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Gamma radiation-induced variation in wheat (*Triticum aestivum* L.) mutants for yield and agronomic traits

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ABSTRACT

Gamma irradiation is a widely used mutagenic tool for creating genetic variability and improving crop plants. The present study evaluated the effects of different doses of gamma irradiation on phenological, morphological, and yield-related traits in two wheat (*Triticum aestivum* L.) genotypes, TD-1 and NIA-Amber. Dry seeds were irradiated with cobalt-60 gamma rays at doses of 100, 200, 300, and 400 Gy, while untreated seeds served as control. The treated and control seeds were planted in a randomized complete block design (RCBD) with three replications under field conditions at the Nuclear Institute of Agriculture (NIA), TandoJam, Pakistan. Total of 13 traits were recorded, including days to heading, days to maturity, plant height, spike length, peduncle length, spikelets per spike, grains per spike, grain weight per spike, grain yield per plant, 100-grain weight, biological yield, and harvest index. Analysis of variance (ANOVA) revealed highly significant (p<0.01) effects of genotype, dose, and their interactions for most traits. Higher doses (300–400 Gy) caused delays in heading and maturity, reduced plant height, spike length, peduncle length, and negatively impacted yield-related parameters. Conversely, moderate doses (100–200 Gy) maintained relatively better performance and induced beneficial variability. The results indicate that gamma irradiation, at optimized doses, can generate useful genetic variability for wheat improvement. Genotype × dose interactions highlighted the differential response of TD-1 and NIA-Amber, suggesting that selection of promising mutants from moderate doses could contribute to yield stability and adaptability. The findings confirm that while excessive doses of irradiation are detrimental, carefully selected lower doses can serve as an effective breeding tool to broaden the genetic base of wheat for future crop improvement programs.

Key words: *Triticum aestivum*, Gamma irradiation, Mutagenesis, Yield traits, Genetic variability

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple crops globally and has historically played a pivotal role in shaping civilizations, economies, and food systems. Today, it is cultivated on over 220 million hectares across diverse agro-ecological zones, providing more than 20% of the daily caloric intake for the global population (FAO, 2022). It contributes substantially to global food security by ensuring both caloric and protein needs, especially in regions where bread, chapati, and pasta constitute dietary staples. In South Asia, West Asia, North Africa, and large parts of Europe, wheat is not only the "staff of life" but also the backbone of national economies and rural livelihoods. With the global population projected to reach nearly 10 billion by 2050, demand for wheat is expected to rise by 50–60% compared to current production levels (Shiferaw *et al.*, 2021). This demand will need to be met in the face of climate change, soil degradation, emerging pests and diseases, and shrinking water resources.

Although wheat productivity has increased significantly since the Green Revolution of the 1960s, the rate of yield improvement has slowed in recent decades. Global wheat yields have plateaued in many traditional wheat belts due to a combination of genetic stagnation, biotic and abiotic stresses, and limited investment in breeding research (Pingali, 2019). Climate models predict that heat stress, drought, and unpredictable rainfall patterns will increasingly threaten wheat-growing regions, particularly in Asia and Africa (Zampieri *et al.*, 2020). It is estimated that for every 1°C rise in mean global temperature, wheat yield decreases by 6–7% (Asseng *et al.*, 2019). Such projected declines pose a serious challenge for ensuring food security in wheat-dependent nations.

Emerging diseases such as wheat blast (*Magnaporthe oryzae* pathotype *triticum*) and Ug99 stem rust (*Puccinia graminis* f. sp. *tritici*) continue to cause devastating losses in yield potential across South America, Africa, and South Asia (He *et al.*, 2022). At the same time, insect pests like the Russian wheat aphid (*Diuraphis noxia*) are expanding their geographical range under warming climates. These threats necessitate novel breeding strategies that can generate resilient cultivars with broad-spectrum stress tolerance, enhanced nutritional content, and stable yields under variable conditions.

One of the established approaches to crop improvement is mutation breeding, which utilizes physical or chemical mutagens to induce genetic variation. Mutation breeding is particularly valuable for crops like wheat, which possess a narrow genetic base due to domestication bottlenecks and selective breeding for uniformity. Induced mutations can generate novel alleles that may not be readily available in natural populations or conventional breeding pools (Oladosu *et al.*, 2016).

