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Carbon Ion Beam-Induced Mutagenesis for the Development of Durable Blast- Resistant Wheat Mutant Lines with High-Yield Potential

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Keywords: Physical mutagenesis, carbon ion beam, variability, blast-resistance, food security, climate change

Posted Date: April 8th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9238075/v1

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Additional Declarations: No competing interests reported.

Abstract

Wheat blast, caused by *Magnaporthe oryzae* pathotype *Triticum* (MoT), is a catastrophic threat to global food security. Given the narrow genetic base of resistance in current elite germplasm, this study evaluated heavy ion beam irradiation as a novel physical mutagenic approach to develop durable blast-resistant wheat lines from two popular varieties, BARI Gom 28 and BARI Gom 30. Seeds were exposed to carbon ion beam doses of 50, 60, and 70 Gy, resulting in total 772 surviving M1 plants. High-throughput screening of M2 progenies via detached leaf assay identified 80 resistant lines, which were further narrowed to 16 promising mutants through artificial inoculation in the M3 generation. Notably, the mutant line BG30(I60)-77-8-3 exhibited immune-level resistance (0% severity), while others-maintained severity below 6.3%, compared to over 70% in parental controls. Beyond resistance, these mutants showed superior agronomic performance; BG28(60)-42-10-3 achieved a twofold yield increase over its parent, supported by a significant rise in effective tillers (25 vs. 11). Principal Component Analysis confirmed that carbon ion beam irradiation effectively decoupled disease susceptibility from yield traits, with moderate doses (50–60 Gy) proving optimal for balancing mutational frequency with plant survival. Their high field resistance and enhanced yield potential make them primary candidates for direct varietal release or as donor parents in speed breeding programs. Taken together, our study demonstrates that carbon ion beam irradiation is an effective tool for developing high-yielding, blast-resistant wheat mutant lines suitable for further breeding and varietal development.

Introduction

Wheat (*Triticum aestivum* L.) is a cornerstone of global food security and a vital cereal crop, serving as a primary source of calories and protein for billions of people worldwide. However, sustainable wheat production is increasingly undermined by a complex array of biotic and abiotic stresses. Among these, fungal pathogens remain a primary constraint, with wheat blast, caused by *Magnaporthe oryzae* pathotype *Triticum* (MoT) emerging as a particularly destructive and rapidly expanding global threat (Islam et al. 2016, 2020; Pequeno et al., 2024; Bhattacharjee et al. 2025).

Wheat blast was first reported in Brazil in 1985 and subsequently disseminated throughout several South American countries (Igarashi et al., 1986; Islam et al., 2020). A major epidemiological shift occurred in 2016 when the disease was identified for the first time in Bangladesh, where it caused severe yield losses of up to 100% across approximately 15,000 hectares (Islam et al., 2016). The pathogen was later detected in Zambia in 2018 (Tembo et al., 2020), marking its further expansion into the African continent. Genomic analyses demonstrated that the outbreaks in Bangladesh and Zambia were attributable to a clonal population belonging to the South American B71 lineage, most likely introduced through international grain trade (Latorre et al., 2023). This rapid transcontinental dissemination underscores the high epidemic potential of MoT and highlights its serious implications for food security, particularly in densely populated regions of South Asia and in emerging wheat-producing areas of Africa.

Conventional breeding approaches have achieved incremental progress in enhancing wheat blast resistance, the durability of this resistance remains uncertain. The frequent emergence of new pathogen races and the remarkable adaptive capacity of MoT continue to undermine resistance deployed in commercial cultivars. Progress has been further constrained by the limited availability of effective resistance sources. To date, only a small number of resistance genes have been identified, many of which exhibit race-specificity and temperature sensitivity, thereby reducing their stability under diverse agroecological conditions (Islam et al., 2020; Bhattacharjee et al. 2025). Among the identified genes, only *Rmg8* has been successfully cloned (Asuke et al., 2024), highlighting the scarcity of well-characterized and deployable resistance factors. However, the efficacy of this gene is significantly compromised at elevated temperatures, exhibiting a marked loss of protection when conditions reach or exceed 30°C. This critical gap in host resistance underscores the urgent necessity to identify and develop novel, broad-spectrum, and durable resistance sources. Furthermore, the immense and complex 17 Gb hexaploid genome of wheat (IWGSC et al., 2018) exacerbates the technical and financial hurdles of gene discovery and cloning, making traditional approaches labor-intensive and prohibitively expensive (Wang et al., 2025). Collectively, these constraints have shifted research focus toward alternative strategies, such as induced mutagenesis, to rapidly generate novel genetic variation and accelerate the breeding of blast-resistant wheat germplasm.

Physical mutagenesis, particularly heavy ion beam irradiation, has emerged as a high-efficiency strategy for generating novel genetic variation and improving complex traits in crops, including wheat (Murai et al., 2013; Abe et al., 2021; Bordoloi et al., 2024). Unlike traditional gamma radiation, ion beams possess high linear energy transfer (LET), which induces a broad spectrum of genomic alterations—ranging from point mutations to significant structural changes such as large deletions, inversions, and chromosomal translocations (Abe et al., 2021). This diverse mutational footprint provides plant breeders with a unique allelic reservoir for selecting superior agronomic and disease-resistance traits. Globally, the impact of mutation breeding is evidenced by the release of over 3,000 crop varieties, underscoring its pivotal role in modern crop improvement (FAO/IAEA Joint Division, 2018).

Compared to conventional hybridization, Ion beam–mediated mutagenesis offers a distinct advantage: the rapid generation of novel variation within an elite genetic background without the logistical delays of crossbreeding or the regulatory hurdles associated with transgenic modifications. Furthermore, induced mutations can be stabilized and phenotypically evaluated as early as the M3 generation, significantly accelerating the development of elite lines with durable resistance to devastating pathogens like wheat blast (Chavez and Kohli, 2020).

In Bangladesh, the high to moderate susceptibility of currently released elite wheat cultivars to *Magnaporthe oryzae* pathotype *Triticum* (MoT) represents a critical vulnerability in national food security. We hypothesized that Ion beam irradiation could induce novel, stable genetic variations conferring robust resistance to MoT while maintaining or enhancing the yield potential of high-quality Bangladeshi varieties. To test this hypothesis, this study aimed to (i) subject two high-yielding elite varieties, BARI Gom 28 and BARI Gom 30, to varying doses of Ion beam irradiation; (ii) advance and

systematically screen the resulting mutant populations under stringent field conditions via artificial MoT inoculation; and (iii) identify and characterize high-yielding, blast-resistant mutant lines suitable for immediate integration into breeding programs or direct varietal development.

Materials and methods

Plant materials and muragenesis

For the development of blast-resistant wheat lines, two elite cultivars released by the Bangladesh Agricultural Research Institute (BARI)—BARI Gom 28 and BARI Gom 30 were selected as parental materials. The seeds were put in a petri dish with a diameter of 35 mm, and treated by high-energy carbon ion beams ($^{12}C^{6+}$, 960 MeV) at the Heavy Ion Research Facility in Lanzhou (HIRFL), China, the doses were set as 50, 60, or 70 Gy respectively with a dosage of 60 Gy/min. The overall experimental workflow, including mutagenesis and subsequent screening for blast-resistant mutant lines, is presented in Fig. 1.

Research site, breeding procedure and agronomic management

Field experiments were conducted at the research farm of Gazipur Agricultural University (GAU), Bangladesh (24.0360° N, 90.3962° E), and at a farmer's field located in the wheat blast–prone district of Meherpur (23.7750° N, 88.6417° E) in southwestern Bangladesh. Laboratory experiments and disease assays were conducted at the Institute of Biotechnology and Genetic Engineering (IBGE) at GAU. Generation advancement was performed using the single spike-to-line method.

