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Genetic Variability and Heritability Estimates in M1–M3 Generations of Kuantan Singingi Local Rice (White Sage Genotype) Induced by Gamma Radiation

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Abstract: Rice (*Oryza sativa* L.) is a critical staple crop in Indonesia, particularly in Kuantan Singingi Regency, where local rice varieties exhibit several advantageous traits but also possess notable limitations. This study aimed to induce genetic variability in a local rice genotype ("White Sage") through gamma ray irradiation, with the goal of selecting promising mutant lines based on their phenotypic and heritable traits. Gamma irradiation at a dose of 300 Gy, previously determined to be effective, was applied to M0 seeds, with untreated seeds (0 Gy) serving as controls. A total of 600 plants per treatment were cultivated in a randomized complete block design (RCBD) with five replications. The experiment was conducted under rainfed conditions in Pebau Hulu Village, Kuantan Mudik District, from April 2024 to April 2025. M1 mutants were evaluated for key agronomic traits, and selected M2 lines were advanced



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using the bulk method. In the M3 generation, nine mutant lines were identified and characterized. Results indicated that most traits observed in M1 and M2 generations, including plant height, number of seedlings, number of productive tillers, flag leaf length, and grain characteristics, exhibited broad genetic variability and high heritability. In the M3 generation, plant height, number of seedlings, productive tillers, flag leaf length, and number of empty grains showed wide genotypic variability. However, the number of filled grains and weight of 100 grains showed narrow genetic variability. High heritability estimates in M3 were observed for the number of seedlings, productive tillers, and number of empty grains, while other traits demonstrated lower heritability. These findings suggest the potential for effective selection of superior mutant lines with improved agronomic performance in future breeding programs.

Keywords: Gamma Ray Irradiation, Genetic Variability, Heritability, Local Rice, Mutagenesis, *Oryza sativa*.

通过伽马射线诱导的宽坦新吉地方稻（白鼠尾草基因型）M1–M3世代的遗传变异与遗传力估算

稻米（*Oryza sativa* L.）是印度尼西亚的重要主粮作物，尤其是在廖内省宽坦新吉县，当地水稻品种具有多种优良性状，但也存在明显的遗传局限性。本研究旨在通过伽马射线诱变对白鼠尾草（White Sage）基因型的地方稻种进行遗传改良，以提高其遗传变异，并筛选出具有优异农艺性状和高遗传力的突变系。

本研究采用前期试验确定的有效剂量——300 Gy伽马射线对白鼠尾草水稻种子（M0代）进行处理，以0 Gy为对照组。每处理设置600株植株，采用随机完全区组设计（RCBD），重复5次。田间试验于2024年4月至2025年4月在宽坦穆迪克地区佩包胡鲁村的旱田条件下进行。

M1代突变体依据主要农艺性状进行观察与初选；M2代采用群体育种法（bulk method）推进，最终在M3代中筛选出9个突变株系进行性状鉴定。研究结果表明，在M1和M2代中，包括株高、分蘖数、有效分蘖数、旗叶长度及籽粒特征等性状均表现出广泛的遗传变异与较高的遗传力。

在M3代中，株高、分蘖数、有效分蘖数、旗叶长度以及空壳粒数等性状展现出广泛的基因型变异，而实粒数和百粒重则表现为较窄的遗传变异。M3代中，分蘖数、有效分蘖数及空壳粒数的遗传力估值较高，而其他性状遗传力较低。研究结果表明，利用伽马射线诱变技术可有效诱导宽坦新吉地方稻种群的遗传变异，为未来的水稻遗传育种提供了优良材料和理论基础。

关键词: 伽马射线诱变；遗传变异；遗传力；地方稻；诱变育种；*Oryza sativa*

1. Introduction

Rice (*Oryza sativa* L.) is one of the most essential staple crops globally, with approximately 40% of Southeast Asia's population relying on it for daily caloric intake. As such, rice plays a pivotal role in the region's social, economic, and political stability. In Indonesia, rice is a critical commodity consumed by over 95% of the population, necessitating continuous development efforts to ensure sustainability and food security [1].

