Research Article

Analysis of Radiosensitivity and M1 Mutation Variance in Double Haploid Rice Induced by Gamma Ray Irradiation

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Abstract | Rice *(Oryza sativa L.)* is a vital food commodity that significantly contributes to global food security. The impacts of climate change pose a threat to crop production, particularly rice, potentially leading to crop failures. A proposed strategy to address this challenge involves inducing mutations in double haploid rice plants to enhance genetic diversity, thereby facilitating the emergence of new, desirable traits. This study aimed to generate highly diverse M1 rice populations. The research employed regression and principal component analyses, focusing on mutant rice lines derived from mutations in the double haploid HS1-28-1-5 lines, subjected to irradiation doses of 200, 400, 600, 800, and 1000 Gy. The findings indicate that the irradiation dose significantly influences rice growth traits, presenting opportunities for the selection of advantageous traits for varietal improvement. Notably, a dose of 200 Gy yielded the greatest variation, whereas higher doses, such as 400 Gy, resulted in a more uniform population, likely due to natural selection for individual tolerance.

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Introduction

Rice (L.) is a vital food commodity that significantly contributes to global food security (Nurkayah *et al*., 2024). According to the Statistics Indonesia, (2023), the total rice production in 2023 is projected to be approximately 30.90 million tonnes, representing a decrease of 645,090 tonnes (2.05 percent) compared to the 2022 production level of 31.54 million tonnes. This reduction is partially attributed to the impacts of climate change, which can adversely affect food crop yields, particularly rice, and may result in crop failures (Priyanto *et al*., 2021). Consequently, climate change poses a substantial challenge to the sustainability and viability of crop cultivation.

Since the onset of the 21st century, climate change has become a prominent topic of discussion, exerting a substantial impact on the agricultural sector, particularly rice cultivation in tropical regions (Priyanto *et al*., 2021). Climate change, whether persistent, sporadic, or enduring, poses a significant threat to the sustainability of agriculture. Persistent effects include rising air temperatures, altered precipitation patterns, increased salinity of groundwater, diminished productivity, and shifts in cropping patterns and indices. Conversely, sporadic effects encompass increased crop failures due to the frequency and severity of extreme weather events such as floods, droughts, and strong winds, as well as outbreaks of pests and diseases (Widiarta, 2016).

Abiotic stresses, such as drought, pose significant challenges to climate management. Drought occurring during the vegetative phase of rice plants results in inhibited root and leaf development, consequently reducing the number of tillers and panicles per hill. Drought during the generative phase exerts a substantial impact on rice yield (Yasrifah *et al*., 2021). As noted by Hariyono (2014), drought during the generative phase, specifically the flowering and grain-filling stages, can lead to yield reductions of up to 50% and 21%, respectively. This is corroborated by Wening *et al*. (2019), who emphasized that the generative phase is critical for rice plants' tolerance to drought stress, with potential yield reductions of 13-35% during the rainy season and 34-59% during the dry season. The decline in productivity and production stagnation necessitates technological innovation to address the challenges posed by climate change (Auliya *et al*., 2024).

Plant breeding is a scientific endeavor aimed at enhancing the genetic attributes of plants to develop superior varieties. This process is pivotal in the agricultural sector, particularly in augmenting the productivity and quality of agricultural products (Ashar *et al*., 2024). Mutation serves as a technique to induce genetic diversity. The induction of mutations through gamma ray irradiation can generate populations with substantial diversity by instigating alterations in the plant's DNA and chromosomes, thereby leading to the emergence of novel characteristics. Individuals resulting from these mutations are termed mutants and are selected to acquire traits that align with breeding objectives. This is due to the random nature of mutations, which precludes the possibility of directing changes to a specific gene or chromosome (Suliartini *et al*., 2022). Anshori *et al*. (2018) conducted research utilizing anther culture, which successfully yielded 56 double haploid (DH2) rice lines with homozygous genetic conditions, rendering these lines ideal for mutation applications. The study aimed to obtain offspring of the M1 rice population with high diversity. Mutations in double haploid rice lines are anticipated to produce considerable genetic diversity, thereby facilitating the selection of traits suitable for the stresses employed in the testing phase.

Materials and Methods

The research will be conducted over a three-month period at the Smart Green House of the Faculty of Agriculture, Hasanuddin University. This study focuses on a rice mutant line derived from mutations in the double haploid line HS1-28-1-5, which was subjected to mutation at doses of 200, 400, 600, 800, and 1000 Gy. Fifty seeds were utilized per dose, resulting in a total of 250 seeds being sown for each line. The study employs regression and principal component analyses for its structure.

