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Agronomic Traits and Yield Component Responses of M2 Wheat Genotypes Derived from Gamma-Ray-Induced Mutation under Tropical Highland Conditions

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Abstract: This study aimed to evaluate the agronomic performance and yield components of 25 M2 mutant wheat genotypes generated through gamma-ray irradiation compared with five tropical wheat varieties used as checks. The experiment was conducted at the Salaran Experimental Station, Satya Wacana Christian University, using an *augmented randomized complete block design* with three replications for the check varieties. The evaluated parameters included agronomic traits and yield components. Data were analyzed using analysis of variance (ANOVA) at a 5% significance level, followed by the *critical difference* (CD) test to compare individual genotypes with the check varieties. The results revealed variation in the responses of several agronomic traits and yield components, with highly significant differences ($p < 0.01$) observed only for 100-grain weight (coefficient of variation [CV] = 0.70%; range = 3.86–4.96 g). The genotypes GR 2-0, GR 4-0, GR 4-2, GR 4-3, GR 7-3, and GR 7-4 exhibited 100-grain weights ranging from 4.63 to 4.96 g, which were significantly higher than those of most check varieties (CD 5% = 0.09 g). This study indicates that gamma-ray irradiation has the potential to enhance genetic variation in M2 wheat populations, particularly for the 100-grain weight trait as an important yield component. These six genotypes represent promising candidates for further selection in the M3 generation within tropical wheat breeding programs in Indonesia.
Keywords: Tropical wheat; mutant genotype; irradiation; gamma ray; yield component

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple crops worldwide and plays a strategic role in global food security due to its contribution to human calorie and protein intake. Global wheat demand continues to increase due to population growth and changes in dietary patterns; however, wheat production in several non-traditional wheat-growing regions remains constrained by environmental limitations and limited genetic resources (Erenstein et al., 2022). In Indonesia, wheat production remains insufficient to meet domestic demand, resulting in a high dependence on imported wheat grain. The development of wheat cultivation in Indonesia is particularly challenging because wheat is not naturally adapted to tropical environments, where high temperature, altered photoperiod conditions, and unfavorable grain-filling environments can negatively affect plant growth and yield formation (Ahumada-Flores et al., 2021). Therefore, improving wheat adaptation under tropical conditions requires integrated approaches involving optimized agronomic management, environmental adaptation strategies, and the development of genetically improved wheat varieties.

The development of wheat varieties adapted to Indonesian environments has been constrained by the limited genetic diversity available for breeding programs. Genetic variation is a fundamental prerequisite for crop improvement because it provides the basis for selection and accumulation of desirable traits, including adaptation, yield potential, and stress tolerance. As wheat is an introduced crop in Indonesia, the available genetic resources remain relatively limited compared with regions where wheat has undergone long-term adaptation and diversification (Firmansyah et al., 2024). Consequently, expanding genetic diversity through artificial variation-generating approaches has become an important strategy to accelerate the development of wheat genotypes with improved adaptation to tropical environments.

One promising approach for broadening genetic variability is induced mutagenesis using physical mutagens, particularly gamma irradiation. Gamma rays generated from cobalt-60 sources can induce random alterations in plant DNA, potentially creating novel genetic variants with desirable phenotypic characteristics (Kiani et al., 2022). Unlike conventional hybridization, which depends on available parental variation, mutation induction provides an opportunity to generate additional variability within existing elite genetic backgrounds. However, the phenotypic consequences of induced mutations are highly dependent on genotype, irradiation dose, and environmental conditions, resulting in diverse responses among mutant populations (Meliala et al., 2016). Therefore, systematic evaluation and selection of mutant generations are essential steps to identify stable and agronomically valuable variants. Gamma-ray mutagenesis has been successfully applied in various crops to expand genetic diversity and generate improved traits related to productivity and environmental adaptation (Oladosu et al., 2016).