According to national statistics, Indonesia produced approximately 52.66 million tons of dry

unhusked rice (GKG), with Riau Province contributing 225.84 thousand tons [1]. Local rice varieties in Kuantan Singingi Regency, Riau, are known for their tolerance to brown planthopper infestation and drought conditions [4][5]. However, limitations such as tall plant height and long maturity periods hinder their broader adoption and productivity [24].

The *White Singgam* genotype is particularly characterized by a long life cycle (approximately 150 days), tall stature (>125 cm), and low productivity (1.59 tons/ha) [24]. To improve these traits, mutation breeding using

gamma-ray irradiation has been proposed as an effective approach. Gamma irradiation is a widely used mutagenic method for increasing genetic variation, allowing plant breeders to select for desirable traits such as reduced plant height, early maturity, and higher yield [3][6][8][13][17].

Following irradiation, the process of obtaining homozygous lines typically spans three generations: M1 (initial generation post-irradiation), M2 (segregating population), and M3 (selection and line stabilization). Homogeneity and trait fixation are commonly achieved in the M3 generation. In Kuantan Singingi, preliminary findings from M1 plantings indicated improved growth and yield traits following gamma-ray exposure, although radiation can also induce deleterious effects depending on dosage and genotype sensitivity [6][12][13][15].

Subsequent generations (M2 and M3) are developed using seeds harvested from selected individuals showing target phenotypes such as shorter stature and earlier flowering. At each stage, phenotypic and genotypic changes are monitored to evaluate variability and heritability of traits [9][10][11][19].

This study aims to generate and characterize mutant lines of *White Singgam* rice from Kuantan Singingi Regency, developed through gamma-ray irradiation. The goal is to identify genotypes with improved agronomic traits and to assess their genetic variability and heritability across M1, M2, and M3 generations.

2. Literature Review

Rice (*Oryza sativa* L.) remains one of the most critical staple food crops worldwide, particularly in Southeast Asia, where it provides the primary caloric intake for a majority of the population. Indonesia, as one of the leading rice-producing countries, has prioritized the improvement of rice varieties, especially local genotypes with high adaptability but limited yield potential [1]. Kuantan Singingi's local rice, for example, possesses valuable traits such as drought tolerance and pest resistance, but its long growth duration and tall plant structure limit its agronomic value [24].

To address these limitations, mutation breeding using gamma irradiation has emerged as an effective strategy to induce genetic variability and generate novel traits [3], [5]. Gamma rays

interact with the DNA of plant cells, inducing point mutations or chromosomal rearrangements that can be stably inherited across generations [27]. This technique is widely applied in rice improvement programs globally due to its capacity to create genetic diversity without disrupting desirable parental traits [28].

In Indonesia, mutation breeding has been successfully applied to local cultivars, including black rice and upland rice varieties, showing improvements in plant morphology, productivity, and biochemical traits [13], [30]. Specifically, studies on M1–M3 generations demonstrate the progression from initial irradiation effects to heritable and selectable traits in later generations. The M1 generation often displays high phenotypic variation due to chimerism and physiological damage, while the M2 and M3 generations are more genetically stable, allowing for effective selection [9], [10], [22].

Numerous researchers have reported significant improvements in yield components, such as plant height, number of productive tillers, grain number, and grain weight in irradiated populations [11], [26], [29]. For instance, Dash & Kujur [3] demonstrated that gamma irradiation of black rice significantly altered physiological traits such as chlorophyll content and antioxidant activity, which are crucial for stress tolerance. Similarly, Putra et al. [30] selected promising M3 black rice mutants with enhanced grain quality and yield performance.

Heritability estimates are critical in determining the effectiveness of selection for a given trait. High heritability in broad sense (H^2) indicates that selection will be effective in improving that trait in subsequent generations [16]. Studies have shown that traits like plant height, number of tillers, and grain yield often exhibit moderate to high heritability in gamma-irradiated rice populations [10], [15], [26]. For example, Marnita et al. [11] and Qadri et al. [16] reported heritability estimates above 0.5 for several agronomic traits, suggesting a strong genetic basis and limited environmental influence.

