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Research Article

Development of novel blast-resistant wheat lines via X-ray induced mutation breeding

Hossain Sohrawardy#, Sanjoy Kumar Paul#, Nur Uddin Mahmud#, Paritosh Chandra Roy, Abdullah Al Mahbub Rahat, Dipali Rani Gupta and Tofazzal Islam*

Institute of Biotechnology and Genetic Engineering, Gazipur Agricultural University, Gazipur, Bangladesh

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ABSTRACT

Wheat blast, caused by the fungus *Magnaporthe oryzae Triticum* (MoT), poses a severe and escalating threat to global food security, particularly as conventional breeding struggles due to scarce genetic resources and the pathogen's rapid adaptation. In this study, X-ray irradiation-induced mutation breeding was employed to develop novel and durable resistance. Seeds of three high-yielding wheat varieties from Bangladesh: BARI Gom 27 (BG27), BARI Gom 29 (BG29), and BARI Gom 33 (BG33), were treated with a single acute dose of 180 Gy. Following irradiation, preliminary screening of the M2 generation using a Detached Leaf Assay (DLA) identified thirty fully resistant lines. To assess the stability and durability of resistance, fourteen selected M2 lines were advanced to the M3 generation and subject to rigorous field screening under artificial MoT inoculation in a blast-endemic environment. This critical field evaluation identified eight superior mutant lines, including BG27(X180)-8-4, BG27(X180)-8-5, and four lines derived from the BG33(X180)-98 series, all of which exhibited complete immunity (0% disease incidence and 0% disease severity) to MoT. To the best of our knowledge, this is the first report of completely blast-resistant M3 wheat lines identified through large-scale, open-field screening using X-ray induced mutagenesis of three high-yielding varieties. These resistant M3 lines represent an invaluable and readily deployable genetic resource for the rapid development of durable, blast-resistant wheat cultivars. Such advancements are crucial for mitigating the growing threat of wheat blast in high-risk regions, including Bangladesh, Zambia, and parts of South America. A further multi-location field trials and genomic analyses of the mutant lines are essential to ensure their stability for deployment as blast-resistant wheat variety, and identify the loss-of-function mutation(s) useful for marker development for molecular breeding.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, serving as a primary food source for billions of people. However, its productivity is increasingly threatened by diverse biotic and abiotic stresses. Among these, wheat blast caused by the *Magnaporthe oryzae Triticum* (MoT) pathotype has emerged as a rapidly spreading and highly destructive fungal disease, posing a major challenge to global food security (Islam et al., 2016; 2020). First reported in Brazil in 1985 (Igarashi et al., 1986), the disease spread aggressively throughout

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*Corresponding author: tofazzalislam@gau.edu.bd, #Equally contributed.

South America (Bhattacharjee et al., 2025). A major shift occurred in 2016 when a severe epidemic hit Bangladesh, inflicting up to 100% yield losses on 15,000 hectares of wheat fields (Islam et al., 2016). By 2018, MoT was also detected in Zambia (Tembo et al., 2020). Genomic studies confirmed that the Bangladesh and Zambia outbreaks were caused by the South American B71 clonal lineage, indicating long-distance transboundary introduction through grain trade and highlighting the pathogen's alarming capacity for intercontinental spread (Latorre et al., 2023).

Conventional breeding programs have achieved some progress toward wheat blast resistance; however, the frequent emergence of new pathogen races and MoT's high evolutionary potential continue to undermine the development of durable resistance. Currently, only a limited number of partial resistance sources are available, many of which are temperature- or race-sensitive and thus lack resilience (Islam et al., 2020). To date, only a single major resistance gene, *Rmg8*, has been successfully cloned (Asuke et al., 2024), and identifying new resistance genes in wheat remains a major challenge due to its large and complex genome, significantly slowing down progress and increasing cost (Wang et al., 2025). Therefore, the diversification of the resistance gene pool is not only important but also urgently required to secure global wheat production in vulnerable regions, particularly across South Asia and Africa (Anh et al., 2018).

Induced mutagenesis represents a powerful and promising alternative for rapidly generating novel blast-resistant germplasm. Physical mutagenesis methods, such as X-ray irradiation, have long been recognized for their ability to introduce a broad spectrum of genetic alterations, including point mutations, deletions, insertions, inversions, and chromosomal rearrangements, ultimately creating useful phenotypic diversity for crop improvement (Bordoloi et al., 2024). Globally, mutation breeding has already contributed to the release of more than 3,000 crop cultivars (FAO/IAEA**,** 2018). Importantly, this approach facilitates the development of improved varieties without genetic transformation, making it particularly relevant for regions with strict genetically modified organism (GMO) regulatory frameworks. Furthermore, desired traits can emerge rapidly in the M2 generation, significantly shortening the breeding timeline for improved resistance and agronomic performance (Chavez and Kohli, 2020).

In Bangladesh, where most high-yielding wheat cultivars remain alarmingly susceptible to blast, the development of durable MoT resistance is critically vital to sustain national wheat production and prevent future catastrophic yield losses as the disease spreads to major wheat-growing areas. Based on this compelling regional and global need to diversify genetic resistance sources, the present study employed X-ray irradiation to induce genetic variation in three elite wheat varieties of Bangladesh, viz. BARI Gom 27 (BG27), BARI Gom 29 (BG29), and BARI Gom 33 (BG33). The primary objective of this work was to screen and identify stable mutant lines exhibiting complete and durable blast resistance through rigorous field-based artificial inoculation with MoT fungus. By expanding the functional resistance gene pool, this work aims to directly contribute to the development of resilient wheat varieties suitable for MoT-endemic agro-ecosystems in Bangladesh and elsewhere.

Materials and Methods

Mutagenesis of wheat seeds

Three elite wheat varieties developed by the Bangladesh Agricultural Research Institute (BARI), namely BARI Gom 27, BARI Gom 29, and BARI Gom 33, were selected for mutation breeding to develop blast-resistant lines. In November 2019, X-ray irradiation was utilized as a physical mutagenic agent, provided by the International Atomic Energy Agency (IAEA) in Vienna, Austria. Based on established literature and prior experimental results, a single 180 Gy dose was applied to the seeds of three wheat varieties. This dosage was selected because levels exceeding 200 Gy are known to be lethal for wheat (Ylli et al., 2024).

Exactly, 500 seeds from each of the three varieties were treated with X-ray. The mutagenesis and screening scheme for blast-resistant mutant lines is depicted in Fig. 1. Wheat seeds obtained from the Bangladesh Agricultural Research Institute (BARI) were transported to the International Atomic Energy Agency (IAEA) in Vienna, Austria, for X-ray irradiation. Following treatment, the M1 seeds were returned to the Institute of Biotechnology and Genetic Engineering (IBGE) at Gazipur Agricultural University (GAU), Gazipur. The seeds were shipped in dry condition and maintained at room temperature throughout transit.

