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Effects of gamma-ray irradiation on agronomic traits and determination of the optimal mutagenic dose (LD_{50}) in the local rice cultivar Mayas (*Oryza sativa* L.)

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Abstract. This study aimed to determine the effects of gamma irradiation on the agronomic characteristics of the local rice cultivar Mayas and to identify the optimal radiation dose that may produce superior mutant lines. Mayas rice seeds were irradiated with gamma rays at doses of 0, 50, 100, 150, 200, 250, and 300 Gy. Gamma irradiation significantly affected seed germination and mortality rates. The highest germination percentage was observed at a dose of 50 Gy (88%), whereas higher doses (250–300 Gy) resulted in reduced germination and increased seed mortality up to 50%. The LD_{50} value was estimated at approximately 295 Gy, indicating that this dose caused 50% seed mortality. Plant height decreased with increasing radiation doses, with the lowest average height observed at 100 Gy (73 cm) compared to the control (125 cm). Conversely, the number of tillers and productive tillers increased under low to moderate radiation doses. The 50 Gy treatment produced the highest number of tillers per clump (50 tillers) compared to the control (13 tillers) and the greatest number of productive tillers (25 tillers) compared to the control (4 tillers), demonstrating the stimulatory effect of gamma irradiation on vegetative growth and yield potential.

1 Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop in Indonesia, serving as the primary staple food and a commodity of high strategic and economic value. As the main food source for the majority of Indonesia's population, rice plays a central role in national food security, thus requiring continuous efforts to enhance its productivity and adaptability. Indonesia is home to a rich diversity of local rice varieties that are distributed across various agroecological zones. These local cultivars represent valuable genetic resources, providing a

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reservoir of alleles that govern key agronomic and adaptive traits, including stress tolerance, yield stability, and grain quality.

The extensive genetic diversity among Indonesia's local rice germplasm offers tremendous potential for breeding new superior varieties [1, 2]. Local cultivars are often characterized by resilience to biotic and abiotic stresses, as well as distinct grain qualities and taste profiles favored by local consumers [3, 4]. However, despite their adaptive advantages, most local varieties exhibit limitations such as low yield potential, long growth duration, and susceptibility to lodging [1]. These drawbacks constrain their widespread utilization in modern rice production systems, underscoring the need for genetic improvement while retaining their adaptive and quality traits.

East Kalimantan is among the Indonesian provinces particularly rich in local rice germplasm, with several unique and promising cultivars adapted to the humid tropical environment [1, 3]. One of the most well-known is Mayas rice, a local cultivar widely grown and highly appreciated for its excellent taste and tolerance to environmental stress [4]. Despite its favorable traits, Mayas rice, like many traditional varieties, suffers from long maturation time and low yield potential, limiting its agricultural competitiveness. Mutation breeding has emerged as a promising strategy to overcome these limitations and generate improved genotypes while maintaining the desirable characteristics of local germplasm.

Mutation breeding involves the deliberate induction of genetic changes, either at the nucleotide (point mutation) or chromosomal level, using physical or chemical mutagenic agents [5]. Among physical mutagens, gamma irradiation is one of the most widely used and effective tools for inducing heritable genetic variation. Gamma rays, a form of high-energy electromagnetic (ionizing) radiation, penetrate plant tissues and interact with DNA molecules, causing molecular alterations that can lead to phenotypic variation. Controlled exposure to gamma radiation can generate beneficial mutations that improve agronomic performance, such as yield potential, growth vigor, and stress tolerance, while maintaining genetic integrity [5, 6].

Numerous studies have demonstrated the successful application of gamma irradiation in improving local rice varieties in Indonesia. For example, irradiation of the local cultivar Ase Banda from South Sulawesi at doses of 200 and 300 Gray produced shorter plants with increased tillering and earlier flowering compared to the non-irradiated control [7]. Similarly, irradiation of Mentik Susu and Umbul cultivars identified three promising mutants, all derived from Mentik Susu. Two mutants exposed to 100 Gy produced 81 and 75 tillers, with 246 and 183 filled grains per panicle, and matured earlier (114 and 112 days), while one mutant irradiated at 300 Gy exhibited 71 tillers, 164 filled grains, and a maturity duration of 130 days [8]. These results highlight the potential of gamma ray-induced mutation to generate beneficial genetic variability for use in rice improvement programs.