Furthermore, molecular and biochemical analyses have provided additional evidence supporting the effectiveness of mutation breeding. Li et al. [8] highlighted the role of key genes in regulating grain composition and plant architecture, which can be modified through mutagenesis. Naibaho et al. [13] used biochemical markers to evaluate M3 mutant lines and found

substantial improvements in photosynthetic efficiency and protein content.

In addition, mutation-induced variability has been supported through cytological and molecular studies that show increased mitotic and meiotic anomalies in early generations, which eventually stabilize in later generations [21], [31]. This stabilizing trend is essential for the development of true-breeding mutant lines with desirable traits.

In conclusion, gamma irradiation has proven to be a powerful tool for expanding the genetic base of rice, especially for improving underutilized local genotypes. Through systematic selection across M1 to M3 generations, it is possible to isolate mutant lines with superior agronomic traits and stable inheritance, thus contributing to national food security and regional rice breeding programs [4], [13], [25].

3. Research Methods

This study was conducted in rainfed lowland fields located in Pebaun Hulu Village, Kuantan Mudik District, Kuantan Singingi Regency, Riau Province, Indonesia. The experiment was implemented over three growing seasons corresponding to three generations of mutant rice: M1 (November 2023–April 2024), M2 (June–October 2024), and M3 (December 2024–March 2025).

Plant Material and Gamma Irradiation

The genetic material used consisted of seeds from the local white Singgam rice genotype. Seeds were subjected to gamma irradiation at a dose of 300 Gy, identified as an effective mutagenic dose in prior studies. A parallel control group (0 Gy) was maintained without irradiation. The purpose of applying gamma radiation was to induce random mutations with the potential to improve key agronomic traits, particularly plant height and growth duration.

Experimental Design and Cultivation

A completely randomized design (CRD) was adopted, with two treatment groups (irradiated and control), each replicated five times. Each replicate included 200 to 1000 plants to ensure sufficient population size for variability estimation and selection.

In the M2 generation, a bulk method was used to plant a mixture of seeds harvested from M1 plants. From this bulk population, 400

individuals were selected for phenotypic evaluation. Based on observed traits, a subset of plants was advanced to the M3 generation. In M3, nine stable mutant strains were established and analyzed.

Standard cultivation inputs such as organic manure, NPK fertilizer, herbicides, and insecticides were applied uniformly across all plots. Tools used included plant protection nets, measuring equipment, and labeling systems to ensure consistent and accurate data collection.

Inclusion and Exclusion Criteria

Inclusion Criteria:

- M2 and M3 plants derived directly from irradiated M1 individuals.
- Plants exhibiting observable variation in one or more target traits (e.g., plant height, maturity).
- Individuals with good overall plant vigor and no visible signs of disease or deformities.
- Plants producing viable seeds suitable for advancing to the next generation.

Exclusion Criteria:

- M1 individuals exhibiting lethal or sterile mutations with no seed production.
- M2 plants with extreme abnormalities or growth defects unrelated to agronomic improvement.
- Any plants affected by environmental stressors (e.g., pest infestation, drought) that could confound trait expression.
- Individuals showing no distinguishable difference from the control group (0 Gy) across all measured traits.

Traits Observed and Data Analysis

Morpho-agronomic traits evaluated included:

- Plant height (cm)
- Number of total tillers and productive tillers
- Length of flag leaf (cm)
- Number of filled and unfilled grains per panicle
- Weight of 100 grains (g)

Quantitative data were subjected to analysis of variance (ANOVA) using an F-test at a 5% significance level to determine statistical differences between treatments. The M2

generation was analyzed descriptively to assess the range of induced variability. In the M3 generation, segregation patterns and genetic stability were analyzed using Chi-square (χ^2) tests to determine conformity to expected ratios.

The combination of selection criteria, structured generational advancement, and statistical testing ensures reliable identification of beneficial mutations and supports the development of improved rice lines for future breeding efforts.

4. Results and Discussion

The results of the fingerprint analysis revealed that gamma ray irradiation had no statistically significant effect on plant height (cm), flag leaf length (cm), and 100-grain weight (g). In contrast, it exerted a significant to highly significant effect on several other traits, including the number of tillers (stems), number of productive tillers (stems), number of filled grains (seeds), and number of empty grains (seeds).