Seeds were sown annually in November, corresponding to the optimal wheat-growing season in Bangladesh. In the M1 generation, seeds were planted at a spacing of 3 cm between individual seeds to ensure adequate population density. Single spikes were harvested individually from each plant. For the M2 population, seeds from each single spike were sown in distinct rows following a single spike progeny row method. Similarly, the M3 population was developed using the same procedure. In case of M2 progenies, spacing was adjusted to 20 cm between plants and 25 cm between rows to facilitate individual plant evaluation and selection.

Fertilizer management followed the recommendations of the Bangladesh Agricultural Research Council (BARC, 2012), with Urea (220 kg ha^{-1}), Triple Super Phosphate (TSP; 150 kg ha^{-1}), Muriate of Potash (MP; 100 kg ha^{-1}), and Gypsum (100 kg ha^{-1}). Two-thirds of the Urea and the full amounts of TSP, MP, and Gypsum were incorporated as basal applications during final land preparation. The remaining one-third of the urea was top-dressed at the time of first irrigation (17–20 days after sowing).

Irrigation management included a light first irrigation to avoid waterlogging, followed by subsequent irrigations at the maximum tillering stage (50–55 days after sowing) and the early grain-filling stage (70–80 days after sowing). Manual weeding was carried out as necessary throughout the growing season. To

prevent mutant lines from bird damage, nylon netting was used as a protective physical barrier over the experimental filed.

Field bioassay for Magnaporthe oryzae Triticum (MoT)

The wheat blast isolate BTJP-4(5) of MoT was obtained from infected wheat spikes collected from blast-affected fields in Bangladesh and maintained on Potato Dextrose Agar (PDA) in the laboratory. For wheat blast field bioassay, the isolate BTJP-4(5) was cultured on PDA media and incubated for 8 days at 25°C to allow sufficient mycelial growth and conidial growth (Gupta et al., 2020). Following incubation, the mycelium was gently scraped from the culture surface to stimulate conidia formation. After 24 hours, the culture plates were washed with 3–5 mL of sterile distilled water containing two drops of Tween 20 per liter to facilitate spore release. The resulting conidial suspension was filtered through two layers of cheesecloth to remove mycelial fragments and adjusted to a final concentration of 5×10^4 conidia mL^{-1} for use in inoculation assays using sprayer machine in the field during flowering stage. The inoculated plants were immediately covered with polyethylene to maintain humidity above 90% and temperature 25–30℃, and the polyethylene was removed 48 hours after inoculation. Inoculation was done in the afternoon. During that period, plants remained in wet conditions. After the first inoculation, the second inoculation was done one week later, according to the first inoculation. Then, wheat blast disease symptoms appeared on spikes, stems, or leaves.

Data recording, calculation of disease intensity and severity

Germination rates were evaluated in the M1 progenies, while resistant lines in the M2 generation were identified using the detached leaf assay (DLA). Disease intensity and severity were further assessed in the M3 mutant progenies. Twenty-five plants were investigated for each M3 progenies. During the reproductive stage of M3 plants, data were collected on key agronomic traits, including plant height, total tiller number, effective tiller number, infected tillers per plant, and spike length. The severity of wheat blast, caused by the fungal pathogen *M. oryzae* pathotype *Triticum* (MoT), was quantified using a standardized disease scoring system.

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). 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, V = value representing the highest score.

Detached leaf assay (DLA)

Wheat leaves were collected from seedlings at the five-leaf stage and placed on plates lined with moist paper towels. Each detached leaf was inoculated with 1 µL of a conidial suspension containing 1×10^5 *M. oryzae Triticum* (MoT) conidia per mL. Following inoculation, plates were incubated at 25°C in complete darkness under 100% relative humidity for the first 24 hours. Thereafter, leaves were maintained at 24°C under a 16 h light/8 h dark photoperiod.

Disease symptoms were evaluated six days post-inoculation (dpi) 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 with mild chlorosis; 4 = grey lesions ringed with necrosis and chlorosis; 5 = extensive grey lesions with chlorosis; 6 = grey sporulating lesions with water-soaking across the entire leaf (Goddard et al., 2020).

The assay was performed independently three times, with three replicate leaves per treatment in each experiment. Lesion length was measured on three leaves per replicate to quantify the severity of wheat blast for each treatment.

Experimental design and statistical analysis

Laboratory and field experiments were conducted using a completely randomized design (CRD) and a randomized complete block design (RCBD), respectively, to evaluate and identify mutant wheat lines exhibiting resistance to wheat blast in comparison to their parents. Data processing, statistical analysis, and visualization were performed using RStudio (version 2025.05.1), IBM SPSS Statistics 25, and Microsoft Excel 2015, providing a robust framework for dataset management and the generation of informative graphical representations.

Mean comparisons among treatments were carried out using Tukey's Honest Significant Difference (HSD) test at a significance level of $p \leq 0.05$. Each treatment of detached leaf assay was replicated five times, and results are presented as mean ± standard error (SE) in the tables and figures.

Results

Germination and survival dynamics of Ion beam-irradiated M1 populations

To evaluate the biological effectiveness of Ion beam irradiation, a total of 2,000 seeds (1,000 per variety) were sown under field conditions to establish the M1 generation. Of these, 772 plants (38.6%) survived

to physiological maturity. In BARI Gom 28 wheat variety, the survival rates of plants subjected to 50, 60, and 70 Gy Gamma irradiation were 36.75%, 51.00%, and 65.00%, respectively. In contrast, in BARI Gom 30 wheat variety, the corresponding survival rates were 44.25%, 14.25%, and 28.50%, respectively. This survival rate suggests that the selected dosages (50, 60, and 70 Gy) were within a range capable of inducing significant genetic variation while remaining below the absolute lethal threshold for both cultivars. Seedling mortality in the remaining 61.4% was attributed to acute radiation-induced damage and the accumulation of deleterious mutations, typical of high-energy physical mutagenesis. Surviving M1 plants exhibited marked phenotypic pleiotropy, including variations in seedling vigor and early growth habits, confirming the potency of Ion beam irradiation in altering the wheat genome (Fig. 2).

Genotype-dependent sensitivity to ion beam dosages

The impact of irradiation on reproductive fitness was quantified by the number of spikes successfully harvested across different treatment levels (Fig. 3). We observed a distinct, genotype-specific response to radiation intensity. In BARI Gom 28, spike production followed a non-linear trend; it initially increased from 147 at 50 Gy to a peak of 204 at 60 Gy, before declining to 130 at 70 Gy. This suggests that for BARI Gom 28, 60 Gy may represent an optimal "sweet spot" for inducing variability without compromising reproductive output. Generally semi-lethal or semi-dwarfing dose is used to describe the appropriate dose for mutation breeding.

Conversely, BARI Gom 30 demonstrated a more linear, dose-dependent sensitivity. The highest reproductive recovery occurred at the lowest dose (total 177 spikes at 50 Gy), with a sharp and identical decline to 57 spikes at both 60 and 70 Gy. This marked reduction at higher doses indicates that BARI Gom 30 possesses a lower radiation tolerance threshold compared to BARI Gom 28. Across all treatments and varieties, a total of 772 independent M1 spikes were collected, forming the foundation for subsequent M2 resistance screening.

Resistance screening of M2 progenies via detached leaf assay (DLA)

The M2 population, developed from the harvested M1 spikes, was screened using a high-throughput Detached Leaf Assay (DLA) to assess resistance against *Magnaporthe oryzae* pathotype *Triticum* (MoT). In BARI Gom 28, the recovery of resistant phenotypes was highest at the 50 Gy and 60 Gy dosages, yielding three and two "Resistant (R)" lines, respectively (Table 1). Interestingly, while the 60 Gy treatment produced the largest screening population (204 plants), the proportion of resistant individuals suggests that the 50 Gy dose may be more efficient for preserving viable, high-quality mutations in this variety. At 70 Gy, no resistant lines were identified, likely due to the combined effects of reduced population size and increased mutational load.