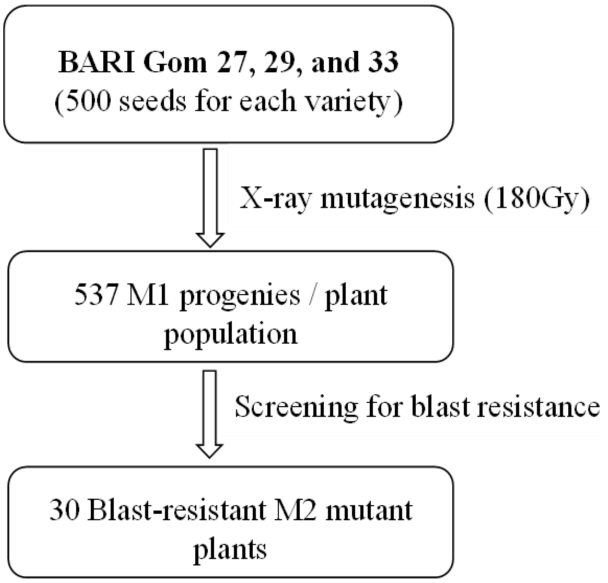


Fig. 1. Scheme of mutagenesis and development of blast-resistant M2 wheat mutant lines.

Research site, breeding methods and agronomic practices

Field experiments were conducted at the research field of Gazipur Agricultural University (GAU) (24.0360° N, 90.3962° E), Bangladesh, and a farmer's field in the blast-prone area of Meherpur (23.7750°N, 88.6417°E) district in south-western Bangladesh. Laboratory work was carried out at the Institute of Biotechnology and Genetic Engineering Laboratory at GAU. The single spike-to-line sowing method was employed for advancing generations. Seeds were sown annually in November, which marks the wheat-growing season in Bangladesh. For the M1 population, seeds were sown with a 3 cm spacing. For the M2 generation, plant spacing was set at 20 cm, and row-to-row distance was maintained at 25 cm. Recommended doses of fertilizers, including Urea (220 kg/ha), TSP (150 kg/ha), MP (100 kg/ha), and Gypsum (100 kg/ha), were applied (BARC, 2012). Two-thirds of the urea and all other fertilizers were applied as basal at the final land preparation. In contrast, the remaining one-third of the urea was top-dressed during the first irrigation (17-20 days after sowing). The first irrigation was light, with excess water drained immediately. Second and third irrigations were applied at the maximum tillering stage (50-55 DAS) and the early stage of grain filling (70-80 DAS), respectively. Weeding was performed manually throughout the wheat growing period. To protect the mutant population from avian damage, a significant risk due to variation in maturity rates, nylon netting was installed as a physical barrier.

Field bioassay

For the bioassay, *M. oryzae Triticum* (MoT) isolate, BTJP-4(5) cultures were incubated for 6 days at 25°C (Gupta et al. 2020). Subsequently, mycelium was gently scraped from the culture plate to promote conidia formation. Twenty-four hours later, the plate was washed with 3–5 mL of sterile distilled water, to which two drops of Tween 20 per liter were added to release the spores. The resulting conidial suspension was filtered through two layers of cheesecloth and adjusted to a concentration of 5×10^4 conidia mL^{-1}.

Recording of data, measurement of disease intensity and severity

Germination rates were recorded from M1 progenies. M2 resistant lines were selected using Detach Leaf Assay (DLA) while disease intensity and severity were assessed in M3 mutant progenies. Data on the number of total tillers, effective tillers, infected tiller $hill^{-1}$, entire length, and infected part of spike, seeds $spike^{-1}$, 1000 grain weight, and grain yield $hill^{-1}$ were gathered during the reproductive period of M3 progenies. During the vegetative phase, data were recorded for the total number of seedlings, the number of infected seedlings per pot, total leaf length, and the length of the infected leaf area. To evaluate disease intensity, wheat blast, a devastating fungal infection caused by MoT, was scored.

The disease intensity (DI) was calculated using the formula:

DI=(Total number of infected plants)/
(Total number of plants observed) × 100

Similarly, blast disease severity was assessed on a five-scale basis, representing the percentage of infection affecting the length of the spike: 0 = No lesions; 1 = 1–25% infection; 2 = 26–50% infection; 3 = 51–75% infection; 4 = 76–100% of the length of the spikes infected by the blast (Suryadi et al., 2013; Goddard et al., 2020). Disease Severity (DS) was calculated as:

$$DS = (n\times v)/(N\times V)\times 100\%$$

Where, DS = disease severity, n = number of spikes infected by the blast, v = value score corresponding to the category of attack, N = total number of spikes observed, and V = value representing the highest score.

Detached Leaf Assay (DLA)

Wheat leaves were detached from five-leaf stage seedlings and placed in plates lined with moist paper towels. Then, detached leaves were inoculated with 1 µl of a conidial suspension containing 1×10^5 MoT conidia/mL, followed by incubation of the plates at 25˚C under 100% relative humidity in the dark for the first 24 h, and thereafter at 24 °C under a 16 h/8 h light-dark photoperiod. At 6 days post inoculation [dpi] leaves were scored for disease symptoms using a 0–6 scale (0 = no visible symptoms, 1 = pin-point brown necrotic lesions, 2 = brown necrotic lesions across the leaf, 3 = brown necrotic lesions and mild chlorosis of the leaf, 4 = grey lesions ringed with necrosis and chlorosis, 5 = extensive grey lesions and chlorosis across the leaf, 6 = grey sporulating lesions and water soaking across the entire leaf. The test was performed three times independently, with 3 replicate samples per run. The resulting length of wheat blast lesions, MoT, was measured from 3 leaves per experiment for each treatment.

Design of experiment and statistical analysis

The experiments in the laboratory and field conditions were performed using a completely randomized design (CRD) and a randomized complete block design (RCBD), respectively, to identify resistant mutant lines against wheat blast compared to parental lines. Data analysis and visualization were performed using RStudio 2025.05.1 and Microsoft Office Excel 2015, which provided a flexible and powerful environment for handling datasets and generating informative graphical representations.

Results

Germination of X-ray irradiated seeds

Five hundred X-ray irradiated seeds for each variety (BARI Gom 27, BARI Gom 29 and BARI Gom 33) were sown in the field. Among these, BARI Gom 27, BARI Gom 29 and BARI Gom 33 of X-ray irradiated seeds were germinated with 32, 75, and 430 seeds, respectively. In total 537 M1 plants (35.8%) successfully survived, indicating that the irradiation doses effectively induced viable mutants. The remaining plants succumbed to lethal doses. Observable variations among surviving plants compared to the control further confirmed the successful induction of mutations by X-ray irradiation, as shown in Fig. 2.

As depicted in Fig. 3, control plants consistently exhibited the highest seed germination (98%) rates compared to all mutant populations. Among the treated varieties, BARI Gom 27, irradiated with 180 Gy, showed the lowest germination rate (6%). On the other hand, 15% and 86% of M1 seeds of BARI Gom 29 and BARI Gom 33, respectively, were germinated under field conditions. Despite the overall reduction, a considerable number of seeds germinated at 180 Gy highlighting the potential for successful mutagenesis without complete loss of viability. Genotypic variations in tolerance to irradiation were clearly observed. For instance, BARI Gom 33 maintained significantly higher seed germination rate (86%) of M1 seeds.

