with 220 Kg urea, 125 kg Triple Super Phospahte (TSP), 120 Kg Muriate of Potash (MoP) , 65 Kg Gypsum, 6 Kg Zinc sulphate, and 6 Kg Boric acid / hectare (Harun-Or-Rashid *et al.*, 2019). During final land preparation, the entire amount of TSP, MOP, and half of the urea were applied. Seeds were planted in three replicates of 3m x 1m plot. Line to line distance was maintained 20 cm and block to block 1m with RCBD design. Seeds of non-irradiated var. BG 26 (control), BG26 (fungicidal spray) and Mutant line BG26M3L4 (obtained from M3 BG26, 300 Gy) were. The plot soil was manually leveled after the seeds were placed on November 15, 2020. The experimental plot was constantly monitored for external threats like as birds, foxes, and other animals. Tagging, iintercultural operation, thinning, gap filling, weeding, and watering were done as required after germination. Three treatments viz. T_1= control (susceptible Var. BG 26), T_2 = 3 spray (45, 55 and 60 DAS) of combined fungicides of half doses of (Nativo 0.3g/L + Filia (1ml/L) and T_3 = mutant line BG26M3L4 (300 Gy) were used with thrice replication. The plants were kept under continued observation for expression of wheat blast symptoms. The DI(%), DS(%), plant height (cm), spikes length (cm), number of grain /spikes, number of grain /plant, 1000 grain weight (g), yield /plant and yield (t/ha) were recorded.

Statistical analysis: The collected data were analyzed by R and MSTAT C software. The mean of all the treatments were compared by critical difference value at 5 % level of significance.

RESULTS AND DISCUSSION: Wheat blast infected leaf, spike and seeds were used for MoT inocula preparation. Severely infected wheat blast samples were collected from farmer's field. Infections occurred at various spots on the leaves, growing spike /head. Bleaching happened from the top to the bottom, regardless of the point of infection and bleached the entire spikes (figure 1a-1d). Pale grey to dark brown discoloration was observed on blast infected wheat seeds (figure 1e). The symptoms of MoT were similar as described by Kohli *et al.* (2011), Chowdhury *et al.* (2017), Singh (2017) and Kabir *et al.* (2021). Typical pyriform, 2-septate hyaline conidia were identified under compound microscope (figure 1g). The fungus grew slowly to develop a typical greyish black to velvety color colony (figure 1h). Solitary, pyriform to obclavate, pale brown or hyaline, smooth 2 septate conidia with beaks of different length (13.2 × 2.8 µm to 20.0 × 5.80 µm spore size) were observed in plenty under compound microscope (figure 1i). In molecular identification, 361 bp amplicon was recorded (figure 1j) while no amplification ws orecoded in negative control. Moist blotter paper and morphological characterization are important for isolation and identification of MoT while pyriform, 2-septate hyaline conidia were identified under compound microscope and 361 bp was identified in molecular confiramtion (Chowdhury *et al.*, 2017).

Out of tested five fungicides Nativo and Filia have significant effect and inhibited 100% the mycelia growth of MoT after 9 days of the artifical inoculation. Two in One, opponent and Azonil arrested the growth of MoT by 45, 80 and 82%, respectively (table 2).

Fungicides	Conc. (ppm)	Mycelial growth (mm)				%Inhibition over control
		24h	48h	72h	216h	
Nativo	600	0.0	0.0	0.0	0.0	100
Filia	2000	0.0	0.0	0.0	0.0	100
Azonil	1000	0.0	2.5	3.0	16.2	82
Opponent	400	0.0	3.0	4.0	18.0	80
Two-in-one	1000	0.0	10.0	12.0	49.5	45
Control	0.0	10.0	20.0	25.0	90.0	-
LSD (0.05)		1.0	3.22	2.52	3.55	

Table 2: *In vitro* mycelial growth inhibition of MoT by different fungicides.