The present study focuses on the induction of mutations in Mayas rice through gamma irradiation at doses of 50, 100, 150, 200, 250, and 300 Gray. The objective is to evaluate the effects of different irradiation doses on key agronomic traits, including growth performance, flowering time, and yield components, and to determine the optimal dose capable of inducing beneficial mutations while minimizing deleterious effects on plant viability. In particular, this research seeks to identify potential mutant lines exhibiting superior agronomic performance, such as shorter growth duration and higher yield potential. The findings of this study are expected to provide valuable insights into the application of gamma irradiation in mutation breeding and contribute to the development of improved Mayas rice cultivars with enhanced productivity and adaptability to the agroecological conditions of East Kalimantan and other tropical regions.

2 Materials and methods

2.1 Plant materials

The plant material used in this study was the local rice (*Oryza sativa* L.) cultivar Mayas, originally collected from Sungai Payang, Loa Kulu Subdistrict, Kutai Kartanegara Regency, East Kalimantan. Gamma-irradiated seeds were used as the experimental material. Mutation induction was performed using physical mutagenesis through gamma-ray irradiation from a Cobalt-60 (^{60}Co) source. The irradiation was conducted using the acute (single-dose) exposure technique at doses of 0, 50, 100, 150, 200, 250, and 300 Gray (Gy). For each dose, 100 grams of Mayas rice seeds were irradiated at the Indonesian Nuclear Technology Polytechnic, Yogyakarta.

2.2 Field experiment

The field experiment was conducted in Karang Tunggal Village, Tenggarong Seberang Subdistrict, Kutai Kartanegara Regency, East Kalimantan, Indonesia. This research was conducted from May to December 2023. The experiment was conducted using a single-plant design, where each irradiated individual (M_1 generation) and its non-irradiated control (wild type) were observed individually without replication. The experiment followed an augmented randomized design. Seeds of Mayas rice were subjected to seven levels of gamma irradiation: 0 Gy, 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, and 300 Gy. For each treatment, 100 seeds were planted in rows, with an inter-row spacing of 50 cm and an intra-row spacing of 30 cm.

The experimental field was plowed twice using a hand tractor until the soil reached a muddy consistency. The surface was then leveled and left at a semi-saturated moisture level while waiting for seedling readiness. Dolomite and farmyard manure were applied after the first plowing to improve soil conditions before transplanting.

Seeds were first visually inspected to remove damaged or empty grains. One hundred viable seeds per treatment were selected and soaked in water for approximately 24 hours to separate filled from unfilled grains. Germination was performed in 500 mL trays filled with soil, and seeds were evenly spread across the surface. Watering was done daily to maintain adequate moisture. Seedlings were grown for 25 days before being transplanted into the experimental field. Transplanting was carried out two weeks after liming. Seedlings aged 25 days were transplanted into puddled soil, with one seedling per planting hole at a depth of 2 cm, following a spacing of 50 × 30 cm. Each treatment row was labeled accordingly. Fertilization involved the application of NPK and urea. A total of 300 kg ha^{-1} of NPK and 100 kg ha^{-1} of urea were applied in three splits: The first (20 days after transplanting, DAT) and second (40 DAT) applications each consisted of 100 kg ha^{-1} NPK and 50 kg ha^{-1} urea. The third application (80 DAT) consisted of 100 kg ha^{-1} NPK. Fertilizer amounts were adjusted proportionally to the experimental plot size.

Routine maintenance included weed control and pest management. Weeding was performed manually every two weeks until the flowering stage. Pest and disease control were conducted as needed using appropriate insecticides. Harvesting was performed manually using a sickle when approximately 80% of the grains had reached physiological maturity, characterized by yellow panicles and yellowing leaves.

2.3 Data collection

The germination percentage was calculated to.... The lethal dose (LD_{50}) is the gamma irradiation dose that causes 50% mortality among treated seeds. Determining LD_{50} is essential for identifying the optimal dose that induces a high mutation frequency with minimal lethality. Plant growth parameters, namely: Plant Height at Flowering, Number of Tillers per Hill, Number of Productive Tillers, Days to Flowering were observed for this study.

2.4 Data analysis

Agronomic data were analyzed descriptively by calculating the minimum, maximum, and standard deviation values to assess the variability of plant characteristics. The results were presented in tabular form and compared with those of the non-irradiated control (wild type) to identify differences and similarities.