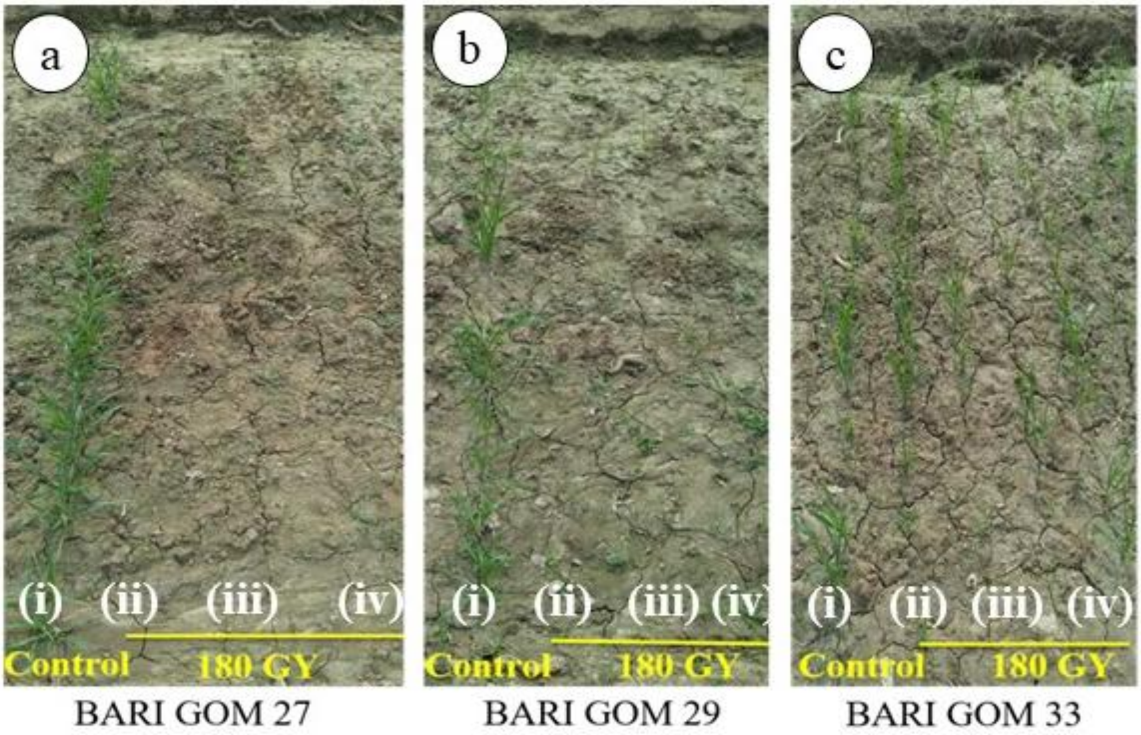


Fig. 2. Germination percentage of M0 (control) and M1 of wheat varieties. (a) BARI Gom 27, (b) BARI Gom 29, (c) and BARI Gom 33 under field conditions, 14 days after sowing. i) M0 seeds (Control), ii), iii) and iv) M1 seeds irradiated at 180Gy.

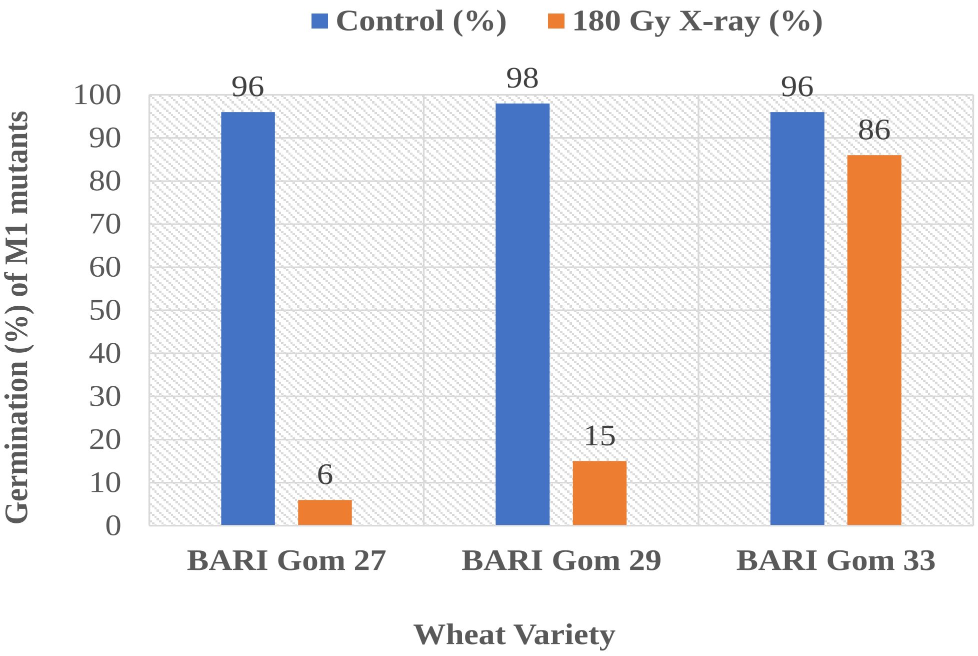


Fig. 3. Germination percentages of 180Gy dose of X-ray irradiation applied to wheat seeds of three varieties of Bangladesh.

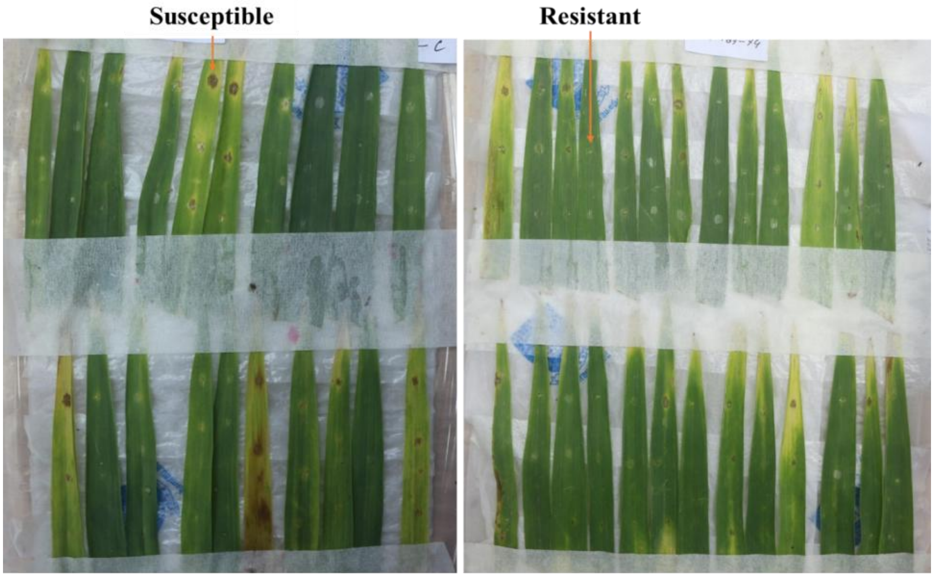
Screening for resistance against wheat blast in X-ray irradiated M2 progenies using the Detach Leaf Assay (DLA) technique

A disease assay was performed on the M2 population derived from 180 Gy X-ray irradiation of three wheat varieties to evaluate initial resistance to the wheat blast pathogen under controlled conditions. From the previously selected lines, 537 individual plants were selected for *in vitro* screening using the Detached Leaf Assay (DLA) based on superior phenotypes. The majority of the tested progenies exhibited susceptibility. Specifically, BARI Gom 27 showed 67.7% susceptibility and 32.3% resistance. BARI Gom 29 recorded 86.7% susceptible and 13.3% resistant plants. BARI Gom 33 displayed the highest level of vulnerability, with 97.7% of plants categorized as susceptible and only 2.3% as resistant. Notably, no plants showed a moderately resistant reaction across any of the tested varieties. Detailed DLA results for these 537 M2 mutant progenies are summarized in Table 1.