For BARI Gom 30, a distinct trend was observed. Despite a smaller population size at the 60 Gy dose (57 plants), two highly resistant lines were successfully identified. This contrasts with the 50 Gy treatment,

which, although yielding 20 "Moderately Resistant (M)" lines, failed to produce any fully resistant (R) phenotypes. This indicates that for BARI Gom 30, a higher energy threshold (60 Gy) may be necessary to trigger the specific genetic alterations required for robust blast resistance.

Across both varieties, a total of 80 lines exhibited improved resistance (73 Moderately Resistant and 7 Resistant) compared to the susceptible parental checks. The DLA visual profiles (Fig. 4) provided a clear qualitative distinction between phenotypes: susceptible leaves were characterized by extensive, water-soaked, sporulating grey lesions and severe chlorosis, whereas resistant mutants exhibited restricted, pin-point necrotic lesions or a complete absence of visible symptoms. These findings validate the efficacy of Ion beam irradiation in expanding the genetic diversity for blast resistance and provided the candidate lines for subsequent M3 field validation.

Table 1
Phenotypic response of M2 wheat mutant progenies to MoT inoculation via Detached Leaf Assay (DLA).

Wheat Varieties	Dose of Gy	No. of Plant Screened	Susceptible (S)	Moderately Resistant (M)	Resistant (R)
BARI Gom 28	50	147	132	12	3
	60	204	184	18	2
	70	130	125	5	0
BARI Gom 30	50	177	157	20	0
	60	57	42	13	2
	70	57	52	5	0

Field evaluation of blast disease resistance in carbon ion beam-induced M3 wheat mutants

To evaluate the disease resistance of M3 mutant lines, wheat plants were artificially inoculated with *MoT* conidia during the flowering stage. Under these natural field conditions of high disease pressure, the non-irradiated parental varieties exhibited extreme susceptibility. Specifically, BARI Gom 28 recorded a disease incidence of 90.91% and a severity of 71.43%, while BARI Gom 30 exhibited a 100% disease incidence and 64.41% severity (Table 2 and Fig. 5). These results confirmed that the parental lines, BARI Gom 28 and BARI Gom 30, both suffered from severe disease symptoms, with incidence levels exceeding 90% and severity surpassing 64%. In contrast, the M3 mutant plants displayed varying levels of disease incidence and severity that were significantly lower than the non-irradiated wild-type plants (Table 2 and Figs. 5 and 6). The irradiated mutant population showed a robust shift toward resistance, with the nearly half of the selected lines maintaining both disease incidence and severity levels below 10% (Table 2). This dramatic reduction in infection frequency and symptom severity indicates that ion beam-induced mutations successfully targeted the genetic pathways governing host-pathogen interactions, leading to enhanced field resistance. However, a few mutant lines such as (BG30(I60)-13-9-

21) exhibited disease incidence and disease severity up to 45 and 33%, respectively. Interestingly, several elite lines demonstrated high-level field resistance, such as BG28(I60)-42-10-6 and BG30(I60)-58-7-11, which recorded severity scores of 5.26% and 5.56%, respectively. A standout result of the mutagenesis was the identification of mutant line BG30(I60)-77-8-3, which displayed complete immunity with 0% incidence and 0% severity. The superior performance of these elite mutant lines to the parental controls, marking a significant advancement in developing durable blast-resistant wheat variety.

Table 2
Comparative evaluation of disease incidence and severity in Ion beam-irradiated M3 wheat mutants artificially inoculated with *Magnaporthe oryzae Triticum* (MoT) conidia under natural field conditions (n = 25).

Parent/ Mutant lines	**Disease incidence (% ± SE) ***	**Disease severity (% ± SE) ***
BARI Gom 28	90.91 ± 0.04a	71.43 ± 0.04a
BG28(I50)-20-7-15	9.09 ± 0.02d	6.67 ± 0.02d
BG28(I50)-26-4-12	27.27 ± 0.03c	18.75 ± 0.03c
BG28(I60)-42-10-3	36.33 ± 0.04b	16.00 ± 0.03c
BG28(I60)-42-10-4	18.17 ± 0.03c	10.00 ± 0.02d
BG28(I60)-42-10-5	36.34 ± 0.04b	13.79 ± 0.03c
BG28(I60)-42-10-6	9.08 ± 0.02d	5.26 ± 0.02d
BARI Gom 30	100.00 ± 0.05a	64.41 ± 0.04a
BG30(I50)-5-2-2	18.19 ± 0.03c	11.12 ± 0.02d
BG30(I50)-5-2-18	36.35 ± 0.04b	12.11 ± 0.02d
BG30(I50)-12-9-8	9.09 ± 0.01d	7.14 ± 0.02d
BG30(I50)-12-9-10	27.27 ± 0.03c	23.07 ± 0.03b
BG30(I50)-21-4-28	36.31 ± 0.04b	15.38 ± 0.03c
BG30(I60)-13-9-21	45.44 ± 0.05b	33.32 ± 0.04b
BG30(I60)-58-7-11	9.09 ± 0.02d	5.56 ± 0.02d
BG30(I60)-77-8-3	0.00 ± 0.00e	0.00 ± 0.00e
BG30(I60)-77-8-8	18.19 ± 0.03c	7.14 ± 0.02d

*Data are presented as mean ± SE of three replications per treatment. Means within a column sharing the same letter(s) are not significantly different, as determined by Tukey's HSD (significant difference) post-hoc test ($p \leq 0.05$).

Note

The mutant line nomenclature follows a structured format indicating the parent variety, irradiation dose, and selection history. For example, BG28(I50)-20-7-15

Agronomic evaluation and yield performance of M3 wheat mutants under wheat blast pressure

To evaluate the impact of carbon ion beam-induced mutagenesis on the agronomic and yield performance of M3 wheat mutants, major phenotypic parameters were recorded alongside disease resistance. The irradiated progenies exhibited significant transgressive segregation for multiple agronomic traits, reflecting the profound influence of ion beam mutagenesis on wheat architecture (Table 3 and Figs. 7 and 8). While random mutagenesis often carries a yield penalty, the majority of the selected M3 lines demonstrated consistent improvements in key yield determinants, such as total tiller number, effective tiller count, and spike length. This concurrent expression of reduced disease incidence and enhanced yield components suggests that ion beam irradiation effectively decoupled the typical biological trade-off between plant defense mechanisms and overall productivity.

Table 3
Effect of Ion beam irradiation of selected M3 generation wheat plants on yield and yield components under field conditions.

Parent/ mutant	Plant height (cm)	Total spikes/ hill	Effective spikes/ hill	Spike length (cm)	Infected spikes/ hill	Average spikelet/ spike	Yield (g)/ plant
BARI Gom 28	97 ± 3.2	14 ± 1.1	11 ± 0.9	15 ± 0.8	10 ± 1.3	17 ± 1.0	10.05 ± 0.74
BG28(I50)-20-7-15	85	15	14	15	1	18	16.15
BG28(I50)-26-4-12	88	16	14	17	3	19	14.52
BG28(I60)-42-10-3	96	25	25	17	4	20	33.08
BG28(I60)-42-10-4	93	20	19	16	2	19	28.03
BG28(I60)-42-10-5	100	29	24	17	4	20	28.92
BG28(I60)-42-10-6	93	19	16	16	1	19	22.15
BARI Gom 30	102 ± 3.5	17 ± 1.3	15 ± 1.1	14 ± 0.7	15 ± 1.5	16 ± 1.1	13.73 ± 0.96
BG30(I50)-5-2-2	83	18	18	17	2	20	22.77
BG30(I50)-5-2-18	90	33	31	17	4	21	41.04
BG30(I50)-12-9-8	87	14	14	17	1	19	18.82
BG30(I50)-12-9-10	88	13	13	16	3	18	18.51
BG30(I50)-21-4-28	89	26	24	18	4	21	29.81
BG30(I60)-13-9-21	94	15	14	17	5	18	13.26
BG30(I60)-58-7-11	93	18	17	18	1	21	22.92
BG30(I60)-77-8-3	94	20	18	19	0	22	25.08

Note: Mutant data are based on single-plant selection.