3 Results and discussion

3.1 Effects of gamma irradiation on germination percentage

The germination test results (Table 1, Figure 1) showed that gamma irradiation significantly affected the germination performance of Mayas rice seeds. The highest germination percentage was recorded at 50 Gy (88%), which exceeded the control (76%). However, as the radiation dose increased beyond this level, a marked decline in normal seed germination was observed, only 37% of seeds germinated normally at 250 Gy, accompanied by a corresponding increase in non-viable (dead) seeds, reaching 55.5%. This dose-dependent response demonstrates the dual, hormetic nature of gamma irradiation: low doses can enhance metabolic activity and early embryonic growth, while high doses impose physiological stress that inhibits germination.

At low doses, gamma irradiation may activate hydrolytic enzymes and stimulate DNA repair mechanisms, which in turn promote water uptake, embryo growth, and radicle emergence. This stimulatory effect results from the temporary enhancement of energy metabolism and enzyme activation that support early vegetative growth. Conversely, higher doses of ionizing radiation generate reactive oxygen species (ROS) and cause chromosomal aberrations that damage meristematic tissues and disrupt cell division, thereby reducing germination and seedling vigor. The sharp decline in germination observed at 250–300 Gy is consistent with deterministic radiation effects, where excessive energy exposure surpasses the cellular repair threshold, resulting in irreversible cell death [6, 9].

These findings corroborate previous research on other rice cultivars, including *Oryza sativa* cv. Situ Bagendit and Mentik Susu, which exhibited similar reductions in germination rate at doses above 200 Gy [8]. The results from the present study therefore confirm that gamma irradiation induces a hormetic response in Mayas rice seeds, moderate exposure (50 Gy) enhances germination through physiological stimulation, while excessive irradiation causes detrimental, dose-dependent cellular damage. This understanding provides an empirical basis for selecting optimal irradiation levels in mutation breeding programs aimed at generating beneficial variation without compromising seed viability.

Table 1. Effect of gamma irradiation dose on germination percentage of local rice cultivar Mayas (*Oryza sativa* L.).

Irradiation Dose (Gy)	Percentage of Germination (%)		
	Normal	Abnormal	Non-viable seeds
0	76	13,5	10,5
50	88	4.5	7.5
100	72	6	22
150	70	6.5	23.5
200	67	10	23
250	37	7.5	55,5
300	42	6.5	51,5

3.2 Determination of lethal dose 50 (LD_{50})

The determination of the LD_{50} value, which represents the radiation dose causing 50% seed mortality, provided essential insight into the radiosensitivity of Mayas rice. Based on the regression between gamma-ray dose and mortality rate (Figure 1), mortality remained relatively low (7–23%) at doses up to 200 Gy but increased sharply at 250 Gy and 300 Gy, both approaching 50%. From these data, the LD_{50} for Mayas seeds was estimated to be between 240 and 260 Gy, indicating a moderate level of radiosensitivity compared to other local *Oryza sativa* cultivars.

Determining the LD_{50} is a crucial step in mutation breeding, as it defines the dose range that effectively induces mutations while maintaining sufficient seed viability for subsequent generation development. Exceeding this threshold often results in severe physiological damage, such as high mortality, sterility, and morphological abnormalities [9]. In the present study, mortality levels above 250 Gy reflect the onset of lethal damage to meristematic tissues, likely caused by double-strand DNA breaks and chromosomal fragmentation.

Comparable findings have been reported for other local rice varieties, such as black rice irradiated at 0–500 Gy, which exhibited a similar dose–response pattern: germination decreased progressively with increasing dose, and complete lethality occurred at 500 Gy, yielding an LD_{50} range of approximately 300–340 Gy [10]. The slightly lower LD_{50} observed in Mayas rice suggests greater radiosensitivity, which may be linked to genotype-specific differences in DNA repair efficiency and physiological resilience.

Overall, the LD_{50} range of 240–260 Gy identified in this study provides an appropriate reference point for optimizing gamma irradiation treatments in Mayas rice mutation breeding. Selecting irradiation doses near, but below, the LD_{50} ensures an effective balance between mutation frequency and plant survival, thereby maximizing the likelihood of generating useful phenotypic variability without causing irreversible physiological damage.