The overall results suggested that although the frequency of resistance was low, irradiation generated useful variability and enabled the identification of resistant individuals within the M2 populations. These resistant plants were advanced for further phenotypic evaluation and stability testing in subsequent generations. A visual representation of the DLA is illustrated in Fig. 4.

Table 1. The results of DLA of X-ray (180Gy) irradiated M2 progenies grown in the field and bioassayed for disease resistance of leaves in controlled conditions.

Wheat Varieties	Number of Plant Screened	Susceptible (S)	Moderately Resistant (MR)	Resistant (R)
BARI Gom 27	32	21	0	10
BARI Gom 29	75	65	0	10
BARI Gom 33	430	421	0	10



BARI Gom 27 progenies irradiated with X-ray

BARI Gom 33 progenies irradiated with X-ray

Fig. 4. Leaf-assay of X-ray irradiated M2 progenies in the laboratory.

Field evaluation of X-ray induced mutants of BARI Gom 27 against wheat blast

Field screening under artificial inoculation conditions revealed a wide range of variation among the M2 mutants of BARI Gom 27 in relation to blast disease response and agronomic performance (Table 2). The control variety BARI Gom 27 exhibited high susceptibility, with a disease incidence (DI) of 84.21% and disease severity (DS) of 50%, indicating strong pathogen pressure in the test environment. In contrast, all evaluated X-ray mutants showed complete resistance to wheat blast, as demonstrated by 0% infected spikes, DI, and DS values. Among the resistant mutants, several lines displayed significant improvement in yield-contributing traits. Mutants BG27(X180)-28 and BG27(X180)-31 exhibited the highest number of total and effective tillers per hill (43 and 47 tillers, respectively), whereas BG27(X180)-14 recorded the tallest plants (107 cm). Additionally, spike length ranged from 11 cm to 18 cm, with BG27(X180)-11 producing the longest spikes. These findings confirm that X-ray mutagenesis successfully generated wheat blast-resistant genotypes while simultaneously improving yield-related traits. Such elite mutants represent promising materials for advancement through subsequent generations and potential inclusion in future varietal development pipelines.

Table 2. X-ray irradiated M2 mutants of BARI Gom 27 showed in suppression of wheat blast disease development in artificially inoculated wheat field.

Sl. No.	Control/ Mutant	Plant height (cm)	Total tillers/hill	Effective tillers/hill	Spike length (cm)	Infected spike	DI (%)	DS (%)
1	BARI Gom 27	93	19	16	10	8	84.21	50
2	BG27(X180)-8	80	16	14	15	0	0	0
3	BG27(X180)-9	82	12	8	16	0	0	0
4	BG27(X180)-10	94	30	30	11	0	0	0
5	BG27(X180)-11	83	14	12	18	0	0	0
6	BG27(X180)-14	107	23	20	11	0	0	0
7	BG27(X180)-16	90	14	12	15	0	0	0
8	BG27(X180)-21	98	29	27	11	0	0	0
9	BG27(X180)-28	94	43	43	12	0	0	0
10	BG27(X180)-30	77	12	10	16	0	0	0
11	BG27(X180)-31	88	47	47	12	0	0	0

DI: Disease Intesity; DS: Disease Severity.

Field evaluation of X-ray induced mutants of BARI Gom 29 under artificial inoculation

The M2 mutants derived from BARI Gom 29 showed substantial improvement in wheat blast disease suppression along with desirable agronomic characteristics under artificially inoculated field conditions (Table 3). The control variety BARI Gom 29 exhibited high disease incidence (80%) and moderate disease severity (37.5%), confirming susceptibility to blast pressure in the screening environment. In contrast, all evaluated X-ray mutants expressed complete resistance to wheat blast, reflected by zero infected tillers, DI, and DS values. Significant improvements in tiller productivity and spike traits were also observed. Mutant BG29(X180)-17 recorded the highest total tiller count (31) and effective tiller count (29), while BG29(X180)-58 also produced a remarkably high total tiller count (36). Spike length varied between 11 cm and 21 cm, with BG29(X180)-43 producing the longest spikes.

Improved plant height was noted in multiple mutants, especially in BG29(X180)-23 and BG29(X180)-17 (108 cm and 107 cm, respectively), surpassing the control. These findings suggest that X-ray mutagenesis effectively generated resistant, high-performing genotypes with enhanced yield attributes. Such elite M2 mutants demonstrate promising potential for further evaluation and varietal development.

Table 3. X-ray irradiated M2 mutants of BARI Gom 29 showed in suppression of wheat blast disease development in artificially inoculated wheat field.

Sl. No.	Control /Mutant	Plant height (cm)	Total tillers/hill	Effective tillers/hill	Spike length (cm)	Infected tillers	DI (%)	DS (%)
1	BARI Gom 29	94	20	16	10	6	80	37.5
2	BG29(X180)-17	107	31	29	11	0	0	0
3	BG29(X180)-19	86	13	9	15	0	0	0
4	BG29(X180)-23	108	25	23	13	0	0	0
5	BG29(X180)-29	83	12	9	16	0	0	0
6	BG29(X180)-30	88	24	23	18	0	0	0
7	BG29(X180)-31	101	21	18	14	0	0	0
8	BG29(X180)-43	80	12	9	21	0	0	0
9	BG29(X180)-47	80	13	10	15	0	0	0
10	BG29(X180)-48	96	23	23	11	0	0	0
11	BG29(X180)-58	94	36	36	13	0	0	0

DI: Disease Intesity; DS: Disease Severity.

Field Performance of X-ray induced mutants of BARI Gom 33 under wheat blast inoculation

The M2 population of BARI Gom 33 developed through X-ray irradiation exhibited substantial improvements in wheat blast resistance and agronomic traits under artificial inoculation (Table 4). The control BARI Gom 33 showed noticeable susceptibility, recording 16.67% disease incidence and 6.39% disease severity, confirming that the environment was suitable for disease expression.

However, all eleven evaluated mutants demonstrated complete resistance, as reflected by zero infected tillers, DI, and DS values. Enhanced agronomic performance was also evident among several mutants. The maximum plant height was observed in BG33(X180)-347 (117 cm) and BG33(X180)-365 (116 cm). Whereas, BG33(X180)-13 and BG33(X180)-15 were comparatively shorter. Spike length varied between 12 cm and 21 cm, with BG33(X180)-33 producing the longest spikes. Additionally, BG33(X180)-365 produced the highest number of effective tillers (9) among the evaluated mutants. These results indicate that X-ray mutagenesis successfully generated multiple resistant mutants with improved yield-related features. The identified mutants are promising candidates for future selection and advancement in wheat blast-resistant breeding programs.