Research implementation

Research implementation included the preparation of planting media, sowing, planting, maintenance, harvesting, and data collection.

Planting media preparation

The planting medium was prepared by loosening the soil with a hoe. The medium comprised soil mixed with husks in a 2:1 volume-to-volume ratio. Subsequently, the soil and husk mixture was transferred into buckets and containers using a shovel. The soil in the buckets was then saturated with water until it was flooded, whereas the soil in the containers was only moistened.

Seeding and planting

Seeds from each variety were soaked in water within small plastic containers for a duration of 24 hours, followed by a drying period of an additional 24 hours. Subsequently, the seeds of each strain were sown in containers for a period of 20 days, utilizing a planting medium composed of a soil and husk mixture in a 2:1 volume-to-volume ratio. Each strain of planted seed was assigned a code corresponding to the mutation name. The planting process was conducted 20 days after sowing (DAS), utilizing soil contained within a bucket.

Plant maintenance

Plant maintenance are followed by weeding, fertilization, and irrigation. Weeding was conducted by manually removing weeds from the containers and buckets. Fertilization was carried out on four occasions: 10 days after planting (DAP) with urea fertilizer, 21 DAP with NPK fertilizer, 45 DAP with Ultradap fertilizer, and 60 DAP with KNO_3 fertilizer. Regular irrigation was implemented to ensure adequate water availability in the buckets as their water volume began to diminish.

Harvesting and data collection

Harvesting may commence when two-thirds of the panicles attain physiological maturity, indicated by the yellowing of the straw, and the rice grains at the base have solidified. The harvesting process adhered to the specific criteria established for each rice line. Manual harvesting was conducted using a sickle. Each plant population (panicle) was placed into a coded sample bag and subsequently stored in sacks, organized according to the rice line. Data collection was conducted both prior to and following the harvesting process, in accordance with these parameters.

Observation parameters and data analysis

The parameters assessed in this study comprised live seedlings (LS), plant height (PH), flag leaf length (FLL), number of productive tillers (NPT), number of grains per panicle (NGP), and the percentage of filled grains per panicle (PFG). The collected data were subjected to regression analysis, followed by principal component analysis (PCA), utilizing R Studio software followed by the use of Factominer package.

Results

The results of the lethal dose (LD50) analysis indicated both linear and quadratic mortality rates corresponding to increasing radiation doses (Figure 1). As depicted in this figure, the mutation dose two weeks after planting (2 WAP) exhibited a linear pattern, represented by the equation -0.1274 gamma ray dose (GRD) + 108.38. This equation is characterized by a coefficient of determination of 0.86 and identifies an LD50 at a dose of 458.24 Gy. In contrast, the lethality response of mutations eight weeks after planting (8 WAP) demonstrated a quadratic pattern, described by the equation $0.0002GRD^2 - 0.3312GRD + 118.38$. This equation also possesses a high coefficient of determination (0.89), with an LD50 of 243 Gy.

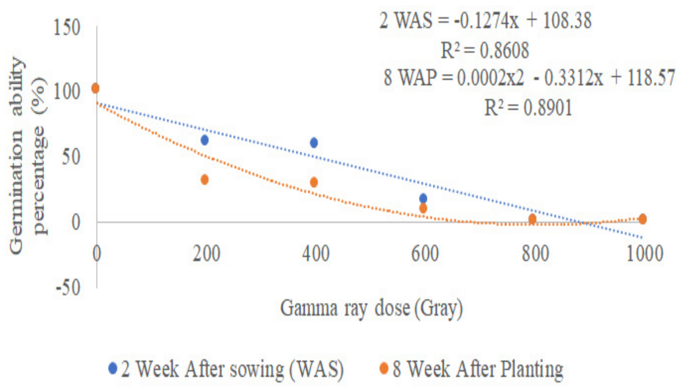

Figure 1: *Lethal dose analysis of gamma irradiation mutations on wild-type lines*

The analysis of the growth response of mutant rice lines revealed that gamma irradiation treatment elicited varied responses across different growth characteristics (Figure 2). The figure highlights nine primary characteristics: plant height, number of productive tillers, percentage of grains per panicle, number of filled grains, number of grains per panicle, weight of 1000 grains, and panicle length. Notably, plant height, percentage of grains per panicle, number of filled grains, and weight of 1000 grains exhibited a pronounced negative linear trend. Specifically, an increase of 1 Gy in radiation dose corresponded with a decrease in these traits. Conversely, the number of productive tillers demonstrated a significant positive correlation with gamma radiation dose, evidenced by a determination value of 80.8%. Additionally, the characteristics of the number of seeds per panicle, panicle length, and flag leaf length followed an upward-opening parabolic pattern (U-shaped) with an R2 value of 1, indicating a quadratic relationship between gamma ray dose and these characteristics. These findings suggest that while low to moderate doses of gamma irradiation can enhance certain morphological characteristics, higher doses tend to adversely affect rice growth and yield.