In control plate, the fungus had profuse growth and it took 9 days to cover the whole plate. Finally, Nativo and Filia were selected for pot experiment. It was observed that Pre-heading stage (52 DAS) was the critical stage for severe wheat blast infection than the other four stages. In pre-heading stage, significantly the highest %DI and %DS were recorded 78% and 85.3% respectively followed by heading stage. The lowest %DI and %DS were observed in tillering stage (figure 1n). Blast disease was recorded at var. BG26 after 16 days of artifical inoculation. Nativo sprayed wheat plants resulted 17% blast severity, 20.2% in Filia, whereas control plot resulted 100% disease severity at 80 DAS. Nativo reduced 83% blast disease severity while 79.8% was reduced by Filia. Combination of Nativo and Filia fungicidal spray resulted 94% reduction of blast severity in wheat (table 3). Our findings are also in association with the results of Kohli *et al.* (2011). Effect of gamma radiation in reducing severity of wheat blast was observed under artificial inoculated condition at pre-heading stage in the net house of BINA during 2020-2021. Blast incidence and severity were observed 16 days after the inoculation and data were recorded till 80 DAS. Wheat plants of non-radiated seeds (0 Gy) of all four varieties resulted highest blast incidence (41.4-100%) and severity (45.8-100%) than the gamma radiated M3 plants. Non-radiated var. BG24 and BG26 showed significantly 100% disease incidence and severity than the other varieties. It was observed that all four doses (150, 200, 250, and 300 Gy) of gamma radiated M3 wheat populations significantly reduced percent blast incidence and severity in all the tested four wheat varieties/ germplasms. However, BG26 showed significantly the lowest %disease incidence (14.2%) and

Treatments	% Disease Severity				Reduction of DS (%)
	71 DAS	74 DAS	77 DAS	80 DAS	
T_0 (Control)	44.2	84.83	99.0	100	-
T_1 (Nativo)	9.00	12.6	15.8	17.0	83.0
T_2 (Filia)	9.33	11.9	16.5	20.2	79.8
T_3 (Nativo+Filia)	1.0	1.38	4.4	6.0	94.0
LSD(0.05)	8.16	14.30	17.55	7.18	-

Table 3: Effect of fungicidal spray as preventive measure in reducing wheat blast severity in an artificial inoculated condition at pre-heading stage of var. BG26.