Table 4. X-ray irradiated M2 mutants of BARI Gom 33 showed in suppression of wheat blast disease development in artificially inoculated wheat field.

Sl. No.	Control/ Mutant	Plant height (cm)	Total tillers/hill	Effective tillers/hill	Spike length (cm)	Infected tillers	DI (%)	DS (%)
1	BARI Gom 33	107	8	6	15	1	16.67	6.39
2	BG33(X180)-13	87	9	7	17	0	0	0
3	BG33(X180)-15	87	8	7	15	0	0	0
4	BG33(X180)-33	104	9	6	21	0	0	0
5	BG33(X180)-34	97	8	6	19	0	0	0
6	BG33(X180)-98	114	9	7	16	0	0	0
7	BG33(X180)-137	104	9	7	12	0	0	0
8	BG33(X180)-279	97	8	8	18	0	0	0
9	BG33(X180)-322	112	9	6	17	0	0	0
10	BG33(X180)-347	117	8	7	15	0	0	0
11	BG33(X180)-365	116	9	9	14	0	0	0

DI: Disease Intesity; DS: Disease Severity.

Stability and yield trials of X-ray irradiated M3 progenies

To assess the performances of the M3 mutant lines, a field trial was conducted compared with the unmutated parent. These findings highlight the potential of forward mutagenesis for improving wheat blast resistance and emphasize the importance of advancing selected lines to later generations (M3) for stability and yield trials. Based on bioassay result, we selected fourteen suitable mutant lines from 750 mutants of BARI Gom 27, BARI Gom 29, and BARI Gom 33. The rest of the mutants from BARI Gom 27, BARI Gom 29, and BARI Gom 33 were discarded due to their susceptibility against wheat blast. The field views of screening for resistance to wheat blast, as well as the yield trial of Gy-irradiated M3 progenies, are shown in Fig. 5. At the end of the experiment, crop residues were burnt to prevent further unexpected expansion of the devastating disease. Crucially, fourteen M3 plants demonstrated resistance against blast infection e.g., BG27(X180)-8-4, BG27(X180)-8-5, BG27(X180)-30-3, BG27(X180)-30-10, BG29(X180)-17-5, BG29(X180)-17-6, BG29(X180)-17-7, BG29(X180)-58-5, BG33(X180)-98-4, BG33(X180)-98-5, BG33(X180)-98-6, BG33(X180)-98-7, BG33(X180)-347-8, and BG33(X180)-347-9.

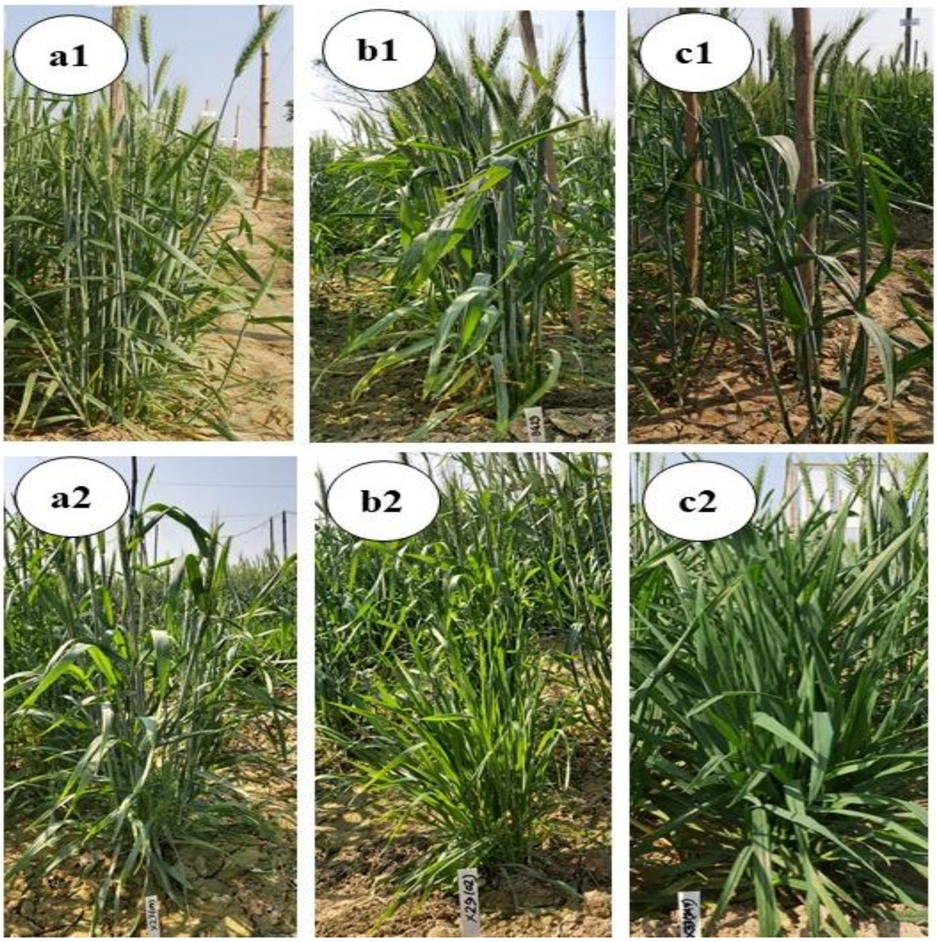


Fig. 5. Variations in plant height and tillering morphology in X-ray mediated wheat mutant populations during M2 generation in field condition. a2, b2, and c2 are mutants of BARI Gom 27, BARI Gom 29, and BARI Gom 33 respectively. a1, a2, and c1 represent as parental lines of BARI Gom 27, BARI Gom 29, and BARI Gom 33 respectively.

Infection manifested as whitening of the spike, usually from the top, on the growing heads of wheat plants during the pre-heading stage in the farmer's fields of Meherpur. On the developing head, infection also occurred at random in various locations, such as the centre, next the tip, or at the base. It was frequently noted that bleaching happened from top to bottom, regardless of the infection site. This indicates that an infection at the base would cause the entire spike to bleach, an infection close to the top would bleach the spike from the top to the point of infection, and an infection in the centre would bleach half of the spike from the top. However, over time, the entire spike in all cases turned silvery white, i.r., completely bleached, as shown in Fig. 6.

Effect of X-ray irradiation on the yield components of the selected plants at the M3 generation

Seventeen wheat genotypes, including three checks (BARI Gom 27, BARI Gom 29, and BARI Gom 33) and fourteen X-ray induced mutant lines, were evaluated for their agronomic and blast disease response traits (Table 5). Significant variation was observed among the genotypes for all studied parameters.

Plant height ranged from 82 cm to 121 cm, where the mutant line BG33(X180)-347-8 and BG33(X180)-347-9 exhibited the tallest plants (121 cm), while BG27(X180)-30-10 produced the shortest plants (82 cm). Variation in tillering ability was also prominent. The highest total and effective tiller counts per hill were recorded in BG27(X180)-8-5 (24 and 22, respectively), indicating improved tiller productivity compared with its control BARI Gom 27. Spike length varied from 12 cm in BG33(X180)-98-7 to 23 cm in BG33(X180)-347-9, with several mutant lines showing longer spikes than their corresponding controls. Thousand-grain weight displayed marked improvements in mutants, with BG27(X180)-30-3 and BG33(X180)-347-9 recordeding the highest values (53.89 g and 49.63 g, respectively), outperforming the control varieties.

