



## **Orientation Of Effective Irradiation Dosage In Local Genetic Rice Improvement (*Oryza sativa* L.) Kuantan Singingi District Using Induced Mutation**

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### **ABSTRACT**

The staple food of the Indonesian population is rice. Utilizing mutation induction in rice research can lead to a significant increase in genetic diversity. This increased genetic diversity in the initial population will streamline the process of selecting desired traits. The primary objective of this study is to identify the most effective dose for inducing genetic diversity in local rice from Kuantan Singingi. The research focuses on the Singgam Putih genotype. Gamma radiation experiments are conducted at the Center for Isotope and Radiation Application (PAIR) BATAN in South Jakarta. Various radiation doses are administered, ranging from 0 Gy to 350 Gy. The planting process takes place in the greenhouses of the Dhabit Farm Experimental Garden in Kari Teluk Kuantan. Data collection includes observations on sprouting percentage, seedling height, number of leaves, and root length. The LD50 value is calculated based on the regression analysis of sprouting percentage. The findings indicate that the rate of sprouted seeds decreases as the gamma irradiation dose increases. Additionally, seedling height and root length decrease with higher doses of gamma irradiation. At the same time, the number of leaves remains unaffected by the radiation dose, which may lead to changes in leaf shape. The LD50 value for the Singgam Putih genotype is determined to be 313.63 Gy. Ultimately, the optimal dose of gamma-ray irradiation is identified as 300 Gy.

Keywords: *Induction of mutation, LD50, irradiation, local rice*

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## 1. INTRODUCTION

Rice (*Oryza sativa*. L) is a crucial staple food in various regions, particularly in Indonesia. According to the Badan Pusat Statistik (2023), Indonesia's rice production is projected to reach 53.63 million tons of GKG. Despite a decrease from the previous year, the demand for rice continues to rise due to the country's growing population. In Riau, rice production was only 209.19 tons of GKG, with Kuantan Singingi Regency contributing 21.36 tons of GKG. To further boost rice production in Kuantan Singingi, utilizing local genetic seeds that are well-suited for the region is essential, as these seeds offer numerous benefits.

Kuantan Singingi Regency showcases a considerable level of local genetic variability in rice. The local genetic characteristics of Kuantan Singingi encompass moderate maintenance needs, excellent adaptability, appealing taste, resilience to pests and diseases, and the capacity to withstand drought stress. However, a downside of this local genetic diversity is the extended lifespan and the plant's tall stature propensity to collapse easily. Hence, to accelerate breeding initiatives, mutation techniques should be applied in local rice breeding (Fajar *et al.*, 2020).

Genetic mutations involve modifications in the genetic material, affecting base pairs, DNA fragments, and chromosomes. These alterations lead to changes in phenotype that subsequent generations can inherit (Tagatorop *et al.*, 2016). Physical mutagens, such as gamma-ray irradiation, can induce mutations. Research has shown that gamma ray irradiation can also cause seed germination by damaging the seed coat layer (Nurrachmamila *et al.*, 2019).

According to Nurrachmamila (2019), the impact of irradiation ranging from 0-300 Gy on germination rate is observed but not deemed significant. Furthermore, the effective dose of gamma-ray irradiation at 200 Gy has the potential to enhance the growth rate, germination, vigor index, relative growth rate, and uniformity of seed emergence,

as stated by Untari *et al.* (2021). However, the required dose of gamma-ray irradiation may vary depending on the specific rice genotype and location. It is important to note that beyond 300 Gy, the utilization of increasing doses of gamma-ray irradiation can result in significant physiological damage to seed height, survival percentage in the field, and production, as highlighted by Harding *et al.* (2012).

The application of irradiation to food crops, particularly rice, aims to diminish the undesirable traits and enhance the desirable characteristics of the plant's phenotype. Consequently, irradiation is extensively employed to induce mutations and develop improved mutant varieties.

The primary objective of this study is to employ gamma-ray irradiation methods on indigenous rice varieties in Kuantan Singingi Regency in order to generate mutants with desirable traits. Viability and vigour assessments were conducted before subjecting the rice seeds from Kuantan Singingi Regency to gamma-ray irradiation. The findings of these tests revealed that the seeds exhibited a germination capacity of approximately 85%. Consequently, the process of inducing mutations through gamma-ray irradiation was pursued to determine the optimal dosage for subsequent research endeavors.