The principal component analysis of all traits elucidates the direction of diversity at each irradiation dose (Figure 3). This analysis delineates the potential impact of each irradiation dose. According to the figure, the 200 Gy dose exhibits the greatest variability, indicative of its highly variable and random mutagenic effects. In contrast, the 400 Gy gamma ray dose yields more uniform results, albeit with an impact on yield. This is posited to result from the influence of high doses on the selection of more tolerant individuals.

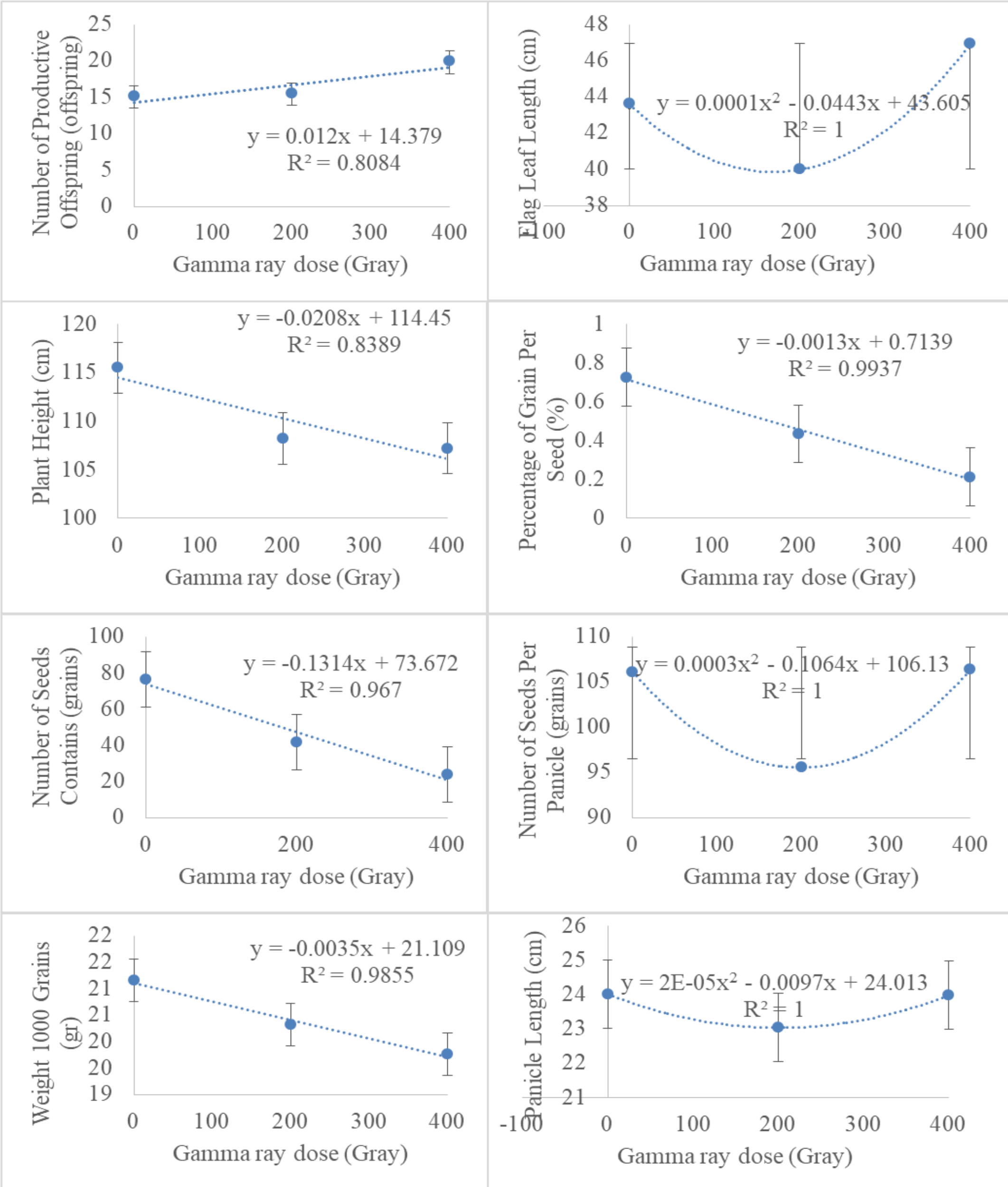

Figure 2: *Analysis of gamma irradiation mutation dose response on growth characteristics*