A substantial reduction in infected tillers was observed in most mutants of BARI Gom 27 and BARI Gom 33, where multiple genotypes showed complete absence of infected tillers and zero blast infection (DI = 0% and DS = 0%). Notably, BG27(X180)-8-4, BG27(X180)-8-5, BG27(X180)-30-3, BG27(X180)-30-10, and four mutants of BARI Gom 33 revealed complete resistance to wheat blast under field conditions. On the other hand, the control varieties exhibited high susceptibility to blast disease, particularly BARI Gom 27 with DI 91.67% and DS 79.80%. Among the BARI Gom 29 mutants, disease incidence and severity were reduced but not completely eliminated; BG29(X180)-58-5 showed the lowest infection within this cluster (DI 14.29%, DS 1.28%). Overall, the study identified several promising mutant lines combining higher yield-related traits with improved blast resistance. Specifically, BG27(X180)-8-5, BG27(X180)-30-3, and BG33(X180)-98-5 were noteworthy for superior tillering, spike length, grain weight, and complete disease resistance, indicating their strong potential in future breeding and varietal advancement programs.

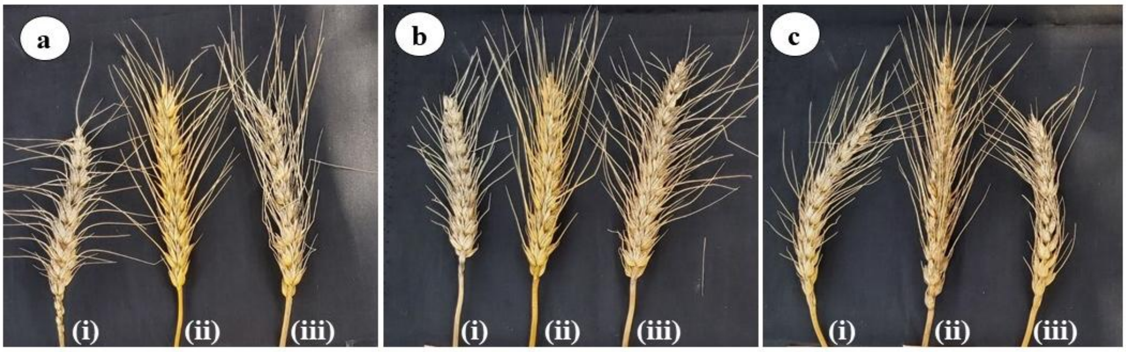


Fig. 6. Pictorial comparison of mutant lines with untreated parents. Resistant reaction indicates by the bright golden color free from gray colored conidial presence in the spikelets. (a), (b) and (c) shows BARI Gom 27, BARI Gom 29, and BARI Gom 33 respectively. In each case, middle one (ii) is the mutant. Conversely, (i) and (iii) are the parental lines.

Table 5. Effect of X-ray irradiation on yield components and suppression of wheat blast disease development of the selected plants from the M3 generation of the BARI Gom 27, BARI Gom 29, and BARI Gom 33 respectively under field conditions.

Sl. No.	Control/ Mutant	Plantheight (cm)	Total tillers/hill	Effective tillers/hill	Spike length(cm)	Infected tillers/hill	1000grain weight	DI (%)	DS (%)
1	**BARI Gom 27**	**98**	**16**	**12**	**15**	**11**	**30.45**	**91.67**	**79.80**
2	BG27(X180)-8-4	87	19	18	14	0	47.46	0.00	0.00
3	BG27(X180)-8-5	93	24	22	13	0	49.54	0.00	0.00
4	BG27(X180)-30-3	91	21	20	16	0	53.89	0.00	0.00
5	BG27(X180)-30-10	82	19	19	15	0	51.23	0.00	0.00
6	**BARI Gom 29**	**100**	**19**	**18**	**16**	**14**	**32.17**	**77.78**	**65.14**
7	BG29(X180)-17-5	96	15	12	16	3	46.15	25.00	3.08
8	BG29(X180)-17-6	98	13	11	15	4	46.35	36.36	4.52
9	BG29(X180)-17-7	88	18	16	17	4	42.16	25.00	4.38
10	BG29(X180)-58-5	84	14	14	17	2	48.97	14.29	1.28
11	**BARI Gom 33**	**110**	**7**	**6**	**19**	**3**	**38.58**	**50.42**	**47.69**
12	BG33(X180)-98-4	102	8	7	18	0	42.48	0.00	0.00
13	BG33(X180)-98-5	105	9	7	18	0	47.58	0.00	0.00
14	BG33(X180)-98-6	101	9	6	17	0	45.63	0.00	0.00
15	BG33(X180)-98-7	100	8	6	12	0	49.25	0.00	0.00
16	BG33(X180)-347-8	121	11	11	20	3	46.26	27.27	1.81
17	BG33(X180)-347-9	121	12	11	23	2	49.63	18.18	0.93

DI: Disease Intesity; DS: Disease Severity.

Principal Component Analysis (PCA) for X-ray irradiated M3 progenies

In the present study, principal component analysis was conducted using seven agromorphological and disease-related traits of wheat genotypes to determine their contribution to overall phenotypic variation. The first two principal components (PC1 and PC2) explained 46.6% and 35.4% of the total variation, respectively, accounting for 82.0% of the cumulative variability (Fig. 7).

PC1 was strongly and positively associated with disease index (DI), disease severity (DS), and infected tillers, indicating that genotypes positioned on the positive axis of PC1 were more susceptible to blast disease. Conversely, effective tillers and total tillers showed a negative loading toward PC1, suggesting these yield-related traits contributed to tolerance. PC2 was primarily influenced by plant height and spike length, which were positively loaded, while thousand-grain weight contributed less strongly along this axis. Genotypes with higher PC2 scores were characterized by better plant architecture traits.

The biplot separated the genotypes into distinct groups. Several mutant lines were positioned in the negative PC1 and positive PC2 quadrants, exhibiting desirable agronomic traits, such as increased effective tillers and improved spike length, while maintaining reduced blast infection levels. In contrast, a few genotypes located on the positive PC1 axis showed higher disease incidence, indicating susceptibility (Fig. 7).