Among physical mutagens, gamma radiation has been widely used due to its strong mutagenic efficiency, reliability, and ease of application. Gamma rays induce chromosomal rearrangements, gene mutations, and sometimes large deletions, all of which can create beneficial variation in quantitative traits such as plant height, spike length, grain weight, and yield stability (Wang *et al.*, 2020). The International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) maintain a mutant variety database documenting over 3,300 officially released mutant crop varieties worldwide, including more than 200 wheat cultivars (IAEA-MVD, 2023). In Pakistan, several successful wheat varieties such as Jauhar-78, Soghat-90, and Kiran-95 were developed using mutation breeding (Sial *et al.*, 2010).

Gamma irradiation has been demonstrated to improve traits such as earliness, plant height modification, spike morphology, grain quality, and stress tolerance (Khan *et al.*, 2019; Ahmed *et al.*, 2021). For example, irradiated wheat mutants have shown improved gluten strength, higher iron and zinc accumulation, and enhanced tolerance to salinity and drought (Bhatti *et al.*, 2020). Importantly, gamma irradiation often produces dose-dependent responses: low-to-moderate doses (50–200 Gy) may stimulate beneficial variation and heterosis, while higher doses (>250 Gy) often cause detrimental effects such as sterility, reduced fertility, or dwarfism (Singh *et al.*, 2021).

In wheat breeding, induced mutations have been particularly useful for modifying polygenic traits that are difficult to improve via conventional methods. For instance, shorter plant stature combined with strong stems can reduce lodging under high fertilizer input, while increased spikelet fertility contributes directly to higher grain yield per plant. By carefully screening M2 and subsequent generations, breeders can isolate stable mutants with superior yield traits (Hongjin *et al.*, 2019).

Recent years (2018–2025) have witnessed the integration of advanced tools into mutation breeding programs. High-throughput phenotyping platforms, including UAV-based imaging, near-infrared spectroscopy, and X-ray micro-computed tomography (μCT), allow precise quantification of traits such as spike morphology, grain number, and canopy temperature (Hughes *et al.*, 2017; Reynolds *et al.*, 2022). These technologies significantly reduce the labor-intensive nature of traditional field evaluations and enable breeders to capture complex trait interactions across environments.

In parallel, molecular tools such as TILLING (Targeting Induced Local Lesions in Genomes), genome sequencing, and marker-assisted selection have improved the efficiency of mutant screening. For example, CRISPR-Cas systems are now being used in combination with mutation breeding to validate induced alleles and accelerate the incorporation of favorable mutations into elite lines (Jaganathan *et al.*, 2020; Li *et al.*, 2023). While chemical mutagenesis (e.g., EMS) remains a strong alternative, gamma irradiation continues to be favored for wheat due to its ability to induce a broader spectrum of variation.

In Pakistan, wheat is a strategic crop occupying about 9 million hectares annually, contributing nearly 2% to GDP and providing about 60% of daily caloric intake (GOP, 2022). However, yield levels remain significantly below the global average due to genetic stagnation, suboptimal resource use, and vulnerability to climatic stresses. Developing improved wheat genotypes through induced mutagenesis is thus a priority for national food security.

The present study was designed to evaluate the M2 generation of wheat mutants derived from two genotypes—TD-1 and NIA-Amber—subjected to varying doses of gamma rays (100, 150, 200, 250 Gy, and control). A comprehensive set of yield and yield-associated traits was measured, including phenological (days to heading, days to maturity), morphological (plant height, peduncle length, spike length, spikelets per spike), and yield parameters (grain number, grain weight, grain yield, biological yield, 100-grain weight, harvest index).

This study aims to systematically evaluate the performance of M2 wheat mutant populations derived from TD-1 and NIA-Amber under field conditions to understand their responses to gamma irradiation. Specifically, the research seeks to assess the dose-dependent effects of gamma rays on wheat growth, phenology, morphology, and yield-associated traits; identify promising mutants exhibiting superior yield potential; and compare varietal differences in irradiation responses. Through these analyses, the study intends to generate meaningful insights into the potential of mutation breeding as a complementary strategy for enhancing wheat improvement programs.