The average values for each morpho-agronomic trait of the Kuantan Singingi local rice genotype (White Sage) are presented in Table 1. Descriptive analysis of the M2 mutant population indicated that gamma irradiation had a statistically significant and highly significant impact on plant height (cm), number of tillers (stems), number of productive tillers (stems), number of unfilled grains (seeds), and the 100-grain weight (g). However, no significant effect was observed for flag leaf length (cm) and the number of filled grains (seeds), as reflected in Table 1.

Furthermore, the results for the M3 mutant population showed that gamma irradiation did not significantly affect plant height, number of tillers, flag leaf length, number of filled and empty grains, or 100-grain weight. However, it had a clear and statistically significant effect on the number of productive tillers, confirming the continued expression of selected traits under gamma-ray treatment.

Table 1. Average response of local rice morphoagronomic characters of Kuantan Singingi to gamma ray radiation at stages 1, 2, and 3 in the field

Morphoagronomic Characters	Mutan 1		Mutan 2		Mutan 3	
	0 Gy	300 Gy	0 Gy	300 Gy	0 Gy	300 Gy
Plant height (cm)	138,00	127,00 ±0,105	132,17	99,66*	132,89	88,89
Number of saplings (stems)	39,00	73,00 *	7,35	14,15**	11,44	10,44
Productive saplings (stems)	6,40	13,00*	6,90	13,50*	10,78	9,89*
Flag leaf length (cm)	78,00	66,00	82,76	71,54	85,00	77,22
Total grain content (Seeds)	196,00**	6,00	200,50	188,40	211,56	191,00
Total grain waste (grain)	28,00	94,40**	51,25	87,40*	3,56	3,78
Production of 100 grains (salt)	2,40	1,04	3,00	1,65**	302,78	229,44
Confidence intervals	0,05		0,05		0,05	

Gamma-ray irradiation at a dose of 300 Gy did not significantly alter the gene structures associated with plant height, flag leaf length, and 100-grain weight in the M1 generation. This observation may reflect the inherent genetic resistance to ionizing radiation or the capacity of antioxidant defense systems to mitigate free radical damage [5]. Hanafy and Akladious [5] reported that gamma irradiation does not always disrupt morphological or physiological traits when gene integrity is maintained.

In the M2 generation, irradiation had no significant effect on flag leaf length and grain number, which could be due to genetic stabilization or the absence of direct radiation exposure. Parikh et al. [17] found that organisms exhibiting robust rRNA-mediated degradation of free radicals displayed enhanced resistance, which supports the hypothesis of acquired genetic tolerance over successive generations.

Plant height analysis confirmed the prevalence of tall phenotypes in M1 mutants, in agreement with Mardiyah et al. [10]. According to

standardized height classifications [19], M1 plants were categorized as "tall" (>141 cm). However, M2 mutants averaged 99.66 cm, aligning with the "short to medium" range, and M3 further decreased to 88.89 cm, nearing the desired semi-dwarf trait. These findings are consistent with prior research indicating that gamma irradiation can progressively reduce plant stature through induced mutations [13], [18].

Gamma irradiation significantly affected the number of tillers and productive tillers in M1 and M2 generations, but only productive tillers in M3. This may be due to the positional effects of gene loci or gene linkage influencing these traits [2], [7]. Ilyas et al. [7] emphasized that mutagen sensitivity varies among cell types, potentially explaining differential trait expression. Furthermore, environmental stress during M3 cultivation may have influenced the phenotypic response, consistent with findings that climatic variables, particularly flooding, can affect rice development [6].

Although flag leaf length did not significantly differ across treatments, this trait remains crucial due to its role in photosynthesis and assimilate partitioning. As noted by Melati et al. [12], longer flag leaves are associated with enhanced yields. The average flag leaf length in all generations approached the ideal value of approximately 50 cm, as suggested by Peng et al. [15].

Significant reductions in the number of filled grains were observed in M1 at 300 Gy, likely due to chromosomal or physiological damage induced by radiation [10]. However, in M2 and M3 generations, differences between irradiated and control plants decreased, indicating possible recovery or adaptation. Similar results were reported by Dash and Kujur [3], who

observed minimal impact of irradiation on final photosynthate allocation in black rice.