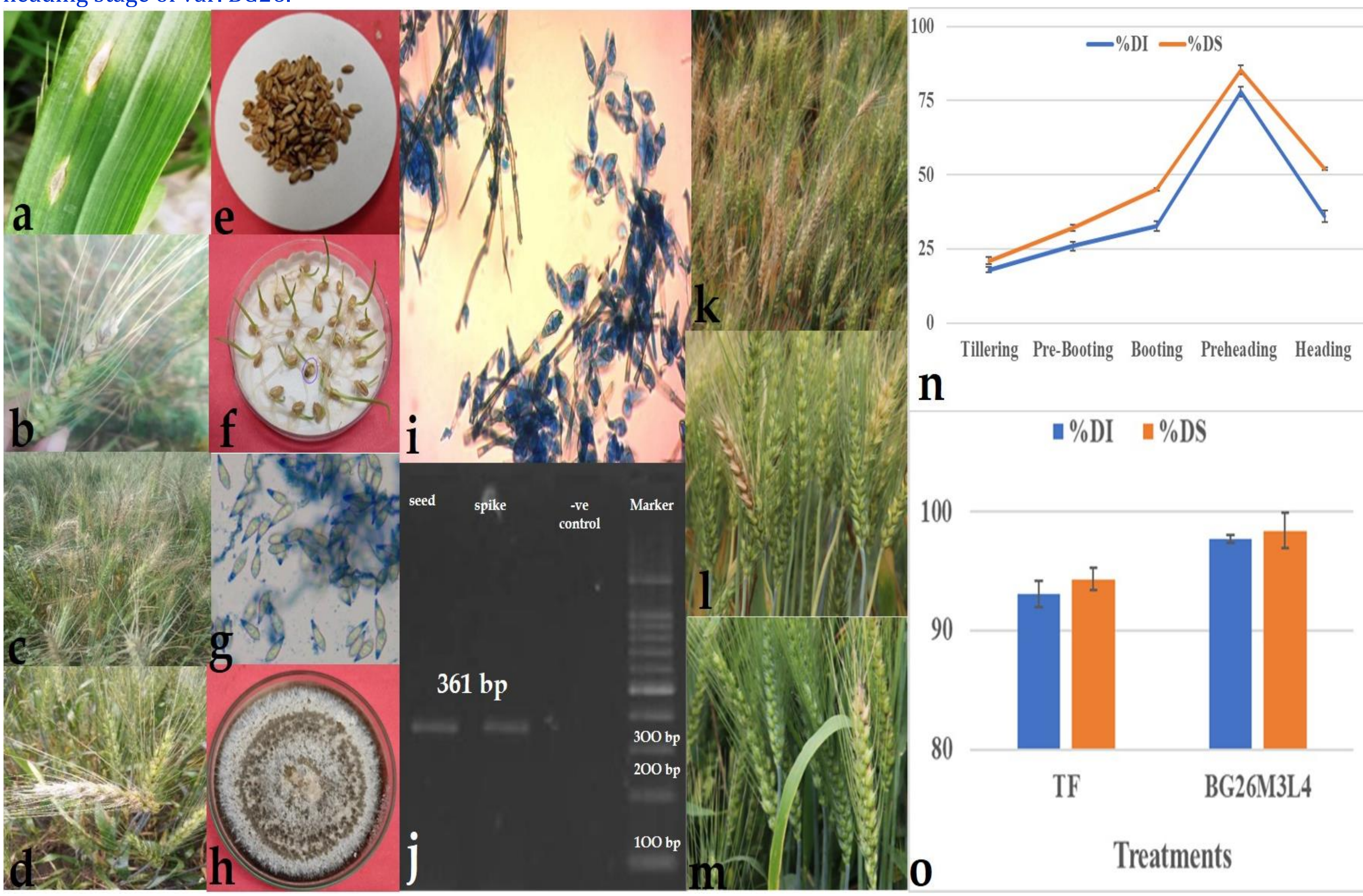

Figure 1: Wheat blast symptoms of eye-shaped spot on the leaf (a), spikelet (b), whitening of spikes from top and bleached wheat ears with straw color (c), blast infected farmers field (d), disease seeds (e), seeds on moist blotter paper (f), conidia of MoT under Steriobinocular microscope (g), fungal colony (h), structures MoT from pure culture (i), PCR amplification (j) and effect of different treatments in field, BG-26 (k), combined fungicide of BG26 (l) and mutant line BG26M3W4 (300 Gy) (m), critical growth stages of wheat {(n; %DI, Lsd (0.05) = 3.205 , Pr(>F)=1.42e-11; for %DS, Lsd(0.05)= 5.231, Pr(>F)=1.12e-09} and reduction of percent disease incidence and severity (o).

severity (8.2%) when applied 300 Gy radiation followed by 250 GY (17% & 13%, respectively) (table 4). This result is supported by Harun-Or-Rashid *et al.* (2019) who found that all the 4 doses of irradiation showed a significant effect on incidence and severity of wheat blast disease in M2 wheat population. Gamma rays are also being widely used as mutation techniques in an attempt to raise abiotic stress tolerance and, disease resistant crop varieties (Katiyar *et al.*, 2022). Among the 10 entries, BG26M3L4 was found significantly the lowest percent disease incidence (2.4%) and severity (2.0%) than the other tested lines at 80 DAS. The non-radiated BG26 was found highly susceptible (100% DI and DS) to wheat blast (table 5). Under natural condition, T_0 (control) non-radiated BG26 showed the highest percent (72%) disease incidence at 80 DAS while the lowest percent blast incidence (1.6%) was recorded in M_3 mutant line BG26M3L4 (300Gy) followed by combined fungicidal treatment T_1 (Nativo+Filia (5%) (table 6). On the other hand, non-radiated BG26 showed the highest percent (64%) disease severity at 80 DAS and M_3 mutant line BG26M3L4 (300Gy) resulted the lowest percent disease severity (1%) followed by combined fungicidal treatment T_1 [(Nativo+Filia), (0.3g+1mL) /L] (3.6%) on 80 DAS (table 6). The highest percent reduction of wheat blast incidence (97.7%) and severity (98.4%) was observed on gamma irradiated (300 Gy) M_3 line BG26M3L4 while combined fungicidal spray treatment (TF) significantly reduced 93.1% DI and 94.3 % DS in var. BG26 over control under natural condition (figure 1o).