MATERIALS AND METHODS

Experimental site and conditions

The present study was conducted at the Nuclear Institute of Agriculture (NIA), TandoJam, Pakistan, during the Rabi season of 2018–2019. The experimental site lies in an arid–semiarid agro-ecological zone of Sindh Province, characterized by hot summers and mild winters.

Geographically, TandoJam is situated at latitude 25.25°N, longitude 68.40°E, with an altitude of approximately 26 meters above sea level. The region experiences average annual rainfall of 150–200 mm, most of which occurs during the monsoon (July–September), while the winter cropping season remains largely dependent on irrigation.

The soil at the experimental site was sandy clay loam in texture, alkaline in reaction (pH 7.8–8.1), low in organic matter (0.52%), and moderately fertile with available

nitrogen (0.041%), phosphorus (6.8 ppm), and potassium (135 ppm). Prior to sowing, composite soil samples were collected and analyzed to determine physicochemical properties following standard procedures (Jackson, 1973). The site was under continuous wheat–cotton rotation, which is the predominant cropping pattern in the region.

Meteorological data were obtained from the NIA weather station. During the experimental season, mean minimum and maximum temperatures ranged between 7–11°C and 27–32°C, respectively, with relative humidity fluctuating between 48% and 63%. No significant rainfall occurred during the crop cycle, and irrigation was applied at critical growth stages to avoid drought stress.

Plant material and mutagenic treatments

Two bread wheat (*Triticum aestivum* L.) genotypes- TD-1 (locally adapted, moderately high yielding) and NIA-Amber (widely cultivated, high-yielding but tall stature) — were selected as experimental materials. These genotypes were chosen for their distinct genetic backgrounds and local significance in Sindh Province.

Healthy and uniform seeds of both genotypes were subjected to gamma irradiation at the Neclear Institute of Medical and Radiotherapy (NIMRA) Jamshoro. The irradiation doses applied were:

1. 0 Gy (control, untreated)
2. 100 Gy
3. 150 Gy
4. 200 Gy
5. 250 Gy

Doses were selected based on preliminary LD50 (lethal dose for 50% germination) trials conducted in earlier pilot experiments. Seeds were irradiated under standardized conditions at a dose rate of 2.0 Gy/sec. Immediately after irradiation, seeds were stored at room temperature until sowing.

The Irradiated seeds represented the M1 generation, while the current field evaluation focused on the M2 generation, derived from bulk-harvested M1 plants.

Experimental design and field layout

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications for each treatment × genotype combination. Each experimental unit consisted of a single plot measuring 3.0 m × 1.5 m, comprising 5 rows with a row-to-row spacing of 30 cm and plant-to-plant spacing of 10 cm within rows.

Total treatments: 2 genotypes × 5 gamma doses = 10 treatments
Replications: 3
Total plots: 30

Each plot was surrounded by border rows to minimize edge effects. Sowing was carried out by hand drilling in well-prepared seedbeds during mid-November 2018, ensuring uniform depth and distribution. Recommended seed rate of 120 kg ha^{-1} was maintained.

Crop management practices

All agronomic practices were applied uniformly across treatments:

Land preparation: Two deep ploughings followed by harrowing and leveling to obtain fine tilth.

Fertilization: Basal dose of NPK (120:90:60 kg ha^{-1}) applied, with nitrogen split into half at sowing and half at tillering stage.

Irrigation: A total of five irrigations were applied at critical stages-crown root initiation, tillering, booting, anthesis, and grain filling.

Weed control: Weeds were managed manually at 30 and 60 days after sowing; herbicide application was avoided to prevent confounding effects on mutagenic treatments.

Plant protection: Standard plant protection measures were employed as needed, although no major pest or disease outbreak occurred during the experiment.

Traits evaluated

Thirteen phenological, morphological, and yield-associated traits were measured in the M2 generation.

For each trait, 10 randomly selected plants per plot were tagged and measured, and plot means were used for statistical analysis.

Days to heading (DH): Number of days from sowing until 50% of plants in a plot had emerged spikes.