## 2. MATERIAL AND METHODS

In September 2023, the Isotope and Radiation Application Center (PAIR) conducted gamma-ray irradiation under the National Nuclear Energy Agency (BATAN). This irradiation took place in Pasar Friday, Jakarta. Subsequently, in October 2023, the M0 planting was carried out at the Green House of the Dhabit Farm Experimental Garden located in Kari Teluk Kuantan.

For this research, the materials utilized were the local rice genotypes from the Kuantan Singingi Regency, specifically the Singgam Putih genotype. Topsoil, sawdust, and burnt cow manure were also employed. The tools used in this study included a ruler or meter,

camera, scissors, permanent marker, pen, seedbed, and labels.

Implementing the Research

1) The seeds are put into 6 plastic bags weighing 250 grams each to be irradiated with gamma rays at the following dose:

R0 : 0 Gy (control/no irradiation dose),

R1 : 150 Gy, R2 : 200 Gy,

R3: 250 Gy,

R4: 300 Gy, and R5: 350 Gy.

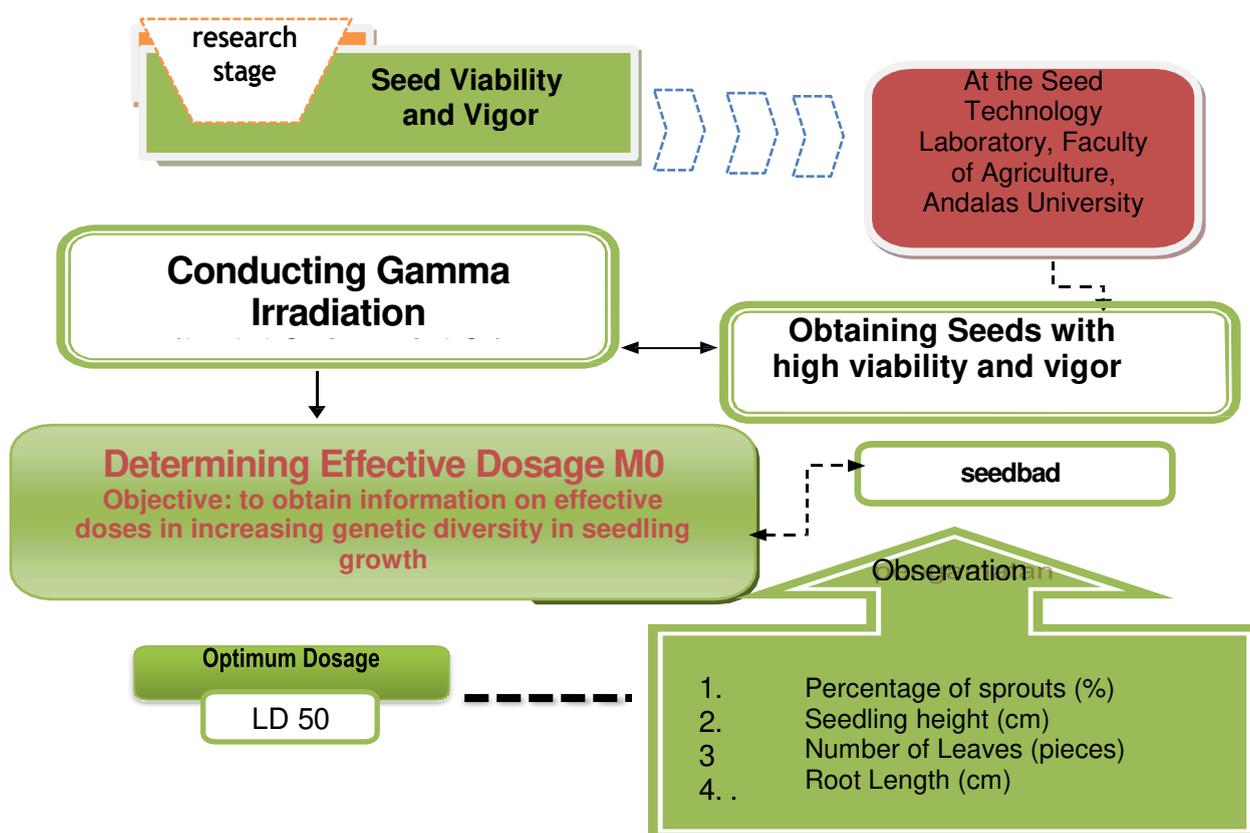
Irradiation exposure is measured in Gray (Gy), where 1 Gy equals 0.01 krad or Jenegri/kg irradiated mass.