Entries/vars.	% Disease incidence					Disease severity (%)				
	0 Gy	150 Gy	200 Gy	250 Gy	300 Gy	0 Gy	150 Gy	200 Gy	250 Gy	300 Gy
G2	41.4	24.6	25.5	30.6	30.2	45.8	40.0	41.0	42.0	42.0
G8	58.1	22.3	24.2	30.8	25.1	51.4	33.0	40.2	43.0	42.0
BG24	100	25.6	24.4	26.4	30.4	100	35.0	38.0	39.0	40.0
BG26	100	18.2	22.2	17.0	14.2	100	18.2	23.0	13.0	8.2
LSD (0.05)	2.086	3.04	2.96	2.062	2.768	1.684	2.73	2.72	2.71	3.277

Table 4: Effect of artificially inoculated MoT at pre-heading stage on gamma radiated M3 wheat populations on the blast incidence and severity under confined condition.

Significantly the lowest number no. of plant height (80 cm), spike length (8.8 cm), no. of grain /spike (42), no. of grains/plant (268), yield /plant (9.12g) and total yield (2.28 t/ha) were recorded in non-treated BG26 var. compared to rest of the treatments. Gamma radiated (300 Gy) mutant line BG26M3L4 had significantly the highest number no. of plant height (102 cm), spike length (12 cm), no. of grain /spike (53), no. of grains/plant (398), yield /plant (21.5g) and total yield (5.37 t/ha) followed by combined fungicidal treated plot (table 7, figure 1 k-m). Although combined fungicidal treatment resulted significantly better agronomic parameters than non-treated treatments but mutant line BG3M3W4 showed best performance on total yield of wheat.

CONCLUSION: The application of combined nd gama irradiation can reduced the losses of wheat caused by MoT.

CONFLICT OF INTEREST: Authors have no conflict of interest.

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Wheat Var.	Disease incidence (%)	Disease severity (%)
Var. BG26	100	100
BG26M3L1	4.51	11.8
BG26M3L2	4.32	8.56
BG26M3L3	3.58	10.7
BG26M3L4	2.40	2.00
BG26M3L5	4.62	5.60
BG26M3L6	2.96	3.90
BG26M3L7	7.22	4.72
BG26M3L8	4.05	3.58
BG26M3L9	11.2	3.28
BG26M3L10	32.5	5.78
Lsd (0.05)	1.393	0.874

Table 5: Incidence and severity of blast in wheat spike of var. BG26 and 10 entries of M3BG26 inoculated with MoT at pre-heading stage under confined condition.

Treatments	Disease incidence (%)				
	68 DAS	71 DAS	74 DAS	77DAS	80 DAS
T_0 (BG26)	33	45.27	52.0	58.2	72.0
T_1 (Nativo + Filia)	2.4	3.25	4.5	4.8	5.0
T_2 (BG26M3L4)	1.0	1.2	1.4	1.6	1.6
LSD(0.05)	2.46	0.88	1.57	2.37	1.14
	Disease severiety (%)				
T_0 (BG26)	30.0	42.1	48.0	55.0	64.0
T_1 (Nativo + Filia)	1.62	2.88	3.2	3.5	3.6
T_2 (BG26M3L4)	1.0	1.0	1.0	1.0	1.0
LSD(0.05)	3.15	2.68	1.08	4.87	1.56

Table 6: Progress of blast incidence and severiety on wheat spike of var. BG26 and BG26M3L4 under natural condition.

Treatments	Plant height (cm)	Spike lengt (cm)	No. of grain/ spike	No. of grains/ plant	1000 grain wt. (g)	Yield/ plant (g)	Yield (t/ha)
T_0 (BG26)	80	8.8	42	268	34	9.12	2.28
T_1	91	9.5	46	352	51	18.4	4.36
T_2	102	12.0	53	398	54	21.5	5.37
Lsd 0.05	6.66	3.35	5.33	53.56	3.05	3.25	0.69

Table 7: Comparison of combined fungicidal treatment and mutant line BG26M3L4 on different agronomic characters under natural condition in BINA during 2021-2022.

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