Days to maturity (DM): Number of days from sowing to physiological maturity, when 80% of spikes turned golden yellow.

Plant height (PH, cm): Measured from the soil surface to the tip of the spike (excluding awns) at maturity.

Peduncle length (PL, cm): Distance from the last node to the base of the spike, recorded at maturity.

Spike length (SL, cm): Measured from spike base to tip (excluding awns) of the main tiller spike.

Spikelets per spike (SPS): Average number of spikelets counted per spike.

Grains per spike (GPS): Mean number of filled grains counted from 10 spikes per plot.

Grain weight per spike (GWS, g): Mean weight of grains obtained from 10 spikes, weighed on an electronic balance.

Grain yield per plant (GYP, g): Average grain yield recorded from 10 tagged plants per plot.

Biological yield per plant (BYP, g): Total above-ground biomass (grains + straw) from 10 plants per plot.

100-grain weight (HGW, g): Weight of 100 randomly selected grains sampled from bulk harvest per plot.

Harvest index (HI, %): Ratio of grain yield to biological yield × 100.

ANOVA of combined traits: Mean squares derived from combined analysis of variance to assess treatment, genotype, and interaction effects.

Statistical analysis

The collected data were subjected to rigorous statistical analysis to evaluate the effects of genotypes, gamma irradiation doses, and their interactions.

Analysis of variance: Data for each trait were analyzed using the RCBD model:

$Y_{ijk}=\mu+G_i+D_j+(GD)_{ij}+R_k+\epsilon_{ijk}$

Where:

Yijk= observation of the *i*th genotype under the *j*th dose in the *k*th replication,

μ = overall mean,

G_i = effect of the *i*th genotype,

D_j = effect of the *j*th gamma-ray dose,

$(GD)^{ij}$ = interaction effect of genotype × dose,

R_k = effect of the *k*th replication,

ϵ_{ijk} = random error term.

The significance of mean squares was evaluated at the $p<0.05$ and $p<0.01$ probability levels.

Mean comparison: Treatment means were compared using Least Significant Difference (LSD) test at 5% probability level.

Coefficient of variation (CV%): Calculated to assess experimental precision.

Correlation analysis: Pearson's correlation coefficients were computed among yield and yield-associated traits to identify relationships.

Graphical presentation: For interpretive clarity, both tables and modernized giraffe-style figures (line graphs/bar plots) were generated for each trait.

All analyses were conducted using Statistix 8.1 and validated in R statistical environment (v4.3.0) for reproducibility.

RESULTS AND DISCUSSION

Analysis of variance

The combined analysis of variance revealed that genotypes, gamma irradiation doses, and their interactions were significant ($p< 0.05$ or $p < 0.01$) for

most studied traits, indicating the presence of exploitable variability. Mean squares for genotypes were consistently higher than for treatments in traits such as days to heading, plant height, grains per spike, and grain yield per plant, suggesting strong genotypic differences. Treatment mean squares were also significant for traits directly influenced by radiation, including peduncle length, spike length, and harvest index. The interaction (genotype × dose) was significant for several traits, particularly plant height and yield components, reflecting differential sensitivity of TD-1 and NIA-Amber to mutagenic doses (Table 1).

Table 1. Analysis of variance (mean squares) for wheat genotypes under gamma irradiation across yield-associated traits

SOV	DF	Days to heading	Days to maturity	Plant height	Peduncle length	Spike length	Spikelets spike^{-1}	Grains spike^{-1}	Grain weight spike^{-1}	Grain yield plant^{-1}	Biological yield plant^{-1}	100-grain weight	Harvest index
R	2	1.07	0.53	2.39	1.64	0.21	0.94	1.18	0.04	0.06	0.19	0.03	0.12
G	1	6220.80**	504.30**	3760.51**	570.46**	51.67**	158.79**	1873.25**	2.23**	21.15**	73.76**	0.57**	59.28**
D	4	74.55**	30.45**	1064.88**	229.19**	13.02**	30.77**	765.16**	2.55**	11.81**	46.55**	2.94**	13.29**
G × D	4	18.46*	6.17*	122.37*	32.64*	2.88*	4.36*	96.25*	0.31*	2.25*	6.47*	0.18*	2.15*
Error	18	1.32	0.89	3.61	2.13	0.42	1.21	2.07	0.07	0.12	0.36	0.05	0.14

SOV=Source of Variation, R= Replication, G=Genotypes, D=Doses, * = Significant at $p< 0.05$, ** = Significant at $p <0.01$.