A marked increase in sterile (empty) grains was observed in M1 at 300 Gy, supporting the hypothesis that radiation induces pollen sterility [10], [21]. Though still elevated in M2, sterility declined in M3, suggesting improved reproductive stability. Siahpoosh et al. [21] reported that while gamma radiation affects meiotic division, its impact on agronomic traits like pod and seed formation can vary. Environmental factors, particularly flood stress, may further exacerbate sterility by disrupting photosynthesis and nutrient flow [14].

The 100-grain weight a direct indicator of productivity was significantly reduced in M1 and M2 under 300 Gy treatment, but no significant difference was found in M3. This stabilization may reflect improved regulation of seed development or compensatory gene expression [27], [31]. According to Umam et al. [23], the weight of 100 grains is a reliable measure of yield potential and depends on assimilate accumulation during seed filling. Additionally, Shi et al. [20] noted that gamma-induced DNA reorganization can influence gene expression, affecting seed protein and starch composition.

The cumulative findings suggest that while gamma irradiation initially disrupted key traits, later generations exhibited trait stabilization and possible adaptation. This supports the value of mutation breeding in enhancing genetic variability and selecting favorable alleles [10], [29]. Estimations of genetic and phenotypic variability, as well as broad-sense heritability, underscore the breeding potential of these induced mutants, particularly for improving agronomic performance in local rice cultivars.

Table 2. Genetic Variability and Phenotypic Expression in Mutants at Each Stage

No	Character	Mutant 1 S ² G	Mutant 1 S ² P	Mutant 1 H ² (%)	Mutant 1 Criterion	Mutant 2 S ² G	Mutant 2 S ² P	Mutant 2 H ² (%)	Mutant 2 Criterion
1	TT	96.83	1125.17	0.09	Low	39441.37	42601.88	0.93	High
2	AND	111.77	130.91	0.85	High	2360.75	2327.75	0.99	High
3	AP	102.59	134.13	0.76	High	2171.25	2195.25	0.99	High
4	PDB	70539.67	85941.33	0.82	High	5508.94	6995.99	0.79	High
5	JGI	90079.13	90933.47	0.99	High	6555.79	9614.63	0.68	High
6	JGH	10726.56	12205.76	0.88	High	650001.00	66360.72	0.98	High
7	100 grains	34.72	36.06	0.96	High	380598.70	382461.63	1.00	High

Descriptions of the observed morpho-agronomic traits are as follows: TT refers to plant height (cm); JA represents the number of saplings (stems); AP indicates the number of productive saplings (stems); PDB denotes flag leaf length (cm); JGI is the number of filled grains (seeds); JGH is the number of empty or unfilled grains (seeds); and “100 biji-bijian” corresponds to the weight of 100 grains (g).

Based on the results presented in Table 2, both genotypic and phenotypic variability play a critical role in plant breeding. Genetic variability provides insight into the extent of diversity within a given population, which is a vital component in the selection and development of superior cultivars. Greater variability within traits suggests a higher potential for identifying desirable genes and selecting for improved agronomic performance [10].

Table 3. Genetic Variability and Phenotypic Variability of Mutant 3

No	Character	S2G	S2G Criterion	S2P	S2P Criterion	H2 value (%)	H2 Criterion
1	TT	206471.29	Broad	5065691.19	Broad	0.04	Low
2	AND	7337.44	Broad	9169.45	Broad	0.8	Tall
3	AP	6662.48	Broad	7306.61	Broad	0.91	Tall
4	PDB	51001.65	Broad	2968610.68	Broad	0.02	Low
5	JGI	- 14672571.44	Narrow	123194872.98	Broad	-0.12	Low
6	JGH	859.88	Broad	764.88	Broad	1.12	Tall
7	100 Biji-bijian	- 14379761.58	Narrow	156170363.58	Broad	-0.09	Low

The results indicate that all observed morpho-agronomic traits in the local rice genotypes from Kuantan Singingi Regency exhibited broad genetic and phenotypic variability, particularly under the influence of gamma-ray irradiation. Phenotypic variability is understood to result from the combination of genetic factors and environmental influences. However, selection efficiency depends largely on the heritability of each trait a measure of how much phenotypic variance is attributable to genetic differences [16].