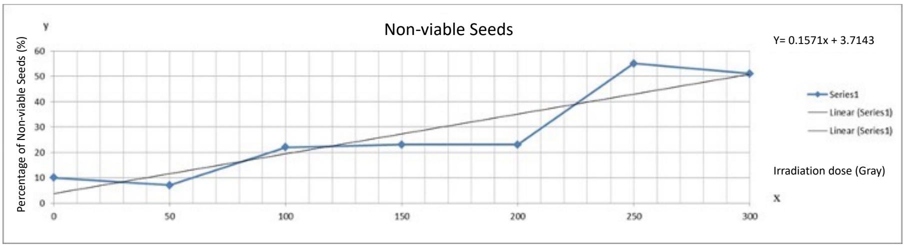

Fig. 1. Regression between gamma-ray dose and mortality rate.

3.3 Plant height

Gamma irradiation had a significant effect on plant height in Mayas rice (Table 2). The tallest plants were observed at a dose of 50 Gy, with an average height of 137 cm, whereas the shortest plants occurred at 100 Gy (102 cm). The control group had a mean height of 125 cm. The stimulatory response at 50 Gy likely results from increased metabolic activity and hormonal regulation, particularly through auxins and gibberellins that promote stem elongation. However, at higher doses, accumulated DNA damage and oxidative stress inhibit mitotic division and cell expansion, thereby reducing plant height.

From an agronomic perspective, an ideal rice height of 90–110 cm is generally preferred because shorter plants are more resistant to lodging, an important trait for maintaining yield stability in modern cultivars. Therefore, the reduction in plant height observed at moderate irradiation doses may be advantageous for breeding semi-dwarf and lodging-resistant Mayas lines. Similar results were reported previously, founding that gamma irradiation at 200 Gy in *Oryza sativa* cv. Ciherang reduced plant height while maintaining good agronomic performance [11]. Thus, controlled application of gamma irradiation represents a promising approach to developing semi-dwarf, high-yielding, and lodging-tolerant rice varieties suited to humid tropical environments.

Table 2. Effect of gamma irradiation dose on plant height of local rice cultivar Mayas (*Oryza sativa* L.).

Plant height	Gamma Ray Irradiation Dose (Gray)						
	control	50	100	150	200	250	300
Minimum value	97	102	73	105	105	110	78
Maximum value	150	158	137	124	131	133	141
Mean	125	137	102	115	116	119	111
Standard deviation	14.2	10.7	23	13.4	7.4	12.1	16.3

3.4 Number of tillers per hill

The number of tillers per hill was strongly influenced by the gamma-ray dose (Table 3). The most vigorous tiller production occurred at 50 Gy and 300 Gy, both yielding up to 50 tillers per hill, whereas the control plants produced only 13 tillers on average. This result indicates that specific irradiation doses can effectively stimulate axillary meristem activity and promote tiller initiation. A secondary increase in tillering at 300 Gy suggests that genetic mutations induced at high radiation levels may trigger atypical but occasionally beneficial growth responses. However, doses exceeding 250 Gy caused irregular and reduced tiller formation, likely due to radiation-induced damage in tiller primordia and disruption of hormonal regulation responsible for shoot branching.

The overall response pattern, an initial increase in tiller number at low to moderate doses followed by a decline at higher doses, is characteristic of a hormetic effect, where sublethal radiation stimulates growth and development, while excessive exposure becomes inhibitory. Similar findings were reported in *Oryza sativa* cv. Mentik Susu, which produced the highest tiller number (81 tillers) at 100 Gy before a sharp decline at higher doses, emphasizing that the optimal dose varies by genotype [8]. Moderate doses of gamma radiation are known to enhance cytokinin-mediated cell division in axillary meristems, leading to more active tiller initiation.

Table 3. Effect of gamma irradiation dose on number of tillers per clump of local rice cultivar Mayas (*Oryza sativa* L.).

Number of tillers per clump	**Gamma Ray Irradiation Dose (Gray)**						
	Control	**50**	**100**	**150**	**200**	**250**	**300**
Min value	3	5	6	7	4	3	3
Max value	30	50	32	7	33	27	50
Mean	13	28	14	7	20	16	11
Standard deviation	9.6	11.2	9.8	0.0	10.1	12.1	13.3

3.5 Number of productive tillers

The trend in productive tiller formation closely followed that of total tiller number (Table 4). The control plants produced an average of four productive tillers, whereas those irradiated at 50 Gy showed a substantial increase, producing up to 25 productive tillers per hill. In contrast, higher irradiation doses (≥200 Gy) markedly reduced the number of productive tillers, with only 1–5 observed at 300 Gy. This pattern indicates that moderate doses of gamma irradiation can enhance reproductive performance, while excessive doses exert a detrimental effect on tiller fertility and panicle formation.