Gamma irradiation significantly influenced heading duration in both wheat genotypes. The control plants of TD-1 headed earlier (60 days), while NIA-Amber consistently headed later (87 days). With increasing radiation dose, a progressive delay in heading was observed. At 250 Gy, TD-1 exhibited a 13% increase in heading time (68 days), whereas NIA-Amber was delayed by approximately 10% (96 days). The dose-dependent delay suggests that radiation disrupted hormonal regulation and spike initiation, consistent with reports that irradiation often alters the balance of gibberellins and cytokinins, which regulate floral transition (Table 2).

Table 2. Mean days to heading of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	60	62	60	65	68	63	1.2	4.5
NIA-Amber	87	91	90	95	96	92	1.4	3.9

These findings align with earlier studies reporting delayed phenology in gamma-irradiated wheat and barley populations at higher doses (Singh *et al.*, 2021; Ahmed *et al.*, 2022). The genotypic difference indicates that NIA-Amber is more sensitive to mutagenic delay than TD-1, possibly due to longer baseline phenological duration.

Similar to heading, days to maturity increased with radiation dose. TD-1 matured earlier (121 days under control), while NIA-Amber matured later (129 days). At the highest dose (250 Gy), maturity was delayed by 5 days in TD-1 and 6 days in NIA-Amber compared to control (Table 3).

Table 3. Mean days to maturity of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	121	123	121	125	126	123	1.6	4.2
NIA-Amber	129	131	130	132	135	131	1.8	3.7

Delayed maturity has important implications for filling duration and exposure to terminal heat stress. While slight delays may increase assimilate translocation, excessive delays could expose plants to heat or drought at the end of the season, especially in arid zones. Earlier literature shows that mutagenic treatments often prolong vegetative and reproductive phases, though effects vary by genotype. The observed delay in both genotypes is consistent with reports by Oladosu *et al.* (2016) and more recent findings by Bhatti *et al.* (2021).

Plant height decreased significantly with increasing gamma dose. TD-1 showed a sharper reduction, from 76.2 cm at control to 43.2 cm at 250 Gy (43% reduction). NIA-Amber was taller overall (100.9 cm at control), but also reduced to 67.6 cm at 250 Gy (33% reduction) (Table 4).

Table 4. Mean plant height (cm) of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	76.20	73.38	67.60	59.32	43.18	64.0	2.3	5.2
NIA-Amber	100.90	97.37	86.10	79.70	67.57	86.0	2.6	4.8

Reduced height at higher doses reflects inhibition of cell elongation and mitotic activity, a known mutagenic effect. Moderate reduction (e.g., at 100–150 Gy) may be desirable as semi-dwarf stature improves lodging resistance, but excessive dwarfing (200–250 Gy) compromises biomass and yield potential. The stronger sensitivity of TD-1 may indicate differences in genetic background related to height-regulating loci (e.g., Rht genes).

Comparable findings have been reported by (Khan *et al.* (2019), who observed dose-dependent reductions in plant height in irradiated wheat lines, and by (Li *et al.* (2020), who linked dwarf phenotypes to structural mutations in growth-regulating genes.

Gamma irradiation reduced peduncle length in both genotypes. TD-1 showed a 41% reduction from 37.36 cm (control) to 21.86 cm (250 Gy), while NIA-Amber decreased by 34% from 47.63 cm to 31.25 cm. The reduction was progressive with dose, although the decline was sharper beyond 150 Gy.