The evaluation of early mutant generations, particularly the M2 generation, is an important stage in mutation breeding programs because segregation and expression of induced genetic variation become more apparent after the M1 generation. Previous studies in wheat have demonstrated that gamma irradiation can generate variation in important agronomic and yield-related traits, including spike characteristics, grain weight, and physiological responses (Li et al., 2022; Indriatama et al., 2016). Shabani et al. (2022) reported that optimized gamma irradiation treatments improved grain weight, fertile floret number, biological yield, and harvest efficiency in wheat. Similarly, Hong et al. (2025) showed that gamma irradiation influenced agronomic performance and antioxidant properties in colored wheat. However, most previous mutation studies in wheat have been conducted under temperate or subtropical conditions, while information regarding the performance of gamma-induced wheat mutant populations under tropical highland environments remains limited. Furthermore, the superiority of mutant genotypes should be evaluated through comparison with locally adapted reference varieties to determine whether the observed phenotypic advantages represent meaningful improvements relative to existing genetic resources.

The evaluation of mutant populations across generations is therefore critical for identifying promising genotypes for subsequent breeding stages. Phenotypic expression in the M2 generation may still be affected by segregation and environmental variation; consequently, selection at this stage should focus on identifying genotypes showing desirable and consistent agronomic characteristics rather than final varietal superiority. Despite previous advances in wheat mutation breeding, limited information is available regarding the agronomic performance and yield component variation of M2 wheat populations derived from Indonesian wheat varieties, including Guri, Dewata, Selayar, and Nias, following gamma irradiation under tropical highland conditions. This information is particularly important for developing wheat breeding materials adapted to Indonesian agroecosystems.

We hypothesized that gamma-ray irradiation generates sufficient genetic variation within M2 wheat populations, enabling the identification of genotypes with improved agronomic characteristics and yield-related traits compared with existing tropical wheat varieties. Therefore, this study aimed to evaluate the agronomic performance and yield components of 25 gamma-induced M2 wheat genotypes under tropical highland conditions in Indonesia and to identify promising genotypes for subsequent selection in wheat breeding programs using five national wheat varieties (Guri 1, Guri 6, Guri 7, Guri 8, and Nias) as reference checks.

METHOD

Experimental Site and Time

The evaluation of gamma-ray-induced wheat genotypes in the M2 population was conducted from May to September 2025 at the Salaran Experimental Station, Faculty of Agriculture and Business, Satya Wacana Christian University, Getasan Village, Semarang Regency, Indonesia. The experimental site was located at an altitude of 1,100 m above sea level (asl). The agroclimatic conditions of the site were characterized by an average temperature ranging from 18–23°C, which is suitable for highland wheat cultivation.

Gamma Irradiation Process and Genetic Materials

Gamma-ray irradiation treatments were performed using a cobalt-60 (^60Co)-based gamma irradiator facility at the Gamma Irradiation Laboratory, Ahmad Baiquni Science and Technology Area (KSTE), National Research and Innovation Agency (BRIN), Yogyakarta, Indonesia. Healthy seeds from several tropical wheat parental varieties, namely Guri 1, Guri 2, Guri 3, Guri 4, Guri 6, Guri 7, Guri 8, Nias, and Selayar, were irradiated with gamma-ray doses of 0, 100, 200, 300, 400, and 500 Gy using a calibrated dose rate to induce genetic variation in the initial population.

The M1 generation populations were subsequently grown under field conditions and allowed to undergo self-pollination. Harvesting and selection were performed using a single-plant selection approach within the M1 population to generate individual-derived M2 seeds. Preliminary selection at the individual plant level was conducted based on germination capacity, plant uniformity, and plant health. This process resulted in the selection of 25 promising M2 mutant genotypes, which were evaluated in the present study.

The genetic materials evaluated consisted of five national wheat varieties used as checks (Guri 1, Guri 6, Guri 7, Guri 8, and Nias) and 25 previously selected M2 mutant wheat genotypes, including GR 1-1, GR 1-2, GR 1-4, GR 2-0, GR 2-1, GR 2-2, GR 2-3, GR 4-0, GR 4-1, GR 4-2, GR 4-3, GR 6-1, GR 6-2, GR 7-1, GR 7-2, GR 7-3, GR 7-4, GR 8-1, GR 8-2, N-1, N-2, N-3, SL-1, SL-3, and SL-4.

Experimental Design

This experimental study employed an augmented randomized complete block design (augmented RCBD) with three replications. The experimental factor consisted of 30 wheat genotypes, including 25 gamma-ray-induced M2 mutant genotypes and five check varieties (Guri 1, Guri 6, Guri 7, Guri 8, and Nias).