Productive tillers are a major determinant of grain yield because each tiller typically bears a panicle that contributes directly to the final yield. The maximum productive tiller count observed at 50 Gy suggests that gamma irradiation at moderate intensity can induce favorable genetic or physiological changes, such as improved panicle initiation or enhanced nutrient allocation to reproductive organs. The minimum number of productive tillers occurred at 150 Gy, emphasizing that beyond an optimal range, irradiation disrupts reproductive organ development. The decline in productive tillers at high doses is likely due to chromosomal aberrations and cellular damage in reproductive tissues, leading to reduced panicle initiation, pollen sterility, or floret abortion. Such deleterious effects are commonly observed when radiation-induced mutagenesis exceeds the plant's physiological repair capacity. Conversely, the improvement at moderate doses supports the hypothesis that low-level irradiation can generate beneficial mutations that increase reproductive efficiency and yield potential [12].

Table 4. Effect of gamma irradiation dose on number of productive tillers per clump of local rice cultivar Mayas (*Oryza sativa* L.).

Number of productive tillers per clump	**Gamma Ray Irradiation Dose (Gray)**						
	Control	**50**	**100**	**150**	**200**	**250**	**300**
Min value	2	2	1	3	2	2	1
Max value	7	25	17	5	16	8	23
Mean	4	12	5	4	7	4	5
Standard Deviation	1.8	5.8	6.0	1.4	5.5	3.5	6.2

3.6 Days to flowering

Gamma irradiation also had a significant effect on the flowering time of Mayas rice (Table 5). The earliest flowering was observed at a dose of 200 Gy, occurring 61 days after transplanting, approximately 11 days earlier than the control plants, which flowered at 72 days. This reduction in flowering duration is agronomically valuable, particularly for breeding programs aimed at developing early-maturing varieties suited to areas with limited or unpredictable growing periods. Earliness allows crops to complete their life cycle before the onset of environmental stress, such as drought or flooding, thereby improving cropping efficiency and yield stability.

The observed reduction in flowering time indicates that gamma irradiation may have induced genetic or physiological changes that accelerate the floral transition. Gamma rays are known to alter meristematic cell division and can induce mutations in genes controlling photoperiod sensitivity and flowering pathways [13]. These mutations may disrupt normal photoperiodic control, leading to earlier initiation of panicle differentiation. The results of this study are consistent with previous reports on local upland rice cultivars such as *Oryza sativa* cv. Sileso, where irradiation at 100–400 Gy shortened the flowering period by 5–10 days [14].

Table 5. Effect of gamma irradiation dose on days to flowering of local rice cultivar Mayas (*Oryza sativa* L.).

Days to flowering (days)	**Gamma Ray Irradiation Dose (Gray)**						
	Control	**50**	**100**	**150**	**200**	**250**	**300**
Min Value	67	62	62	63	61	64	66
Max value	100	85	87	67	73	73	77
Mean	72.1	66.5	72.2	65	66.4	69.7	66
Standar Deviation	8.1	3.9	10.5	2.8	3.4	4.9	5.2

While variability was observed among treatments, the consistent trend toward earlier flowering at moderate doses, particularly at 200 Gy, underscores the potential of gamma irradiation as an effective tool to induce earliness in rice breeding. This trait is especially advantageous in rainfed and drought-prone agroecosystems, where early-maturing varieties can escape terminal water stress and enable multiple cropping cycles per year. Therefore, the acceleration of flowering time in Mayas rice following irradiation at 200 Gy provides a promising direction for developing locally adapted, short-duration cultivars that align with sustainable and efficient tropical rice production systems.

3.7 Superior agronomic traits of irradiated Mayas rice

The integrated analysis of agronomic traits (Table 6) revealed that specific doses of gamma irradiation produced mutant lines with distinctly improved characteristics in Mayas rice. The 50 Gy treatment yielded plants with balanced and desirable agronomic performance, including moderate height (102 cm), high tillering capacity (up to 50 tillers per hill), and an increased number of productive tillers (25), along with an earlier flowering time of 62 days. Similarly, the 200 Gy treatment produced lines that flowered earliest (61 days after transplanting) while maintaining satisfactory tillering capacity (33 tillers per hill). These results indicate that low to moderate doses of gamma rays (50–200 Gy) are particularly effective in generating beneficial mutations that enhance growth and reproductive performance without severely compromising plant vigor.