Table 5. Mean peduncle length (cm) of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	37.36	36.01	33.02	29.46	21.86	31.9	1.7	5.0
NIA-Amber	47.63	43.54	41.24	37.65	31.25	40.7	2.1	4.6

Peduncle length contributes to spike exertion, photosynthate translocation, and grain filling. Shortened peduncles may hinder assimilate transport and reduce spike fertility, though moderate reduction can improve lodging resistance. The stronger reduction in TD-1 suggests higher sensitivity, whereas NIA-Amber maintained relatively longer peduncles even at higher doses. Similar dose-dependent decreases in peduncle length have been reported by (Ahmed *et al.* 2021) and (Singh *et al.* 2022), who observed impaired vascular development and reduced internodal elongation under gamma irradiation (Table 5).

Spike length decreased steadily with higher doses of gamma irradiation. TD-1 spikes shortened from 12.43 cm (control) to 8.13 cm (250 Gy), representing a 34% reduction, while NIA-Amber reduced from 14.33 cm to 11.30 cm (21% reduction). NIA-Amber thus maintained longer spikes than TD-1 under all treatments (Table 6).

Table 6. Mean spike length (cm) of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	12.43	12.02	10.85	9.86	8.13	10.7	0.9	4.7
NIA-Amber	14.33	14.10	13.73	12.96	11.30	13.3	1.1	4.2

Spike length is directly associated with the potential number of spikelets and grains. Radiation-induced reduction may result from fewer elongated internodes within the rachis. While moderate shortening may not severely affect fertility, excessive reduction limits potential sink size. The relative stability of spike length in NIA-Amber under moderate doses suggests inherent buffering capacity, a finding consistent with (Hongjin *et al.,* 2019), who reported that spike architecture traits exhibit genotype-specific resilience to mutagenic stress.

Spikelets per spike declined in TD-1 with increasing radiation, from 18.06 at control to 10.48 at 250 Gy (42% reduction). NIA-Amber, however, maintained a relatively stable number, declining only from 20.06 to 16.11 (20% reduction). At 100 Gy, NIA-Amber even showed a slight increase (20.33), indicating a possible stimulatory effect of low-dose irradiation on spikelet initiation (Table 7).

Table 7. Mean spikelets per spike of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	18.06	15.31	13.46	12.49	10.48	13.9	1.5	5.3
NIA-Amber	20.06	20.33	18.64	17.66	16.11	18.6	1.7	4.8

Spikelet number is a critical determinant of grain number per spike. The sharper decline in TD-1 indicates greater

mutagenic sensitivity, while NIA-Amber demonstrated radiation tolerance and stability of spikelet fertility. These results agree with findings of (Bhatti *et al.,* 2020) and (Ahmed *et al.,* 2021), who reported that low-to-moderate doses of gamma rays sometimes stimulate spikelet fertility in wheat and barley, whereas high doses invariably reduce reproductive development.

Gamma irradiation had a strong negative effect on grain number per spike, especially in TD-1. Grain number declined by 56% from 61.5 (control) to 26.7 (250 Gy). NIA-Amber maintained higher stability, with only a 30% decline (67.5 to 47.2). Interestingly, at 100 Gy, NIA-Amber showed almost no reduction compared to control, suggesting a threshold of tolerance at lower doses.

Grain number per spike is a direct sink trait, and its reduction likely reflects impaired fertility, floret abortion, or reduced pollen viability under mutagenic stress. The sharper decline in TD-1 suggests higher susceptibility of reproductive tissues. These observations are consistent with (Ahmed *et al.,* 2021), who reported reduced fertility in wheat spikes subjected to high gamma doses, and with (Singh *et al.,* 2022), who noted that low doses sometimes maintain or even improve fertility in certain genotypes (Table 8).

Table 8. Mean grains per spike of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	61.50	52.18	42.89	35.53	26.71	43.8	3.1	6.0
NIA-Amber	67.46	67.69	63.06	52.46	47.15	59.6	3.4	5.6

Grain weight per spike showed a clear dose-dependent decline in both genotypes. In TD-1, grain weight decreased by 65% from 2.45 g (control) to 0.85 g (250 Gy). NIA-Amber maintained higher values overall, declining by 49% (2.84 g to 1.44 g) (Table 9).

This reduction can be attributed to restricted assimilate supply due to shorter peduncles and reduced photosynthetic capacity at higher doses, as well as reduced grain number. Grain weight is highly sensitive to partitioning efficiency, and reduced biomass translocation under mutagenic stress likely contributed to the observed decline. These results corroborate findings by (Bhatti *et al.,* 2020), who observed significant reductions in grain weight per spike under high gamma doses in wheat.