Seeds used in the experiment were selected based on visual assessment to ensure uniformity and good health. Each genotype was planted in a 30 × 30 cm polybag containing a growing medium consisting of soil, manure, and rice husk in a 1:1:1 ratio. Three seeds of each genotype were planted per polybag. The tested genotypes were not replicated across blocks, whereas the check varieties were replicated in each block to estimate experimental error. This design enabled statistical evaluation of mutant genotype performance relative to the check varieties.

Observation Parameters

The observed parameters included agronomic traits and yield components as follows:

1. Plant height: Plant height was measured after spike emergence. Measurements were taken from the soil surface to the tip of the highest spike and expressed in centimeters (cm).
2. Number of tillers: The number of tillers was counted during the late vegetative stage, before spike emergence.
3. Number of productive tillers: Productive tillers were counted when tillers began to produce spikes.
4. Spike length: Spike length was measured from the base to the tip of the spike, excluding awns, and expressed in centimeters (cm).
5. Days to spike emergence: Days to spike emergence were recorded from planting until the first spike became visible from the flag leaf sheath. Data were expressed as days after planting (DAP).
6. Days to flowering: Days to flowering were recorded from planting until the appearance of the first flower.
7. Days to harvest: The duration to harvest was calculated from planting until the plants were harvested.
8. Grain-filling duration: Grain-filling duration was calculated as the difference between days to harvest and days to flowering.
9. 100-grain weight: The weight of 100 seeds was measured using a digital balance.

Data Analysis

The collected data were analyzed using an F-test or analysis of variance (ANOVA) at a 5% significance level to determine the effect of genotype on each evaluated agronomic trait and yield component. Further analysis was conducted by comparing the tested genotypes with the check varieties using the critical difference (CD) test at a 5% significance level.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) results for all agronomic traits and yield components of the 25 gamma-ray-induced M2 wheat genotypes and five check varieties are presented in Table 1.

Table 1. Summary of analysis of variance (F-values) and coefficients of variation (CV) for agronomic traits and yield components of wheat genotypes

Trait	Genotype	Test Genotype	Check Variety	Test Genotype vs. Check	CV (%)
Days to heading	52.42 ns	49.92 ns	41.74 ns	115.05 ns	8.66
Days to flowering	23.54 ns	20.55 ns	41.74 ns	22.43 ns	5.05
Days to maturity	15.98 ns	15.03 ns	22.57 ns	12.33 ns	3.28
Grain-filling duration	28.00 ns	14.81 ns	96.44 ns	68.01 ns	15.16
Plant height	45.77 ns	35.36 ns	110.32*	37.58 ns	9.43
Number of tillers	1.08 ns	1.16 ns	0.68 ns	0.67 ns	15.08
Productive tiller number	0.70 ns	0.73 ns	0.70 ns	0.08 ns	16.54
Spike length	4.26 ns	4.25 ns	5.35 ns	0.09 ns	23.39
100-grain weight	0.17**	0.10**	0.56**	0.12**	0.70

Note: ns = not significantly different; * = significant at the 5% probability level; ** = highly significant at the 1% probability level.

The analysis of variance presented in Table 1 showed that most agronomic and phenological traits did not differ significantly among the evaluated M2 wheat genotypes. Highly significant differences ($p < 0.01$) were detected only for 100-grain weight across all evaluated sources of variation, whereas plant height differed significantly ($p < 0.05$) only among the check varieties. In contrast, days to heading, days to flowering, days to maturity, grain-filling duration, number of tillers, productive tiller number, and spike length showed no significant differences among the tested genotypes. These results indicate that, under the conditions of this experiment, 100-grain weight was the trait that most clearly differentiated the M2 mutant population.

The pronounced variation in 100-grain weight is consistent with previous evidence that induced mutagenesis can generate substantial phenotypic variability in grain-related traits of wheat. Gamma irradiation produces random genetic changes, and the magnitude of the resulting phenotypic responses depends on the genotype, irradiation dose, and trait evaluated (Kiani et al., 2022). Rana et al. (2024), in a multi-year study of bread wheat exposed to gamma rays and ethyl methanesulfonate, also reported substantial induced variability in agronomic traits and demonstrated that mutagenic treatments can broaden the genetic base available for selection.