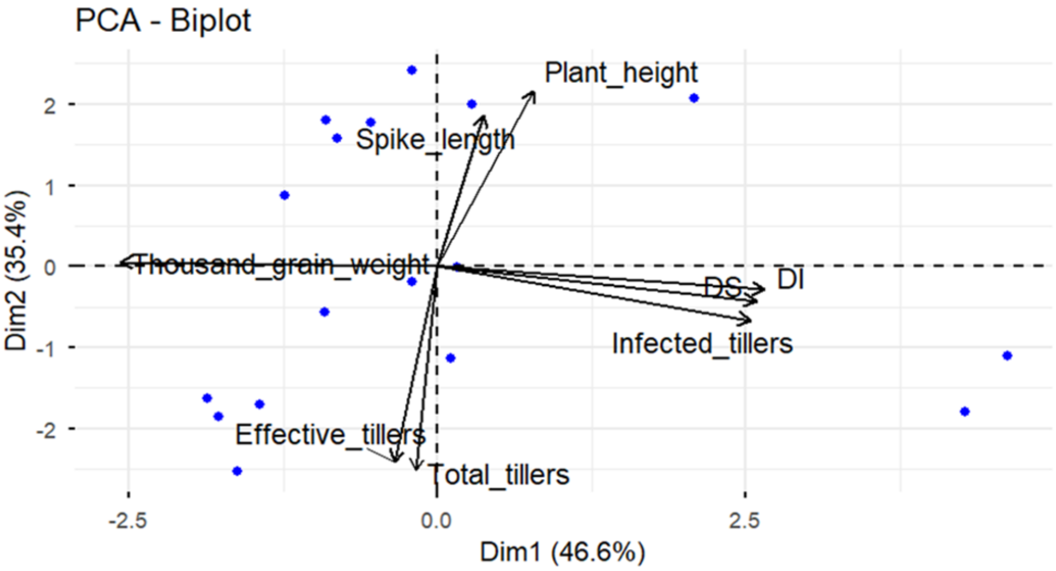


Fig. 7. Principal component analysis for X-ray irradiated M3progenies.

Discussion

The success of mutation breeding hinges on balancing the rate of mutation induction with subsequent plant survival and fertility (Bado et al., 2015; Hassine et al., 2023). In the current study, we demonstrated that X-ray irradiation at 180 Gy effectively induced genetic variation in three elite wheat varieties of Bangladesh for the critical trait of blast resistance. Consistent with established literature on radiation mutagenesis (Hassine et al., 2023), we observed a drastic decline in seed germination and survival across wheat varieties due to irradiation of seeds with 180Gy of X-ray, except BARI Gom 33 (Verma et al., 2017), suggesting that the dose successfully approached the lethal threshold necessary to induce significant chromosomal and genetic alterations. The observed genotypic variation in tolerance, exemplified by BARI Gom 33 maintaining relatively higher germination, underscores the importance of optimizing mutagenic doses on a cultivar-specific basis for a robust mutation breeding program. The resulting phenotypic variability among the surviving M1 and M2 progeny confirmed the successful induction of heritable mutations in wheat for screening for blast resistance (Bado et al., 2015; Hassine et al., 2023; Rana et al., 2025). The current study, encompassing both field and laboratory screening, represents the first identification of several blast-resistant, high-yielding M3 mutant lines. These promising lines serve as valuable genetic resources for developing durable resistance against the destructive wheat blast disease.

Field-validated complete resistance and novelty in M3 genotypes

The ultimate goal of our study was to identify mutant lines with stable, durable resistance to the highly destructive wheat blast fungus, *M. oryzae Triticum* (MoT). The initial M2 Detached Leaf Assay (DLA) served as a high-throughput, preliminary screen (Suryadi et al., 2013), identifying individuals with strong seedling-stage resistance, albeit at a low frequency (e.g., only 2.3% resistance in BARI Gom 33). The identification of these resistant lines in the M2 population, which is a typically segregating generation, suggested the action of stable, strong-effect induced mutations. To rigorously validate the stability and durability of this resistance, 14 selected lines were advanced to field screening at high artificial MoT inoculation pressure in the M3 generation.

Our M3 field trial results represent the most significant novelty of this study: eight superior mutant lines derived from BARI Gom 27 and BARI Gom 33 [e.g., BG27(X180)-8-4, BG33(X180)-98-7, and others] exhibited complete immunity, recording 0% disease incidence (DI) and 0% disease severity (DS) against MoT (Table 5). This outcome contrasts sharply with the high susceptibility observed in the control parents, where BARI Gom 27 showed a DI of 91.67% and a DS of 79.80%. This achievement is a landmark, as it is the first reported successful identification of completely immune M3 plants generated from a large-scale, open-field screen utilizing X-ray-induced mutagenesis in these elite wheat varieties of Bangladesh. This complete, stable, field-level immunity is critical for developing durable resistant varieties, especially given the history of devastating outbreaks in blast-prone regions like Meherpur, Bangladesh (Islam et al. 2016). While physical mutagenesis has been successfully applied to develop resistance to other fungal diseases, such as powdery mildew in blackgram (Tamilzharasi et al., 2023), our success in generating absolute field resistance to MoT provides a uniquely powerful genetic resource.

Simultaneous improvement of resistance and agronomic performance

A core objective of mutation breeding is to ensure that the induced resistance is not linked to detrimental agronomic traits. Our combined M3 screening demonstrated that the resistance was often integrated with superior yield-contributing characters. We used Principal Component Analysis (PCA) to evaluate both disease resistance and yield traits simultaneously, a proven multivariate approach for identifying key selection criteria. The PCA biplot positioned desirable mutants in the quadrant characterized by high agronomic performance (positive PC2, influenced by plant height and spike length) and low disease metrics (negative PC1, strongly associated with total and effective tillers, and inversely related to DI and DS). Specifically, several promising mutant lines successfully combined these desirable traits. For instance, the completely resistant line BG27(X180)-8-5 recorded the highest number of total and effective tillers (24 and 22, respectively), significantly outperforming the control. Furthermore, BG27(X180)-30-3 and BG33(X180)-347-9 recorded the highest thousand-grain weight (53.89 g and 49.63 g, respectively), demonstrating that the induced mutations resulted in beneficial pleiotropic effects or tightly linked positive traits.

In a global context, our findings strongly validate the power of physical mutagenesis as a tool for creating novel resistance in wheat, offering a critical alternative to traditional breeding methods constrained by a limited number of resistance genes (Surovy et al., 2024), such as the recently cloned *Rmg8* (Asuke et al., 2024). The identified M3 mutant lines, which possess complete, stable, field-level immunity, provide an invaluable and immediate genetic resource for national and international wheat breeding programs. The complete resistance observed in these lines offers a unique opportunity for future genomic studies, including targeted transcriptomic analysis of back-crossed populations to map and identify the induced resistance gene(s), providing new insights into the genetic basis of durable resistance against the evolving threat of MoT. Further advancement through selfing and multi-location field trials is the next essential step for developing these elite lines into resilient, commercially viable varieties. Crucially, the discovery of this absolute resistance phenotype offers a unique opportunity for future genomic studies (e.g., MutMap or transcriptomics) to precisely map and identify the underlying induced resistance gene(s), providing new, essential insights into the genetic basis of durable resistance against the evolving global threat of MoT (Wang et al. 2025).

Conclusion

In a global context, our findings strongly validate the power of physical mutagenesis (specifically X-ray irradiation) as a highly effective tool for generating novel and vital resistance traits in elite wheat cultivars, supporting its long-standing contribution to mutant variety development worldwide. While previous studies have successfully used physical mutagens to engineer resistance to other diseases, such as yellow rust, our research represents a significant step forward: the successful induction and stable identification of eight completely immune M3 mutant lines to the devastating wheat blast fungus (*M.oryzae Triticum*) under rigorous, high-pressure field conditions. These promising M3 lines, which demonstrated 0% disease incidence and 0% disease severity, provide an invaluable and immediate genetic resource for national and international wheat breeding programs. Their stable, complete field resistance will enable the rapid development of durable blast-resistant varieties through continued selfing and/or backcrossing. Comprehensive genomic analysis of the candidate mutants and their respective parents could facilitate the discovery of susceptibility (S) genes and loss-of-function mutations. These findings are essential for developing molecular markers that enable rapid, marker-assisted breeding of blast-resistant wheat varieties.