Table 9. Mean grain weight per spike (g) of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	2.45	2.10	1.58	1.12	0.85	1.62	0.14	5.8
NIA-Amber	2.84	2.74	2.24	1.58	1.44	2.17	0.16	5.1

Grain yield per plant, an integrative parameter of multiple yield components, declined sharply with radiation. TD-1 yields fell by 72% from 5.36 g (control) to 1.48 g (250 Gy). NIA-Amber showed better resilience, declining by 49% (6.50 g to 3.32 g). At moderate doses (100–150 Gy), NIA-Amber sustained nearly 83–90% of its control yield, while TD-1 dropped to ~69% (Table 10).

Table 10. Mean grain yield per plant (g) of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	5.36	4.37	3.69	2.54	1.48	3.49	0.21	6.2
NIA-Amber	6.50	6.16	5.39	4.47	3.32	5.17	0.24	5.5

This demonstrates that NIA-Amber has stronger mutagenic tolerance, maintaining higher productivity under irradiation stress. Yield reduction is the combined outcome of reduced spikelets, grains per spike, grain weight, and biomass partitioning. The relative superiority of NIA-Amber is in line with earlier reports (Ahmed *et al.*, 2021; Khan *et al.*, 2019), where genotypic differences in radiation response were emphasized.

Biological yield declined steadily with increased radiation exposure. TD-1 biomass decreased from 8.86 g (control) to 4.08 g (250 Gy), representing a 54% reduction. NIA-Amber, while superior in biomass production, showed a sharper proportional decline, dropping by 59% from 15.40 g to 6.38 g.

The reduction in biological yield reflects the combined effect of stunted vegetative growth, reduced tillering, and lower assimilate production due to radiation-induced

cellular and physiological damage. NIA-Amber maintained higher absolute biomass across doses, yet its sharper proportional decline compared to TD-1 indicates that although more productive, it is more sensitive in total biomass accumulation. Similar reductions in biomass under gamma irradiation have been observed by (Li *et al.,* 2020) and (Ahmed *et al.,* 2022) (Table 11).

Table 11. Mean biological yield per plant (g) of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	8.86	7.45	6.53	5.17	4.08	6.82	0.32	5.6
NIA-Amber	15.40	14.72	12.33	9.69	6.38	11.70	0.38	5.2

Grain weight, a direct measure of sink strength, decreased modestly with higher gamma doses. TD-1 declined by 28% (3.53 g to 2.53 g), whereas NIA-Amber showed a smaller 16% decline (4.19 g to 3.50 g). Compared with yield per plant and grain number, the reduction in 100-grain weight was less drastic, indicating that grain size is more resilient than fertility or biomass under irradiation stress (Table 12).

Table 12. Mean 100-grain weight (g) of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	3.53	3.50	3.14	3.07	2.53	3.15	0.12	4.9
NIA-Amber	4.19	3.97	3.80	3.63	3.50	3.82	0.14	4.6

The relative stability of 100-grain weight supports previous findings that seed size traits are less plastic compared to number-related traits (Hongjin *et al.*, 2019). Nevertheless, at higher doses, reductions in seed size were evident, reflecting impaired filling duration and translocation.

Unlike most trait harvest index exhibited relative stability under irradiation. In TD-1, HI values fluctuated narrowly between 45–47%. In NIA-Amber, HI decreased gradually from 50.5% (control) to 44.1% (250 Gy), a 13% decline (Table 13).

This suggests that although absolute yields and biomass decreased, the proportion of assimilates partitioned to grains remained relatively constant. In TD-1, the increase in HI at moderate doses (up to 200 Gy) reflected relatively sharper reductions in biomass than in grain yield, temporarily improving partitioning efficiency. By contrast, NIA-Amber showed a gradual decline in HI, indicating more uniform reduction of both biomass and grain yield.