More recent evidence further supports the responsiveness of grain-related traits to gamma irradiation. Hong et al. (2025) reported considerable variation in agronomic performance, including seed-weight-related characteristics, among gamma-ray-derived wheat mutants, demonstrating that induced mutations can generate potentially useful variation for wheat improvement. Similarly, advanced gamma-ray-induced wheat mutant populations have shown substantial variation in plant height, spike characteristics, thousand-grain weight, and grain yield, confirming that induced mutagenesis can provide valuable material for subsequent breeding and selection.

The CV values provide additional information on the relative experimental variability of the evaluated traits. The lowest CV was observed for 100-grain weight (0.70%), followed by days to maturity (3.28%) and days to flowering (5.05%), whereas spike length (23.39%), productive tiller number (16.54%), grain-filling duration (15.16%), and number of tillers (15.08%) showed considerably higher CV values. A low CV indicates relatively small residual variation in relation to the trait mean and therefore suggests greater experimental precision for 100-grain weight under the present conditions. However, the low CV should not by itself be interpreted as evidence that 100-grain weight is predominantly genetically controlled or has high heritability. Such conclusions require direct estimates of genetic and environmental variance, heritability, or stability across environments. The interpretation of CV as a direct measure of biological stability can be misleading because CV depends on both the variance and the magnitude of the trait mean.

Conversely, the relatively high CV values for spike length and productive tiller number suggest greater residual or microenvironmental variability for these characters under the experimental conditions. Agronomic field and pot experiments can be affected by spatial and microenvironmental heterogeneity, which may reduce the precision with which treatment or genotype effects are detected. In the present study, this source of variation may be particularly relevant because the M2 materials represent an early segregating generation and the test genotypes were evaluated using an augmented design rather than complete replication. Thus, both biological segregation and experimental variation may have contributed to the relatively high CV values observed for several traits.

The lack of significant differences for most traits should therefore not be interpreted as evidence that gamma irradiation had no biological effect. M2 populations are genetically heterogeneous, and radiation-induced mutations may segregate among individual plants, producing trait responses that are not sufficiently uniform to generate significant population-level differences. Rana et al. (2024) demonstrated that early-generation mutant wheat populations can display substantial within- and among-population variability, while later selection is required to identify stable and heritable mutant phenotypes. Accordingly, the significant response observed for 100-grain weight in the present study provides a useful initial basis for identifying promising M2 genotypes, but confirmation in the M3 and subsequent generations is necessary before this trait can be considered a reliable selection criterion for tropical wheat improvement.

Performance of Vegetative Traits

The vegetative traits evaluated in this study comprised plant height, number of tillers, and productive tiller number. Individual comparisons between the M2 genotypes and the check varieties based on the 5% critical difference (CD) test are presented in Table 2. The results provide an overview of the variation in vegetative performance among the gamma-ray-induced M2 genotypes and allow the identification of genotypes that differed significantly from the check varieties.

Table 2. Performance of vegetative traits among wheat genotypes

Genotype	Plant Height	Number of Tillers	Productive Tillers
GR 1-1	57.07	5.93	4.77
GR 1-2	59.41	6.54	4.39
GR 1-4	49.58	5.26	4.15
GR 2-0	48.75	6.43	5.66
GR 2-1	49.67	5.77	4.07
GR 2-2	52.43	5.58	4.76
GR 2-3	48.95	5.95	5.31
GR 4-0	59.13	7.89	5.96
GR 4-1	58.25	6.55	5.60
GR 4-2	48.22	6.59	5.11
GR 4-3	52.92	6.84	5.90
GR 6-1	68.08+cde	5.68	4.75
GR 6-2	65.42+c	9.64+abcd	7.49+cde
GR 7-1	48.79	5.42	4.41
GR 7-2	45.29	4.72	3.80
GR 7-3	47.05	8.19	6.39
GR 7-4	47.61	5.88	5.00
GR 8-1	51.42	6.05	4.78
GR 8-2	48.23	5.27	4.20
N-1	52.05	5.81	4.63
N-2	50.73	6.50	4.85
N-3	51.77	5.99	5.46
SL 1	48.50	5.50	4.74
SL 3	46.94	4.68	3.81
SL 4	48.61	6.56	4.37
Guri 1	55.40	6.50	5.62
Guri 6	64.04	6.41	5.52
Guri 7	48.24	5.63	4.79
Guri 8	50.84	5.54	4.55

Genotype	Plant Height	Number of Tillers	Productive Tillers
Nias	52.82	5.61	4.81
CD (5%)	14.59	2.69	2.41

Note: (+) indicates significantly higher performance than the corresponding check variety: (a) Guri 1, (b) Guri 6, (c) Guri 7, (d) Guri 8, and (e) Nias based on the 5% critical difference (CD) test. Values without symbols indicate no significant difference compared with all check varieties.