Parent/ mutant	Plant height (cm)	Total spikes/ hill	Effective spikes/ hill	Spike length (cm)	Infected spikes/ hill	Average spikelet/ spike	Yield (g)/ plant
BG30(I60)-77-8-8	97	28	27	17	2	21	37.04
BG30(I60)-02-4-30	89	16	15	19	1	22	21.83
Note: Mutant data are based on single-plant selection.							

The agronomic superiority of these mutants was evidenced by substantial yield increases relative to their parental controls. Within the BARI Gom 28-derived population, BG28(I60)-42-10-3 emerged as the highest-yielding line, achieving a two-fold increase in productivity (33.08 g/plant vs. 10.05 g/plant in the parent) (Table 3). This gain was functionally linked to a superior plant architecture, specifically a 127% increase in effective tillers (25 vs. 11) and a significantly elongated spike that was 2 cm longer than the parent (Fig. 7). Similarly, the BARI Gom 30-derived mutants demonstrated exceptional productivity shifts. Lines BG30(I50)-5-2-18 and BG30(I60)-77-8-8 exhibited the most profound yield enhancements, exceeding 150% of the parental yield. These gains were strongly correlated with a doubling of effective tiller numbers—31 and 27, respectively, compared to 15 in the parent—and improved spike morphology as well as resistance to the disease incidence and severity.

Notably, the mutant line BG30(I60)-77-8-3 emerged as a premier candidate for future breeding, as it successfully achieved the critical objective of combining absolute immunity (0% disease incidence) with a substantial yield advantage (Fig. 8). The highest yield increase (nearly 200% over wild type BG30) was recorded in BG30(150)-5-2-18 followed by BG30(160-77-8-8 were linked to their higher number of effective tillers. This elite line was characterized by superior spike morphology, reaching a length of 19 cm, alongside a robust tillering capacity. Collectively, these results confirm that ion beam irradiation serves as a high-precision tool for generating superior wheat genotypes that simultaneously outperform their parents in both grain yield and disease resistance. The phenotypic stability observed in these mutants confirms their readiness for advanced multi-location trials and their potential as climate-resilient germplasm to mitigate the *MoT* threat.

Multivariate Evaluation of M3 Mutant Progenies via Principal Component Analysis (PCA)

To visualize the global variation and interrelationships among agronomic and pathological traits, a Principal Component Analysis (PCA) was performed on the M3 mutant progenies. The first two principal components (PC1 and PC2) accounted for 81.3% of the total multivariate variance (51.7% and 29.6%, respectively), providing a high-confidence representation of the dataset (Fig. 9). PC1 was primarily defined by yield-contributing attributes, with Thousand Grain Weight (TGW) and Spike Length (SL) exhibiting strong positive loadings and acute-angled vectors, indicating a highly significant positive

correlation between these traits. Total tillers (TT) and effective tillers (ET) also contributed positively to this axis. In contrast, disease-related variables—including Disease Incidence (DI) and Infected Tillers (IT)—displayed vectors oriented in the opposite direction (negative loadings) along PC1. This spatial separation statistically confirms a strong negative association between blast severity and yield components, effectively partitioning the high-yielding, resistant mutants from the susceptible parental checks.

PC2 primarily captured variations in plant architecture, influenced largely by Plant Height (PH) and tillering dynamics independent of grain weight. The biplot reveals a clear divergence of several M3 mutant lines from the parental clusters (BARI Gom 28 and 30). The positioning of elite mutants in the quadrant associated with high ET, SL, and TGW, and away from the DI vector, underscores the success of Ion beam irradiation in generating novel genetic diversity. This multivariate approach successfully validated the selection of promising lines that combine superior yield architecture with robust disease suppression.

Discussion

This study provides the first evidence that heavy carbon Ion beam-mediated mutagenesis can effectively bypass the genetic bottlenecks currently hindering wheat blast resistance in South Asia, South America and Africa. By deploying varying doses of high-energy Ion beams, we successfully generated a novel cohort of mutant lines that exhibit a rare combination of near-total immunity to *M. oryzae* pathotype *Triticum* (MoT) and significantly enhanced grain yield architecture. Most notably, the identification of mutants with a two-fold increase in effective tillers, achieved alongside absolute disease suppression suggests that Ion beam irradiation can decouple the traditional evolutionary trade-off between plant immunity and reproductive fitness. These lines represent an immediate and invaluable genetic resource for the deployment of durable, high-yielding, wheat varieties in regions under severe blast pressure. A large body of literature is available on physical mutagenesis as an effective breeding method for disease resistance and improvement of yield increase of crop plants (Abe et al. 2021; Gaikwad et al. 2026). Over the years, this technique has facilitated the development of numerous improved cultivars possessing desirable traits, including dwarf plant architecture, early maturity, enhanced nutritional quality, tolerance to abiotic and biotic stresses, and resistance to major pests and diseases (Shu et al., 2012; FAO/IAEA Joint Division, 2018; Gaikwad et al. 2026).

In the current study, we demonstrated that Ion beam irradiation serves as a potent, dose-dependent physical mutagen capable of inducing substantial phenotypic and pathological divergence in wheat. The differential germination and survival rates observed in the M1 generation indicate a clear threshold for physiological stress, where moderate doses (50–60 Gy) proved optimal for balancing mutational frequency with plant viability. This sensitivity aligns with broader mutation breeding literature including development of novel wheat mutants by Ion beam (Abe et al. 2021). Abaza et al. (2020) noted that while various mutagens like Gamma rays and EMS can improve agronomic traits, the survival of the initial population remains a primary bottleneck for generating useful variability. Similarly, in rice, high-energy

treatments have been shown to drastically reduce survival and plant height, with an estimated LD_{50} of approximately 287 Gy (Ahumada-Flores et al., 2021). The superior recovery of spikes at 50–60 Gy in both BARI Gom 28 and BARI Gom 30, compared to the sharp decline at 70 Gy, corroborates the principle that excessive genomic instability at high doses impairs reproductive development. Unlike traditional Gamma radiation, Ion beams induce complex DNA lesions, including clustered double-strand breaks and chromosomal rearrangements (Shu et al., 2012; FAO/IAEA Joint Division, 2018). Our study confirms that within the 50–60 Gy range, these lesions are sufficiently repaired to allow for the generation of novel, stable alleles conferring resistance without compromising the plant's structural integrity.

The identification of blast-resistant mutants in both M2 and M3 generations further validates our integrated screening pipeline. By utilizing the Detached Leaf Assay (DLA) for initial M2 screening, we achieved high-throughput elimination of susceptible individuals under controlled conditions—a methodology standardized by Chavez and Kohli (2020) for its reliability. The discovery of 80 resistant or moderately resistant M2 lines, particularly from the 50–60 Gy treatments, strongly suggests that Ion beam irradiation generated novel resistance alleles that were previously absent in the parental gene pool. Subsequent field validation in the M3 generation confirmed the stability of this resistance, with the emergence of "immune" lines such as BG30(I60)-77-8-3 representing a significant breakthrough. While previous studies, such as Goddard et al. (2020), identified multiple QTLs for blast resistance, they noted that seedling and head blast resistance are often controlled by distinct loci. In our study, the mutants that maintained zero disease severity from the seedling stage through to the reproductive stage suggest the induction of broad-spectrum or stage-independent resistance mechanisms. Given the rapid adaptability of *M. oryzae* pathotype *Triticum* (MoT) and the global scarcity of effective resistance genes (Islam et al., 2016; Latorre et al., 2023; Asuke et al., 2024), these mutants represent vital genetic resources for future molecular characterization and the mapping of new resistance-associated loci.