The phenotypic improvements observed in these treatments likely result from induced mutations affecting genes involved in hormonal regulation, growth processes, and reproductive development [12]. Gamma irradiation has been reported to induce a wide range of genetic variations, promoting greater phenotypic diversity within local germplasm. This genetic diversification provides opportunities to identify and select promising mutant lines with superior agronomic potential [15]. In this study, the variation across key traits demonstrates the capacity of gamma irradiation to modulate multiple physiological pathways simultaneously, resulting in plants that are shorter, more prolific, and earlier maturing than the control.

Across all observed parameters, plant height, number of tillers, number of productive tillers, and days to flowering, gamma irradiation induced clear phenotypic differentiation relative to the control (125 cm height, 13 tillers, 4 productive tillers, and 72.1 days to flowering). Irradiated plants exhibited several advantageous modifications: plant height decreased to a range of 73–110 cm at doses between 100 and 250 Gy, producing semi-dwarf plants with reduced lodging risk; tiller number increased substantially, especially at 50 Gy and 300 Gy (up to 50 tillers per hill), reflecting enhanced meristematic activity and possible genetic diversification; the number of productive tillers rose significantly at 50 Gy (up to 25 per hill), directly correlating with improved yield potential; and flowering time advanced to 61 days at 200 Gy, a trait advantageous in short-season or rainfed environments.

Overall, these findings suggest that 50 Gy and 200 Gy represent the most promising irradiation doses for improving Mayas rice. The 50 Gy dose primarily enhanced vegetative and reproductive vigor, yielding more tillers and productive panicles, whereas the 200 Gy dose effectively promoted early maturity without reducing growth performance. Together, these traits, semi-dwarf plant stature, high tillering capacity, and early flowering, constitute an optimal combination for improving productivity and cropping efficiency under the humid tropical conditions of East Kalimantan. Future work should focus on advancing the mutant lines derived from these treatments to the M_2 and M_3 generations, coupled with molecular characterization, to confirm trait stability and identify underlying genetic alterations responsible for the enhanced phenotypes.

Table 6. Superior mutant lines and key agronomic traits of Mayas rice (*Oryza sativa* L.) under different doses of gamma irradiation.

Dose	Plant height (cm)	Number of tillers per clump	Number of productive clump	Days to flowering (DAT)
Control	125	13	4	72.1
50	102	50	25	62
100	73	32	17	62
150	105	7	5	63
200	105	33	16	61
250	110	27	8	64
300	78	50	23	66

4 Conclusion

Gamma irradiation successfully induced phenotypic variability in the local rice cultivar Mayas, leading to observable differences in germination, plant height, tillering capacity, and flowering time. The results demonstrated that low to moderate doses of gamma rays (particularly 50 Gy and 200 Gy) were the most effective in improving key agronomic traits. Promising mutant lines were identified from the irradiated populations of Mayas rice. At 100 Gy, plant height decreased to 73 cm, notably lower than the control average of 125 cm, indicating potential for lodging resistance. The number of tillers per hill increased dramatically at 50 Gy and 300 Gy (up to 50 tillers), while the number of productive tillers peaked at 25 under 50 Gy, substantially higher than the control average of 4. Moreover, plants irradiated at 200 Gy exhibited earlier flowering (61 days after transplanting), approximately 11 days earlier than the control (72.1 days), confirming its effectiveness in promoting genotypic modifications related to growth duration.

Conversely, higher irradiation doses (≥250 Gy) negatively affected germination, plant growth, and yield components, reflecting the detrimental impact of excessive radiation on plant physiology and genomic integrity. Therefore, the optimal irradiation range for mutation induction in Mayas rice is 50–200 Gy, as it provides the best balance between mutation frequency and plant survival.

Overall, this study confirms that gamma-ray mutagenesis is a powerful tool for generating genetic diversity and improving the agronomic performance of local rice germplasm. The mutant lines obtained from 50 Gy and 200 Gy treatments show strong potential as breeding materials for developing semi-dwarf, high-yielding, and early-maturing Mayas rice varieties suited to the agroecological conditions of East Kalimantan. Further evaluation through multi-generation selection and molecular characterization is recommended to identify stable mutant lines with desirable genetic and agronomic traits.

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