2) The dose of each irradiation treatment is by seedling in rows of 1000 seeds. In each seedbed,

there are 200 seeds.

3) The seeds are sown in a seedbed containing media that is ± 5 cm thick; the size of the seedbed is 50cm x 30 cm x 13 cm. The plant media used are topsoil, compost and sawdust in a ratio of 1:1:1.

This research used a non-factorial Completely Randomized Design (CRD). Where is the Singgam Putih (SP) genotype which is local rice from Kuantan Singingi Regency which is treated with gamma ray irradiation at R0 : 0 Gy (Control/no radiation dose), R1 : 150 Gy, R2: 200 Gy, R3 : 250 Gy, R4 : 300 Gy and R5 : 350 Gy. Each treatment



**Figure 1.** Orientation of Effective Irradiation Doses in Genetic Improvement of Local Kuantan Singingi Rice (*Oryza sativa* L.) Using Induced Mutations

The observed parameters in this experimentation are:

1. Percentage of Sprouts (%)

Germination percentage (%) is a parameter often used to measure seed vigor. Germination percentage is the number of regular seeds on the last day of the germination test

and is associated with normal seed potential under field conditions. The germination percentage was observed 11 and 21 days after sowing (HSS). According to ISTA (2024), germination capacity is calculated using the formula:

$$\% \text{ Sprouts} : \frac{\text{Number of Germinating seeds}}{\text{Number of Germinated seeds}} \times 100\%$$

## 2. Seedling Height (cm)

Seedling height is usually used as an indicator of genotypic response to mutagens. The effect of irradiation on seedling height in this study was observed at 21 DAP.

## 3. Root Length (cm)

Root length was observed by measuring the base to the tip of the root at the age of 21 DAP.

## 4. Lethal Dose Value 50 (LD50)

The LD50 value of gamma rays for the Singgam Putih genotype was calculated based on the percentage of sprouts obtained. The data from the latest observations were analyzed using variance analysis using the F test. If the calculated F of the treatment was greater than the F table of 5%, then it was continued with the Duncan Multiple Range Test (DMRT) using STAR (Statistical Tool for Agricultural Research) software, while the Lethal Dose value was 50 (LD50) was analyzed using SPSS.

## 3. RESULT AND DISCUSSION

At the seedling phase, the study conducted an experiment where gamma-ray irradiation was administered at various doses ranging from 0 Gy to 350 Gy. The effects of radiation on germination percentage, seedling height, number of leaves, and root length were then compared using Table 1. The findings of this study reveal the diverse biological responses of rice plants to oxidative stress caused by both acute and chronic irradiation, as well as the survival mechanisms employed by these plants to overcome such stress.

1. Percentage of Sprouts (%) The Singgam Putih genotype rice seedlings germination in generation M0 was monitored 21 hours after sowing. The control group exhibited the highest germination percentage at 85.50% (Table 1). The 0-300 Gy irradiation treatment showed a significant difference from the highest dose treatment of 350 Gy. This difference can be attributed to the increased cell damage at higher doses, leading to a decrease in germination percentage.

Table 1: Germination and Seedling Growth Responses to Various Gamma Irradiation Dose Treatments at the M1 Stage

Irradiation Dose (Gy)	Growing percentage (%)	Seed Height (Cm)	Number of Leaves (Pieces)	Root Height (Cm)	LD50
0	85.50 a	13.40 a	4.80 a	6.06 a	Equation $Y = -1.1668x + 919.92$ LD50 = 313.63 Gy
150	81.20 ab	13.01 b	4.06 a	5.32 a	
200	75.60 bc	11.45 b	4.08 ab	4.46 ab	
250	64.60 cd	10.41 b	4.02 ab	3.88 b	
300	51.20 cd	10.14 b	3.98 b	3.52 bc	
350	48.30 d	6.56 c	3.90, b	3.00 c	
KK	0.45%	0.80%	0.27%	0.77%	