As shown in Table 2, the M2 wheat genotypes exhibited considerable variation in vegetative traits, particularly in plant height and tillering capacity. Plant height ranged from 45.29 cm in GR 7-2 to 68.08 cm in GR 6-1. Genotypes GR 6-1 (68.08 cm) and GR 6-2 (65.42 cm) showed significantly higher plant height than several check varieties, whereas GR 7-2 exhibited the lowest plant height. In terms of tillering traits, GR 6-2 showed the highest number of tillers (9.64) and productive tillers (7.49), both significantly higher than the check varieties. These results indicate that gamma-ray-induced mutagenesis generated useful phenotypic variation in vegetative characteristics, particularly those associated with plant architecture and tillering potential.

The variation in plant height among mutant genotypes is consistent with previous studies demonstrating that gamma irradiation can generate diverse morphological and agronomic responses in wheat. Gamma rays induce random genetic alterations that may affect multiple developmental processes, resulting in variation in plant architecture depending on genotype background, irradiation conditions, and trait-specific sensitivity (Ahumada-Flores et al., 2021; Kiani et al., 2022). Genome-wide analysis of gamma-ray-induced wheat mutants has also confirmed that irradiation can generate diverse genomic changes, providing new genetic resources for wheat improvement (Li et al., 2022).

Although the molecular basis of plant height variation was not directly evaluated in this study, differences among genotypes may be associated with genetic changes affecting pathways involved in stem elongation and plant architecture. Plant height in wheat is largely regulated by genes involved in gibberellin signaling, particularly the Reduced height (Rht) loci, which influence internode elongation, plant stature, and lodging resistance (Peng et al., 2021). Previous studies on gamma-ray-induced wheat mutants have demonstrated that mutations affecting dwarfing-related loci can modify plant height while maintaining favorable yield-related traits (Wang et al., 2023). Therefore, the contrasting plant height observed among GR 6-1, GR 6-2, and GR 7-2 likely represents induced genetic variation, although confirmation through molecular analysis would be required to identify the specific genes involved.

Although taller genotypes such as GR 6-1 and GR 6-2 showed vigorous vegetative growth, greater plant height does not necessarily indicate superior agronomic performance. Excessive plant height may increase susceptibility to lodging, whereas optimized plant stature can improve standability and maintain yield potential. Previous studies have demonstrated that reduced-height alleles can improve lodging resistance without causing yield penalties when appropriate genetic backgrounds are maintained (Voss-Fels et al., 2019; Wang et al., 2023). Therefore, the shorter stature observed in GR 7-2 may represent useful breeding variation, but its value should be evaluated together with lodging resistance and yield-related traits.

The superior tillering performance of GR 6-2 suggests that this genotype may have potential for further selection. However, tiller number and productive tiller number are complex quantitative traits influenced by multiple genetic factors, hormonal regulation, and environmental conditions. Tillering in wheat is regulated by complex genetic networks involving genes associated with axillary bud initiation and hormonal pathways, including auxin, cytokinin, and strigolactone signaling (Li et al., 2021). Previous studies have also shown that gamma irradiation can modify tillering capacity and other yield-related traits, although the magnitude of response varies among genotypes and environments (Shabani et al., 2022). Therefore, the high tillering capacity of GR 6-2 should be further validated under replicated field conditions and evaluated together with spike fertility, grain number, and grain yield.

The interpretation of vegetative variation in this study should consider that the evaluated materials represent the M2 generation, in which genetic segregation remains active. Early-generation mutant populations commonly contain a broad range of phenotypic variation, and favorable traits require subsequent selection and stabilization before being incorporated into breeding programs. Mutation breeding studies emphasize that the M2 generation is primarily used for identifying putative mutant phenotypes, whereas confirmation of trait stability generally requires evaluation across later generations (Parry et al., 2009). Similar observations were reported by Rana et al. (2024), who demonstrated substantial induced variability in gamma-ray-derived wheat populations and highlighted the importance of continuous selection for identifying stable and heritable mutant traits.