In Mutant 1, all traits displayed broad genetic and phenotypic variability, but plant height showed low heritability, suggesting strong environmental influence on this trait. Heritability is considered high when the value exceeds 0.5, moderate between 0.2 and 0.5, and low if below 0.2 [15].

In contrast, observations from Mutant 2 showed high heritability across all measured traits, implying a stronger genetic control over these parameters. In Mutant 3, heritability values varied: high heritability was observed for the number of saplings, number of productive saplings, and the number of unfilled grains. In contrast, traits such as plant height, flag leaf length, number of filled grains, and the weight of 100 grains exhibited lower heritability, suggesting

greater environmental influence or the effects of advanced generational stability.

These findings underscore that traits with high heritability and wide genetic variability particularly the number of productive saplings and grain yield-related components are ideal targets for selection. High heritability implies that the trait’s phenotypic expression is primarily governed by genetic factors and is therefore more reliably passed on to subsequent generations [11].

The comparison of genotypic and phenotypic variances further confirms the efficiency of gamma-ray mutagenesis in inducing useful variability in local rice genotypes. The use of a 300 Gy irradiation dose previously identified as effective appears particularly potent in enhancing genetic variability and improving selection response in key agronomic traits, such as plant architecture and reproductive yield components [10].

5. Conclusion

This study demonstrated that gamma-ray irradiation at a dose of 300 Gy effectively induced significant genetic variability and high heritability across most morpho-agronomic traits in local rice genotypes from Kuantan Singingi Regency. Broad genotypic variability was observed in all

characters, and heritability values were predominantly high, indicating that these traits are largely governed by genetic factors. This suggests a strong potential for selection and genetic improvement, particularly in characters such as the number of productive saplings and grain yield components.

However, plant height was an exception, displaying relatively low heritability, which implies a stronger environmental influence or instability in the expression of this trait under mutagenic stress. Despite this, the emergence of semi-dwarf phenotypes and early-maturing mutants in the M1–M3 generations under the 300 Gy treatment demonstrates the potential of mutation breeding as a valuable tool in developing improved rice cultivars adapted to local conditions.

Limitations

This study was limited to observations under rainfed field conditions in a single agroecological zone, which may not fully capture genotype $\times$ environment interactions. Additionally, the research focused only on early-generation (M1–M3) selections without molecular validation, which could have enhanced the precision of mutant characterization. The absence of controlled environmental parameters (e.g., irrigation, temperature, and pest control) may also have influenced the expression of certain traits, particularly plant height and seed filling.

Recommendations for Future Research

Further investigation is needed in the subsequent generations (e.g., M4 and M5) to confirm the genetic stability and agronomic performance of selected mutants. Molecular characterization through marker-assisted selection (MAS) or whole-genome sequencing is recommended to identify candidate genes associated with desirable traits. In addition, multi-location trials should be conducted to evaluate genotype $\times$ environment interactions and to assess the adaptability and yield performance of the mutant lines across diverse ecological zones.

Long-term breeding programs should also explore the integration of mutation breeding with conventional and genomic-assisted selection approaches to accelerate the development of high-yielding, stress-tolerant local rice varieties tailored to regional farming systems.

Declarations

Authors' Contribution

Conceptualization, G. M., A. S., G., Y.; methodology, G. M., A. S., G., Y.; software, G. M., A. S., G., Y.; validation, G. M., A. S., G., Y.; formal analysis, G. M., A. S., G., Y.; research, G. M., A. S., G., Y.; resources, G. M., A. S., G., Y.; data curation, G. M., A. S., G., Y.; original drafting-drafting, JG. M., A. S., G., Y.; drafting-revising and editing, G. M., A. S., G., Y.; visualization, G. M., A. S., G., Y.; supervision, G. M., A. S., G., Y.; project administration, G. M., A. S., G., Y.. All authors have read and accepted the published version of the manuscript.

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