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Authors contribution

Conceptualization, writing-review, editing, and supervision, TI; investigation, visualization, writing-original draft, and editing, HS, SKP, NUM; methodology and software, HS, AAMR; investigation and formal analysis, HS, SKP, PKP, NUM, DRG; writing-review and editing, project administration, and funding acquisition. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

Anh VL, Inoue Y, Asuke S, Vy TT, Anh NT, Wang S, Chuma I and Tosa Y. *Rmg8* and *Rmg7*, wheat genes for resistance to the wheat blast fungus, recognize the same avirulence gene *AVR-Rmg8*. *Mol. Plant Pathol.* 2018; 19: 1252-1256.

Asuke S, Morita K, Shimizu M, Abe F, Terauchi R, Nago C, Takahashi Y, Shibata M, Yoshioka M, Iwakawa M and Kishi-Kaboshi M. Evolution of wheat blast resistance gene *Rmg8* accompanied by differentiation of variants recognizing the powdery mildew fungus. *Nat. Plants.* 2024**;** 10: 971-983.

Bado S, Forster BP, Nielen S, Ali AM, Lagoda PJL, Till BJ and Laimer M. Plant mutation breeding: current progress and future assessment. *Plant Breed. Rev.* 2015; 39: 23-88.

Bangladesh Agricultural Research Council (BARC).Fertilizer Recommendation Guide-2012. BARC, Dhaka; 2012.

Bhattacharjee P, Ali J and Islam T. Management of wheat blast disease in Bangladesh caused by *Magnaporthe oryzae* pathotype *Triticum*. *Plant Health Cases*, 2025; phcs20250025.

Bordoloi D, Sarma Dand Das BK. Comparative sensitivity and relative biological effectiveness of gamma-rays, X-rays and electron beams in aromatic Joha rice derived from different locations in Assam state. *Cereal Res. Commun.* 2024; 52: 57-71.

Chavez A and Kohli MM. Screening wheat germplasm for blast resistance: methods and protocols. In: *Wheat Blast.* 1st eds. *CRC Press*; 2020. pp. 149-162.

FAO/IAEA Joint Division. FAO/IAEA international symposium on plant mutation breeding and biotechnology.Book of Abstracts (IAEA-CN-263). Vienna, Austria, 27-31 August, 2018.

Goddard R, Steed A, Chinoy C, Ferreira JR, Scheeren PL, Maciel JL, Caierão E, Torres GA, Consoli L, Santana FMand Fernandes JM. Dissecting the genetic basis of wheat blast resistance in the Brazilian wheat cultivar BR 18-Terena. *BMC Plant Biol.* 2020; 20(1): 398.

Gupta DR, Surovy MZ, Mahmud NU, Chakraborty M, Paul SK, Hossain MS, Bhattacharjee P, Mehebub MS, Rani K, Yeasmin Rand Rahman M. Suitable methods for isolation, culture, storage and identification of wheat blast fungus *Magnaporthe oryzae Triticum* pathotype. *Phytopathol. Res.* 2020; 2: 30.

Hassine M, Baraket M, Marzougui Nand Slim-Amara H. Screening of the effect of mutation breeding on biotic stress tolerance and quality traits of durum wheat. *Gesunde Pflanzen.* 2023; 75: 837-846.

Igarashi S,Utiamada CM, Igarashi LC, Kazuma AHand Lopes RS. Occurrence of *Pyricularia* sp. in wheat (*Triticum aestivum* L.) in the State of Paraná. *Fitopatol. Bras.*1986; 11: 351-352.

Islam MT, Croll D, Gladieux P, Soanes DM, Persoons A, Bhattacharjee P, Hossain MS, Gupta DR, Rahman MM, Mahboob MGand Cook N. Emergence of wheat blast in Bangladesh was caused by a South American lineage of *Magnaporthe oryzae*. *BMC Biol.* 2016; 14(1): 84.

Islam MT, Gupta DR, Hossain A, Roy KK, He X, Kabir MR, Singh PK, Khan MA, Rahman M and Wang GL. Wheat blast: a new threat to food security. *Phytopathol. Res.* 2020; 2: 28.

Latorre SM, Were VM, Foster AJ, Langner T, Malmgren A, Harant A, Asuke S, Reyes-Avila S, Gupta DR, Jensen Cand Ma W. Genomic surveillance uncovers a pandemic clonal lineage of the wheat blast fungus. *PLoS Biol.* 2023; 21(7): e3002052.

Rana A, Rana V, Bakshi Sand Kumar Sood V. Isolation and characterization of gamma rays induced mutants for improved agro-morphological performance and harder grain texture in wheat (*Triticum aestivum* L.). *Int. J. Radiat. Biol.* 2025; 101: 85-100.

Surovy MZ, Dutta S, Mahmud NU, Gupta DR, Farhana T, Paul SK,Win J, Dunlap C, Oliva R, Rahman Mand Sharpe AG. Biological control potential of worrisome wheat blast disease by the seed endophytic bacilli. *Front. Microbiol.* 2024; 15: 1336515.

Suryadi Y, Susilowati DN, Riana E and Mubarik NR. Management of rice blast disease (*Pyricularia oryzae*) using formulated bacterial consortium. *Emir. J. Food Agric.* 2013; 25: 349-357.

Tamilzharasi M, Kumaresan D, Thiruvengadam V, Jegadeesan, Souframanien J, Latha TKS, Boopathi N Mand Jayamani P. Development and characterization of gamma ray and EMS induced mutants for powdery mildew resistance in blackgram. *Int. J. Radiat. Biol.* 2023; 99(8): 1267-1284.

Tembo B, Mulenga RM, Sichilima S, M′siska KK, Mwale M, Chikoti PC, Singh PK, He X, Pedley KF, Peterson GL, Singh RP and Braun HJ. Detection and characterization of the fungus (*Magnaporthe oryzae* pathotype *Triticum*) causing wheat blast disease on rain-fed wheat (*Triticum aestivum* L.) in Zambia. *PLoS One.* 2020; 15**:** e0238724.

Verma AK, Reddy KS, Dhansekar P and Singh B. Effect of acute gamma radiation exposure on seed germination, survivability and seedling growth in cumin cv. Gujarat Cumin-4. *Int. J. Seed Spices,* 2017; 7: 23-28.

Wang Y, Wang X, Zhang L, Zhakupova K, Ayala F, Ouyang Y, Lu J, Athiyannan N, Wulff BB and Krattinger SG. An optimized disease resistance gene cloning work flow for wheat. *Nat. Commun.* 2025; 16(1): 4904.

Ylli A, Mitrushi F, Elezi F and Ylli F. Optimization of X-ray irradiation dose for induced mutation in bread wheat varieties cultivated in Albania. *Rap Proc.* 2024; 9: 57-62.