Overall, the vegetative variation identified in this study, particularly the high tillering capacity of GR 6-2 and the contrasting plant architecture among mutant genotypes, provides valuable preliminary material for tropical wheat improvement. However, these traits should be considered promising selection indicators rather than confirmed breeding advantages until their stability, heritability, lodging response, and contribution to yield performance are validated in the M3 generation and subsequent multi-environment evaluations.

Performance of Growth Phase Traits

The growth phase traits evaluated in this study included days to heading, days to flowering, days to maturity, and grain-filling duration. The individual comparison of the gamma-ray-induced M2 genotypes with the check varieties based on the 5% critical difference (CD) test is presented in Table 3. This analysis was conducted to identify mutant genotypes that exhibited distinct phenological responses compared with the reference varieties.

Table 3. Performance of growth phase traits among wheat genotypes

Genotype	Days to Heading	Days to Flowering	Days to Maturity	Grain-filling Duration
GR 1-1	55.94	65.73	104.07	38.33
GR 1-2	55.94	67.33	102.67	35.33
GR 1-4	61.94	73.73	105.07	31.33
GR 2-0	65.94	71.33	106.67	35.33
GR 2-1	70.14	73.93	110.27	36.33
GR 2-2	55.94	71.73	101.07	29.33
GR 2-3	66.14	68.93	106.27	37.33
GR 4-0	72.14	76.93+e	110.27	33.33
GR 4-1	69.94	73.33	107.67	34.33
GR 4-2	69.94	73.33	105.67	32.33
GR 4-3	74.14+ae	76.93+e	111.27	34.33
GR 6-1	66.94	77.73+e	109.07	31.33
GR 6-2	59.14	64.33	107.67	43.33+d
GR 7-1	59.14	76.93+e	104.27	27.33

Genotype	Days to Heading	Days to Flowering	Days to Maturity	Grain-filling Duration
GR 7-2	77.14+ae	80.93+ace	107.27	26.33
GR 7-3	69.94	73.33	105.67	32.33
GR 7-4	74.94+ae	73.33	108.67	35.33
GR 8-1	64.94	71.73	106.07	34.33
GR 8-2	71.94	77.73+e	105.07	27.33
N-1	51.94	61.33	94.67	33.33
N-2	63.94	65.73	98.07	32.33
N-3	63.94	69.73	98.07	28.33
SL 1	72.14	70.93	101.27	30.33
SL 3	68.14	73.93	104.27	30.33
SL 4	56.94	73.33	105.67	32.33
Guri 1	57.67	68.67	105.67	37.00
Guri 6	64.94	72.67	109.33	36.67
Guri 7	64.00	70.00	105.00	35.00
Guri 8	65.00	76.00	102.67	26.67
Nias	57.67	66.33	108.67	42.33
CD (5%)	16.236	10.57	10.09	14.96

Note: (+a), (+b), (+c), (+d), and (+e) indicate significantly higher values than Guri 1, Guri 6, Guri 7, Guri 8, and Nias, respectively, based on the 5% critical difference (CD) test. HST = days after planting. Values without symbols indicate no significant difference compared with all check varieties.

As presented in Table 3, the gamma-ray-induced M2 wheat genotypes showed variation in phenological traits, including days to heading, days to flowering, days to maturity, and grain-filling duration. Days to heading ranged from 51.94 days (N-1) to 77.14 days (GR 7-2). Genotypes GR 4-3, GR 7-2, and GR 7-4 showed significantly delayed heading compared with Guri 1 and/or Nias, while several genotypes, including GR 4-0, GR 4-3, GR 6-1, GR 7-1, GR 7-2, and GR 8-2, exhibited delayed flowering compared with specific check varieties. In contrast, days to maturity did not differ significantly between mutant genotypes and check varieties. Grain-filling duration varied among genotypes, with GR 6-2 showing the longest duration (43.33 days), significantly longer than Guri 8, whereas GR 7-2 showed the shortest duration (26.33 days).