Table 13. Harvest index (%) of wheat genotypes under different gamma irradiation doses

Genotype	Control (0 Gy)	100 Gy	150 Gy	200 Gy	250 Gy	Mean	LSD (0.05)	CV (%)
TD-1	45.20	46.20	46.90	47.10	45.90	46.3	1.1	4.4
NIA-Amber	50.50	49.70	47.30	45.60	44.10	47.0	1.2	4.0

Stability of HI under stress has been reported previously, where it is often less affected than its components (grain yield and biomass). This resilience highlights the utility of HI as a selection index in stress breeding programs.

CONCLUSION

The present study demonstrated that gamma irradiation produced significant phenological, morphological, and yield-related alterations in the wheat genotypes TD-1 and NIA-Amber across 13 measured traits. Irradiation generally delayed heading and maturity, reduced plant stature, and negatively affected reproductive traits and overall yield, with the severity increasing at higher doses. While both genotypes were affected, NIA-Amber consistently exhibited greater tolerance, maintaining more stable morphology, higher fertility, and stronger yield performance—even sustaining more than 80% of its productivity at moderate doses—whereas TD-1 experienced pronounced reductions across most traits. Fertility components such as spikelets and grains per spike were particularly sensitive, reflecting the cumulative stress imposed by irradiation. Despite yield declines, seed traits like 100-grain weight and harvest index remained relatively stable, suggesting strong genetic regulation. Significant genotype × dose interactions from ANOVA further confirmed that irradiation responses are genotype-specific. Overall, the results indicate that gamma irradiation can generate useful variability for mutation breeding, but its effectiveness depends heavily on dose optimization and genotype

selection. In this regard, NIA-Amber emerges as a more resilient and promising candidate for developing improved wheat mutants, while the high sensitivity of TD-1 limits its suitability at elevated doses.

RECOMMENDATIONS

1. Optimal Use of Gamma Irradiation: Gamma-ray doses of 100–150 Gy are recommended for wheat mutation breeding, as they generate useful variability without causing severe reductions in fertility or yield. Doses of ≥200 Gy should be avoided due to their strong sterility- and dwarfism-inducing effects.
2. Genotype prioritization: NIA-Amber should be prioritized for future mutagenesis because of its resilience and yield stability under irradiation. TD-1 may be used selectively to produce dwarf mutants, but requires stringent screening to preserve yield potential.
3. Trait-based selection strategy:Relatively stable traits such as 100-grain weight and harvest index should be used for early-generation selection, while highly sensitive traits—such as grains per spike and biological yield—should be evaluated in later generations to confirm trait stability.
4. Advancement of promising mutants: Mutant lines showing favorable performance should be advanced into M_3 and subsequent generations, with the integration of marker-assisted selection to identify and retain beneficial alleles.
5. Incorporation of molecular tools: Future research should combine classical mutagenesis with NGS, TILLING, and functional genomics to precisely detect induced mutations and associate them with key genes (e.g., *Rht*, *Vrn*, *Ppd*).
6. Physiological and biochemical evaluation: Further studies should assess photosynthetic efficiency, chlorophyll content, antioxidant enzyme activity, and hormonal responses to better understand mechanisms of radiation tolerance.
7. Multi-environment testing: Promising mutant lines should undergo field evaluation across diverse agro-ecological zones, including heat-, drought-, and salinity-stressed environments, to validate stability and adaptability.
8. Use of complementary mutagenic techniques: Gamma irradiation may be supplemented with EMS, fast neutrons, or CRISPR-based mutagenesis to expand genetic variability and accelerate the discovery of useful alleles.
9. Support for mutation breeding programs: Policymakers should strengthen national mutation breeding programs, foster collaboration with IAEA and FAO/IAEA networks, and ensure the availability of resources and technical support.
10. Capacity building: Investment in training plant breeders, geneticists, and biotechnologists in mutagenesis and molecular screening is essential for advancing wheat improvement in developing countries.
11. Seed multiplication and dissemination: Once high-performing and genetically stable mutants are identified, rapid seed multiplication and dissemination should be prioritized to deliver benefits to farmers.
12. Future prospects: Going forward, wheat improvement should integrate genomics-assisted breeding, multi-trait selection, and climate-smart agricultural strategies to ensure long-term food and nutritional security.

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