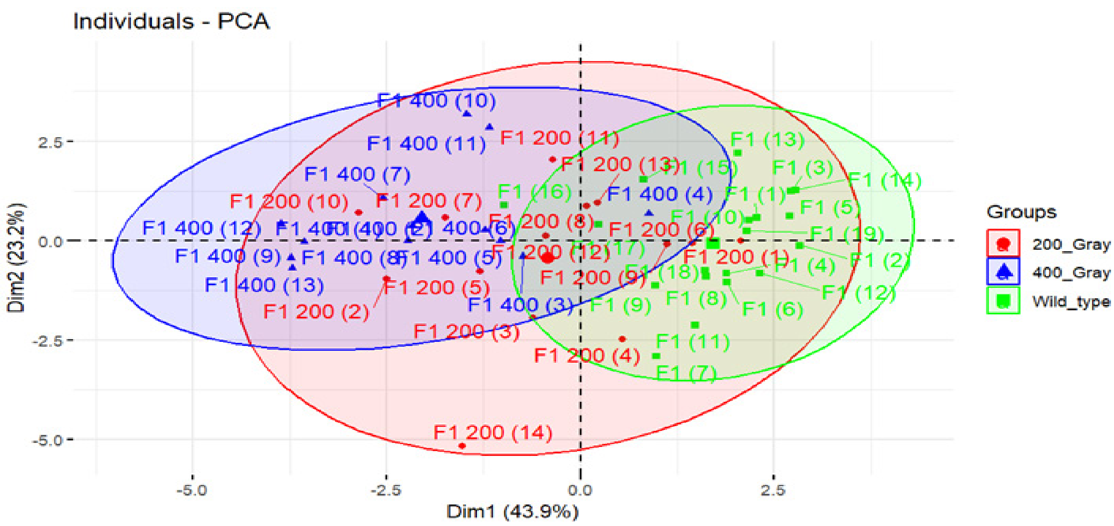

Figure 3: *Biplot analysis of principal components of growth characters of M1 mutant rice based on gamma irradiation dose*

Discussion

Gamma irradiation, with doses ranging from 0 Gy to 1000 Gy, significantly influences seed germination and seedling development. The germination and growth response of rice to each irradiation dose is variable. Notably, a dose of 0 Gy (control) exhibits a distinct response in terms of the germination percentage of irradiated seeds. As the radiation dose increases, there is a corresponding decline in both germination and seedling growth. Prabhandaru and Saputro, (2017) have indicated that radiation can induce damage at the chromosomal and DNA levels in seeds, thereby impacting the physiological and biochemical processes of plants. An increase in irradiation dose is associated with elevated protein content and a reduction in total carbohydrate and energy content, leading to abnormal germination.

Plant mortality following irradiation is attributable to the deterministic effects of gamma radiation. These deterministic effects arise from cell death induced by radiation exposure, leading to physical mutations (Budi *et al*., 2015). The threshold doses, both high and low, are intricately linked to the radiosensitivity of plant genotypes. Radiosensitivity refers to the degree of a plant's sensitivity to radiation. This sensitivity varies significantly among different genotypes and plant conditions during irradiation. Radiosensitivity can be quantified using the LD50 value, which represents the dose that results in the death of 50% of the irradiated plant population (Warman *et al*., 2015). At two weeks after planting (2 WAS), the LD50 value indicated a moderate plant mortality rate of 458.24 Gy, whereas at eight weeks after planting (8 WAP), the LD50 value demonstrated a relatively low plant mortality rate of 243 Gy. This variation may be attributed to the moisture content of the seeds prior to irradiation. The lethal impact of ionizing radiation on cells leads to the cessation of mitotic activity, resulting in the loss of genetic material, which in turn causes reduced germination and seedling growth rates, and can even lead to the lethality of plant material (Warman *et al*., 2015). This observation aligns with the assertion by Prabhandaru and Saputro, (2017) that high-dose gamma radiation can influence electrons within the nucleus, causing a transformation of normal ions into positive ions and the release of energy (ionization) as it traverses tissue, thereby inducing chemical changes in plants and reducing seed germination. This is further corroborated by Budi *et al*. (2019), who noted that the presence of oxygen and water molecules (H_2O) in irradiated material affects the ionization process of free radicals formed, thereby influencing the radiosensitivity of plant cells. The greater the oxygen and H_2O content in the irradiated material, the more free radicals are generated, rendering the material more sensitive.