The variation in heading, flowering, and grain-filling duration indicates that gamma irradiation generated phenotypic diversity in developmental traits among the M2 population. Similar variation in phenological responses has been reported in gamma-ray-induced wheat mutants, although the magnitude of variation depends on genotype background and environmental conditions (Ahumada-Flores et al., 2021; Kiani et al., 2022). Gamma-induced mutations may alter developmental processes associated with reproductive timing; however, the specific genetic mechanisms underlying these responses require further molecular validation (Li et al., 2022).

The absence of significant differences in maturity duration suggests that this trait was relatively stable under the experimental conditions. This pattern is consistent with previous studies showing that phenological traits in early mutant generations may remain variable due to ongoing genetic segregation, while environmental conditions can influence their expression (Shabani et al., 2022; Rana et al., 2024). The longer grain-filling duration observed in GR 6-2 may provide potential advantages for assimilate accumulation; however, extended grain filling does not necessarily translate into higher yield because grain productivity depends on the interaction among grain number, grain weight, and environmental conditions (Vicentin et al., 2024).

Because these materials represent the M2 generation, the observed phenological differences should be considered preliminary indicators of induced variation rather than stable breeding advantages. Further evaluation in the M3 generation and across contrasting environments is required to confirm trait stability and determine their breeding potential (Mba, 2013; Parry et al., 2009).

Performance of Yield-Related Traits

Spike length and 100-grain weight were evaluated as important yield-related traits in this study. As presented in Table 4, spike length ranged from 4.24 cm (GR 7-2) to 10.87 cm (GR 4-0), with GR 7-2 also showing reduced performance in several vegetative traits, suggesting a stronger phenotypic response to mutagenesis. For 100-grain weight, most mutant genotypes exceeded the values of the check varieties Guri 8 and Nias, although Guri 6 showed the highest overall value (5.11 g). Among the mutant genotypes, GR 4-0 exhibited the highest 100-grain weight (4.96 g) and was significantly higher than four of the five check varieties. These results indicate that gamma-ray-induced mutagenesis generated substantial variation in 100-grain weight, highlighting its potential as a key selection trait in wheat improvement programs.

Table 4. Performance of yield-related traits among wheat genotypes

Genotype	Spike Length	100-Grain Weight
GR 1-1	7.65	4.20+de
GR 1-2	9.35	4.59+cde
GR 1-4	6.97	3.98
GR 2-0	9.32	4.63+acde
GR 2-1	7.56	4.04
GR 2-2	7.69	4.19+d
GR 2-3	7.86	4.47+cde
GR 4-0	10.87	4.96+acde
GR 4-1	9.52	4.40+de
GR 4-2	6.35	4.88+acde
GR 4-3	8.29	4.69+acde
GR 6-1	7.73	4.30+de
GR 6-2	10.18	4.19+d
GR 7-1	6.76	3.97
GR 7-2	4.24	3.94
GR 7-3	7.23	4.84+acde
GR 7-4	6.52	4.72+acde
GR 8-1	7.70	4.09
GR 8-2	7.05	3.86
N-1	8.88	4.05
N-2	7.78	4.21+de
N-3	8.49	4.26+de
SL 1	6.87	4.32+de
SL 3	7.44	3.96
SL 4	8.20	4.14+d
Guri 1	8.79	4.53
Guri 6	9.95	5.11
Guri 7	6.80	4.35
Guri 8	7.07	4.03

Genotype	Spike Length	100-Grain Weight
Nias	7.36	4.11
CD (5%)	5.42	0.09

Note: (+a), (+b), (+c), (+d), and (+e) indicate significantly higher values than Guri 1, Guri 6, Guri 7, Guri 8, and Nias, respectively, based on the 5% critical difference (CD) test. Values without symbols indicate no significant difference compared with all check varieties.

The absence of significant differences in spike length agrees with Shabani et al. (2022), suggesting that this trait is strongly influenced by environmental variation. The high CV for spike length (23.39%) further indicates substantial microenvironmental effects. In contrast, the low CV for 100-grain weight (0.70%) suggests higher experimental precision and supports its potential as a reliable selection trait, consistent with reports of high heritability for grain weight-related traits in gamma-irradiated wheat (Indriatama et al., 2016) and the effectiveness of mutagenesis in expanding grain-weight variation (Hussain et al., 2021).