One of the impactful findings of this research is the successful integration of blast resistance with superior agronomic performance especially significant increase in effective tiller numbers of the disease-resistant M3 mutants (Tables 2 and 3 and 6–8). In traditional breeding, resistance often imposes a "fitness cost" or yield penalty. However, our multivariate analysis revealed a strong negative association between disease severity and yield traits (Fig. 9). This indicates that in our M3 population, productivity and resistance were simultaneously improved rather than being antagonistic. This phenomenon of "yield-positive" mutation has been observed in wheat by Ion beams (Abe et al. 2021) and other cereals. For example, Farid et al. (2020) and Hassine et al. (2023) reported wheat mutants that exhibited enhanced resistance to multiple fungal pathogens while maintaining or improving grain quality. The outstanding performance of elite lines like BG28(I60)-42-10-4 and BG30(I60)-77-8-3, which combined absolute disease suppression with increased effective tiller numbers and grain weight highlights the precision of Ion beam mutagenesis (Tables 3 and 4; Fig. 6–8). By generating structural variations that improve plant architecture alongside immunity, this approach provides a practical, non-transgenic solution for wheat improvement. Ultimately, these results suggest that Ion beam-induced mutagenesis can significantly accelerate the development of climate-resilient, high-yielding wheat varieties, offering a robust defense against the transboundary threat of wheat blast in Bangladesh and

other at-risk regions globally. Further molecular characterization of these mutants will likely reveal loss-of-function mutant alleles (Guo et al. 2021) that underpin wheat blast resistance, facilitating the development of diagnostic molecular markers for the rapid introgression of resistance into elite breeding lines.

Conclusion

This study establishes Ion beam irradiation as a transformative strategy for overcoming the genetic bottleneck of wheat blast resistance in elite South Asian highly blast susceptible cultivars. By exposing the popular cultivars, BARI Gom 28 and BARI Gom 30 to varying Ion beam doses (50–70 Gy), we successfully generated a novel mutant population that decouples the traditional evolutionary trade-off between disease immunity and reproductive fitness. Our multi-stage screening pipeline, transitioning from high-throughput detached leaf assays in the M2 generation to stringent field inoculation in the M3, identified 16 elite mutant lines with profound resistance profiles. The novelty of these findings is highlighted by the emergence of the "immune" line BG30(I60)-77-8-3, which maintained 0% disease severity under high inoculum pressure, and BG28(60)-42-10-3, which achieved a two-fold yield increase primarily through a 127% rise in effective tiller production. Principal Component Analysis (PCA) confirmed that moderate irradiation doses (50–60 Gy) are optimal for inducing high-frequency, viable mutations that simultaneously improve yield architecture and suppress *M. oryzae* Triticum (MoT) infection. From a global perspective, these 16 stable mutants represent an immediate genetic resource for safeguarding food security in blast-prone regions. Their superior agronomic performance and robust field resistance position them as primary candidates for direct varietal release or as elite donor parents in speed-breeding programs. Ultimately, this research provides a proven framework for utilizing high-energy physical mutagenesis to rapidly develop climate-resilient, high-yielding wheat varieties capable of resisting the transboundary spread of the devastating MoT pathogen.

Declarations

CONFLICT OF INTEREST

The authors declared no competing interests.

ETHICS STATEMENT

The study was conducted on plant materials and did not involve human participants or animals; therefore, ethical approval was not required.

DISCLOSURE STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FUNDING

The authors are thankful to International Atomic Energy Agency (IAEA)/FAO for funding this research. HS is thankful thankful to Prime Ministers Scholarship Funding for Ph D Fellowship during this work.

Author Contribution

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

Acknowledgement

The authors sincerely acknowledge the funding provided by the International Atomic Energy Agency, Vienna through a CRP to TI. HS is thankful to the Government of Bangladesh for granting the esteemed Prime Minister Fellowship (to HS), which facilitated the completion of this study. Our deep gratitude to Prof. Libin Zhou, Institute of Modern Physics, Chinese Academy of Sciences for Carbon ion beam treatment in this study.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

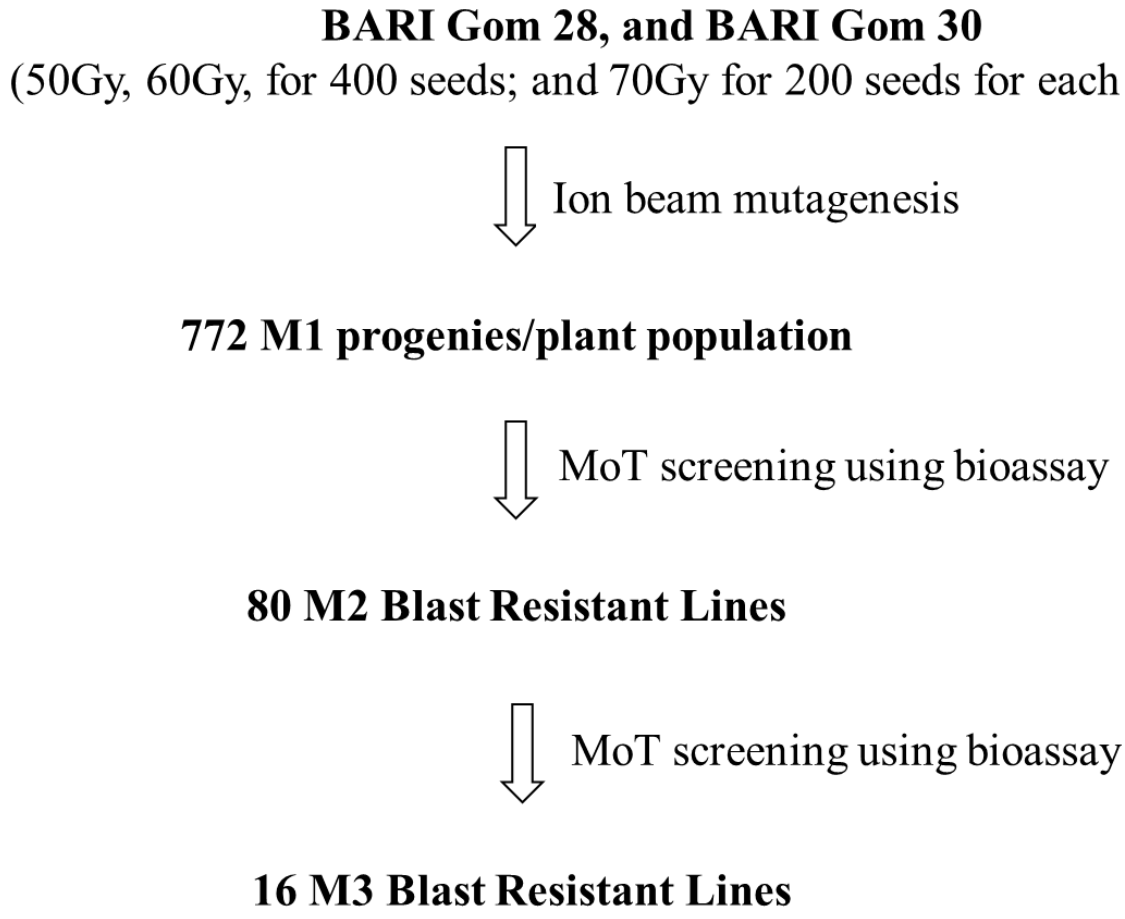


Figure 1

Schematic representation of the mutagenesis process and the development of blast-resistant M3 wheat mutants.