The response patterns predominantly exhibit negative linear trends and upward-opening parabolic trends (Figure 2). Plant characteristics that demonstrate negative linear patterns with increasing irradiation doses include plant height, percentage of grains per panicle, number of filled grains, and weight of 1000 grains. This suggests that increasing irradiation doses initially exert a positive influence on growth traits up to an optimal point. However, surpassing this dose results in a significant decline in trait values. Gamma irradiation can induce genetic variation through DNA damage; however, excessive exposure may lead to lethal or sublethal effects on plant tissues. According to Budi *et al*. (2019), characteristics that support production exhibit a negative linear pattern as the irradiation dose increases, due to the influence of plant radiosensitivity, which results in physical damage such as seed emptiness per panicle. The response of the number of productive tillers displays a positive linear pattern, indicating that higher radiation doses correlate with an increased number of tillers produced. According to Mardiyah *et al*. (2022), the number of productive offspring is believed to be susceptible to the effects of irradiation because the controlling gene is located near the centromere.

Induced mutations are anticipated to minimize physiological damage while maximizing genetic alterations. The primary aim of inducing desired genetic diversity through mutation induction is to reduce chromosomal aberrations, physical damage, and sterility, as well as to regulate the production of desired mutations (Budi *et al*., 2019). An additional indicator of mutation occurrence is the broader diversity demonstrated by the range values in the radiation dose treatment, which exhibit a wider range compared to the control treatment. Higher radiation doses lead to greater differences in plant height ranges. The greater the range variation, the more extensive the diversity and the broader the genetic diversity (Yunus *et al*., 2017).

The plant response exhibited an upward-opening parabolic pattern in the characteristics of flag leaf length, number of seeds per panicle, and panicle length. The results for the 0 Gy dose were nearly identical to those for the 400 Gy dose. However, the 200 Gy dose yielded lower results compared to both the 0 Gy and 400 Gy doses. According to Mardiyah *et al*. (2022), mutation induction through seed irradiation induces physical mutations and can penetrate plant seeds to the chromosomal layer, resulting in morphological changes in the plant.

PCA plots serve as an effective tool for identifying structures and patterns within multivariate data, thereby facilitating the interpretation and analysis of PCA results. The observed separation indicates that gamma irradiation exerts a significant influence on rice growth traits, with varying doses leading to distinct patterns of variation. Specifically, a dose of 200 Gy appears to induce greater variation, potentially enhancing the likelihood of obtaining superior mutant lines. Conversely, a dose of 400 Gy exhibits a more targeted effect, albeit with the risk of reducing variation. These findings align with the research conducted by Warman *et al*. (2015), which asserts that an irradiation dose of 200 Gy results in less physical damage compared to higher doses, suggesting that 200 Gy is an effective dose for producing the desired mutants.

Conclusions and Recommendations

The present study demonstrates that gamma irradiation of double haploid rice lines can induce substantial genetic diversity in the M1 generation. The relatively moderate LD50 value (±243–458 Gy) suggests a high radiosensitivity of the seeds employed. An increase in the irradiation dose adversely affected several growth traits, including plant height, percentage of filled grains, number of filled grains, and 1000-grain weight, while other traits exhibited a quadratic relationship. Principal Component Analysis (PCA) indicated that a dose of 200 Gy generated the greatest diversity, potentially yielding superior mutant lines, whereas a dose of 400 Gy tended to produce more uniform populations, albeit with a lower density. Overall, the irradiation dose significantly influences the modification of rice growth traits and offers opportunities for the selection of desirable traits for varietal improvement.

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Novelty Statement

The study found that a dose of 200 Gy produced the greatest variation, while higher doses, such as 400 Gy, produced a more uniform population due to the possible natural selection of individual tolerance.

Author's Contribution

Muh. Fikri Al Qautzar: Formal analysis, Investigation, Data Curation, Original draft, Visualisation.
Muhammad Fuad Anshori: Conceptualisation, Methodology, Validation, Formal analysis, Source, Data Curation, Visualisation, Funding acquisition.
Abd Haris Bahrun: Conceptualisation, Validation.
Bambang Sapta Purwoko: Conceptualisation, Methodology.
Iswari Saraswati Dewi: Conceptualisation, Validation.
Wijaya Murti Indriatama: Conceptualisation, Methodology.
Novaty Eny Dungga: Validation.
Reskiana Rahman: Source.

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