The genotypes GR 2-0, GR 4-0, GR 4-2, GR 4-3, GR 7-3, and GR 7-4 showed higher 100-grain weight than most check varieties and represent promising materials for further selection. However, their yield superiority remains to be confirmed because grain yield per plant was not directly measured, and grain weight may involve trade-offs with grain number (Vicentin et al., 2024). Figure 1 further supports the selection potential of GR 4-0, GR 4-2, GR 7-3, and GR 7-4, which consistently showed higher 100-grain weight values than most check varieties.

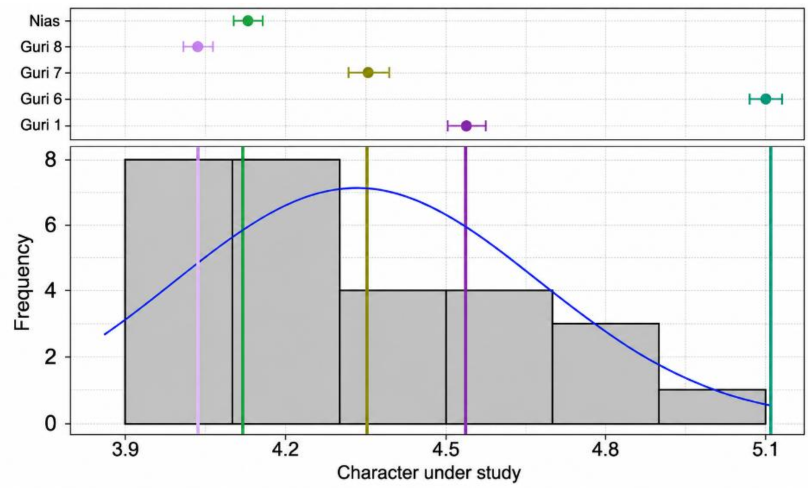

Figure 1. Comparison of 100-grain weight (g) among gamma-ray-induced M2 wheat genotypes and check varieties

The distribution of 100-grain weight among the tested genotypes and check varieties confirms that several mutant genotypes possess favorable grain weight characteristics. Nevertheless, further evaluation is required before these genotypes can be considered superior breeding materials because grain weight represents only one component of yield potential.

This study has several limitations that should be considered when interpreting the results and designing subsequent research. First, the evaluation was conducted at only one location and during a single growing season; therefore, genotype × environment interactions across different locations and seasons remain unknown. Second, the use of polybags as the growth medium differs from open-field conditions, and therefore agronomic performance under field conditions may differ from the results obtained in this experiment.

In addition, the tested genotypes did not receive complete replication within each block, unlike the check varieties. Consequently, experimental error estimation for the tested genotypes was indirectly assessed through the augmented design approach. Therefore, future studies should include multi-location and multi-season evaluations, field-based trials, and the inclusion of original parental genotypes as comparative controls during subsequent M3 generation selection.

CONCLUSION

Gamma-ray-induced mutation in wheat generated observable phenotypic variation among M2 genotypes, particularly in the 100-grain weight trait, which showed the highest consistency within the evaluated population. The genotypes GR 2-0, GR 4-0, GR 4-2, GR 4-3, GR 7-3, and GR 7-4 exhibited higher 100-grain weight compared with most check varieties and represent promising candidates for further evaluation and selection in the M3 generation. In addition, genotype GR 6-2, which showed a longer grain-filling duration, may serve as a potential genetic resource for improving phenological traits. Overall, this study contributes new mutant genetic materials that can support the development of tropical wheat breeding programs in Indonesia, although further validation across environments and subsequent generations is required to confirm the stability and agronomic potential of the selected genotypes.

RECOMMENDATION

Based on the findings of this study, the mutant genotypes GR 2-0, GR 4-0, GR 4-2, GR 4-3, GR 7-3, and GR 7-4 are recommended for further evaluation in the M3 generation, particularly based on their superior performance in 100-grain weight. Multi-location adaptation trials under various tropical highland environments are necessary to assess the stability of their performance and to determine genotype × environment interactions. Furthermore, future studies should consider using more controlled growth conditions, such as greenhouse experiments, to minimize the effects of microclimatic variation on the stability of phenotypic expression.

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