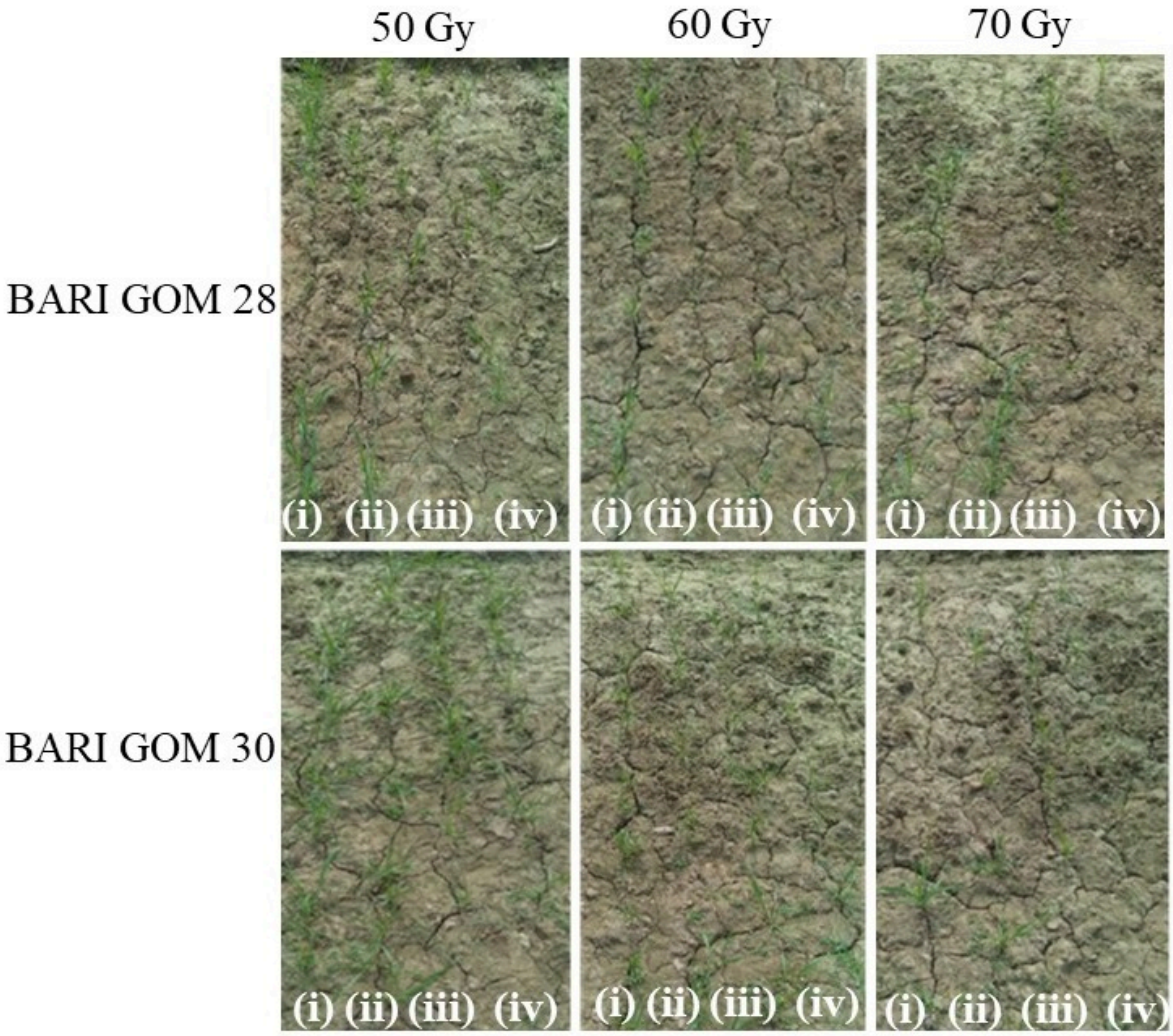


Figure 2

Germination picture of the M1 progenies of wheat varieties BARI Gom 28 and BARI Gom 30 under field conditions, recorded 14 days after sowing.

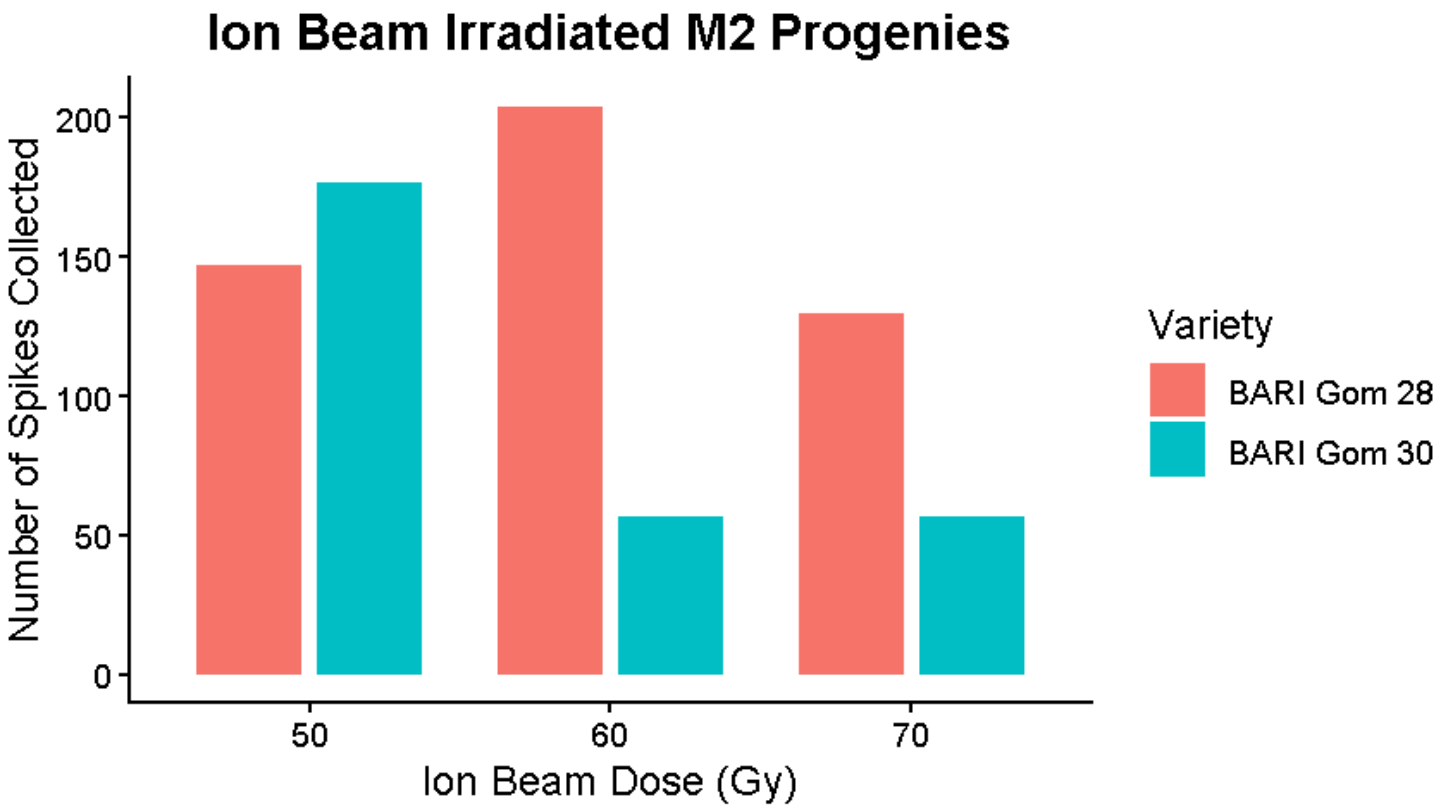


Figure 3

Number of spikes collected from M1 progenies of different doses of Ion beam irradiation.



Figure 4

Representative phenotypic responses of Ion beam-irradiated M2 wheat progenies to *Magnaporthe oryzae Triticum* (MoT) infection in a detached leaf assay.



Figure 5

Representative images illustrating the phenotypic response of mutant lines and their respective untreated parental varieties following inoculation. Resistance is characterized by bright golden spikelets without visible gray conidial growth. (a) BARI Gom 28 and its derived mutant line; (b) BARI Gom 30 and its derived mutant line. In each panel, (ii) denotes the mutant line, while (i) and (iii) indicate the corresponding parental controls.

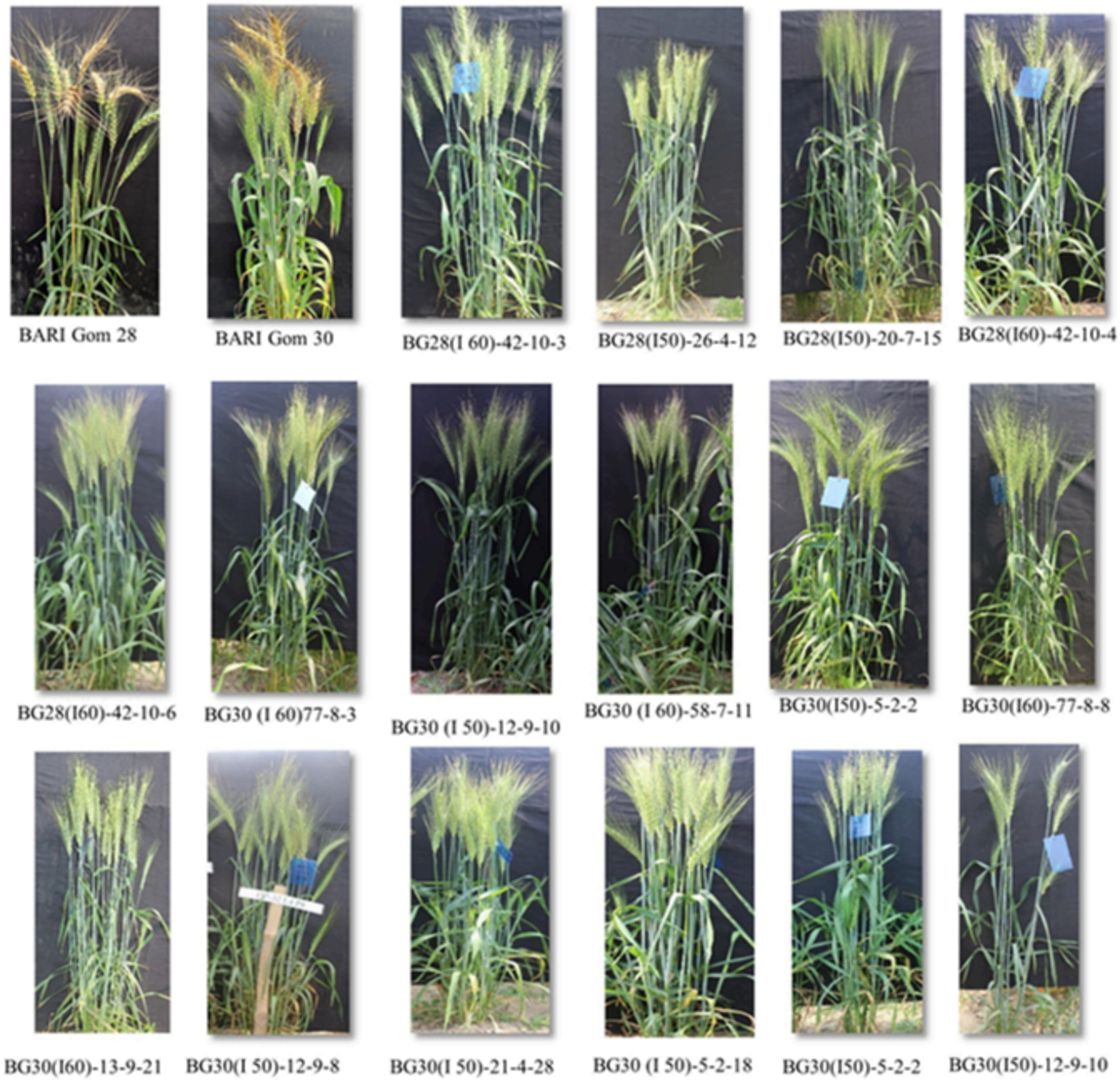


Figure 6

Ion beam–irradiated selected M3 mutant lines derived from BARI Gom 28 and BARI Gom 30 (see Table 3) following artificial inoculation with *Magnaporthe oryzae* pathotype Triticum (MoT) at 60 DAS in a farmer's field at Meherpur, Bangladesh. Plants were covered with polyethylene to maintain >90% humidity for 48 h, and photographs were taken 15 days after inoculation.

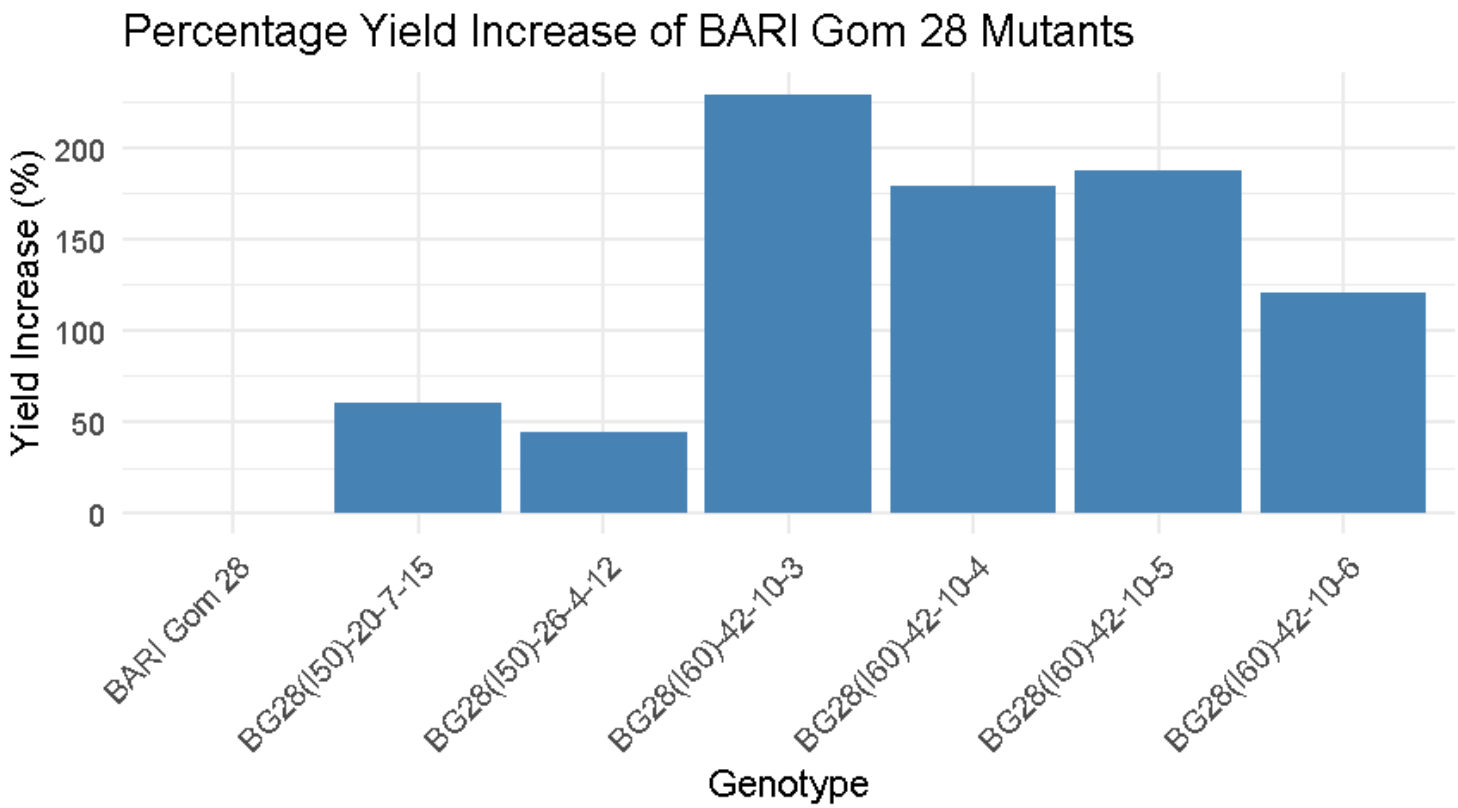


Figure 7

Percent yield increase of single-plant mutants relative to the parent, BARI Gom 28.

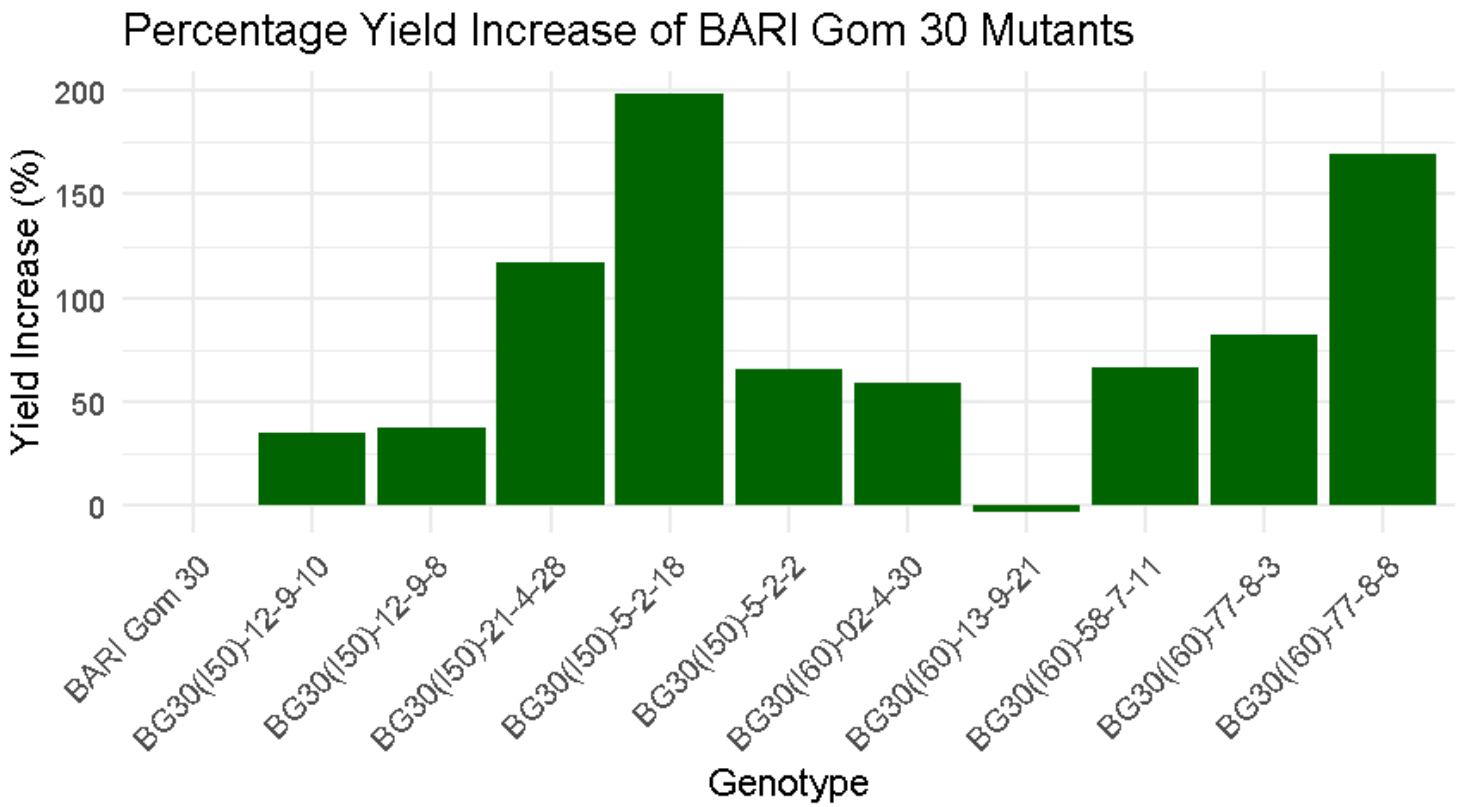


Figure 8

Percent yield increase of single-plant mutants relative to the parent, BARI Gom 30.

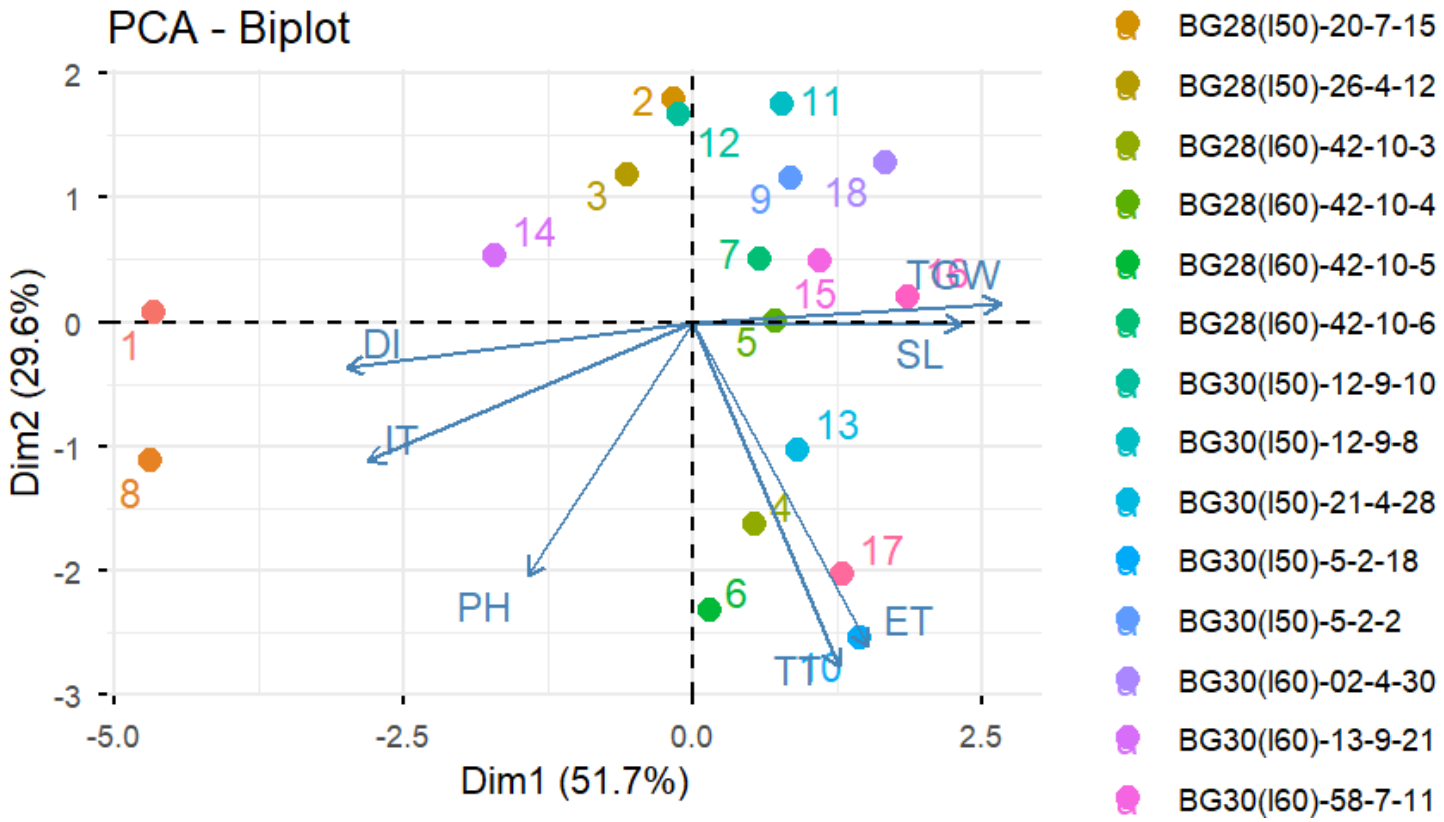


Figure 9

Principal component analysis of the traits of M3 progenies subjected to Ion beam irradiation. PH=Plant Height, TT=Total Tiller, Et=Effective Tiller, SL=Spike Length, TGW=Thousand Grain Weight, IT=Infected Tiller, DI=Disease Incidence.