Note: Numbers in the same column followed by the same letters are not significantly different in the 5% DMRT Test

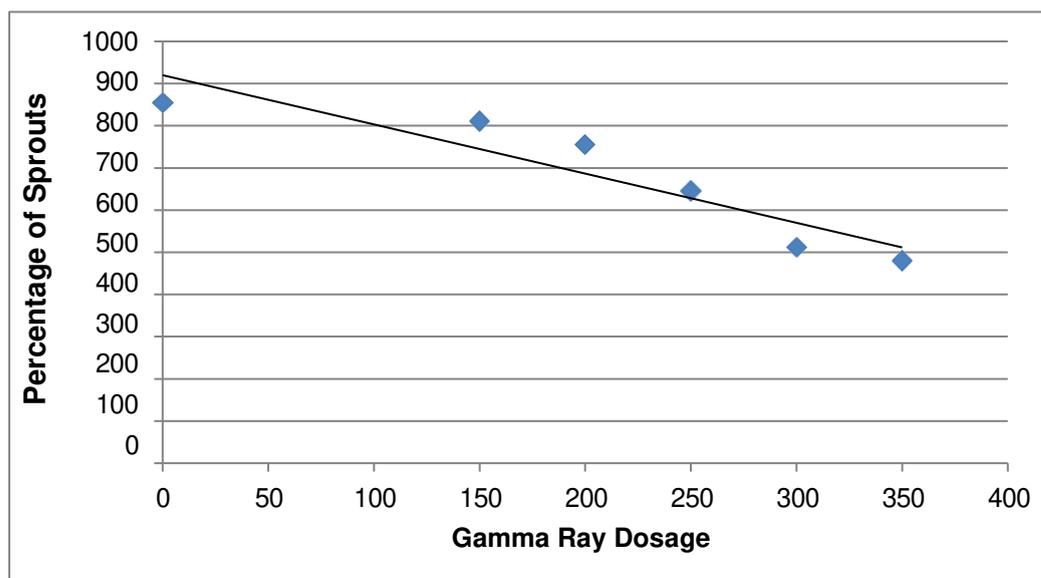
Gamma-ray exposure leads to various outcomes across different dosage levels, as highlighted by Jaipo *et al.* (2019). The most substantial effects are typically observed at the highest dosage, resulting in primarily inhibited

growth, which is why this dosage is employed for sterilization purposes. Conversely, the lowest dosage is commonly utilized to promote and enhance plant germination, whereas the ideal dosage is frequently applied for

mutation induction through breeding techniques. The research findings indicated a notable reduction in germination percentage for the white lion genotype at a dosage of 350 Gy.

According to Tatar *et al.* (2020), the study suggests that mutations occurring in irradiated seed cells at a dosage of 350 Gy create favorable conditions for earlier germination and seedling development compared to other dosages. Conversely, Sari *et al.* (2023) found that the germination percentage of the Madang Pulau and Banang Kuniang genotypes significantly decreased at a dosage of 400 Gy, unlike the Putih Papanai variety. The research also indicates that 100, 200, and 300 Gy dosages increased sprouting percentages (97%, 95%, and 90%, respectively) compared to the control group (87%). However, at dosages of 400, 500, and 600 Gy, there was a notable decrease in the percentage of sprouts.

Jaipo *et al.* (2019) suggested that applying low doses of gamma-ray radiation can enhance enzyme activity, promote the growth of young embryos, and stimulate cell division, which proves advantageous for plant growth during the vegetative phase. Damage induced by mutagens can significantly impact the M<sub>0</sub> generation, as evidenced by alterations in seed germination rates. Furthermore, gamma ray irradiation has been shown to escalate the frequency of damage, particularly at the chromosomal level, as the dosage of irradiation increases. This phenomenon is attributed to the ionization of molecules or atoms, leading to modifications in the DNA of the irradiated material. According to VIENNA (1977), chimaeras' emergence after the irradiation of multicellular meristems hinders the reproductive process in plants utilize.



**Figure 2.** Effect of irradiation on germination percentage in local varieties of Kuantan Singingi Regency generation M<sub>0</sub>

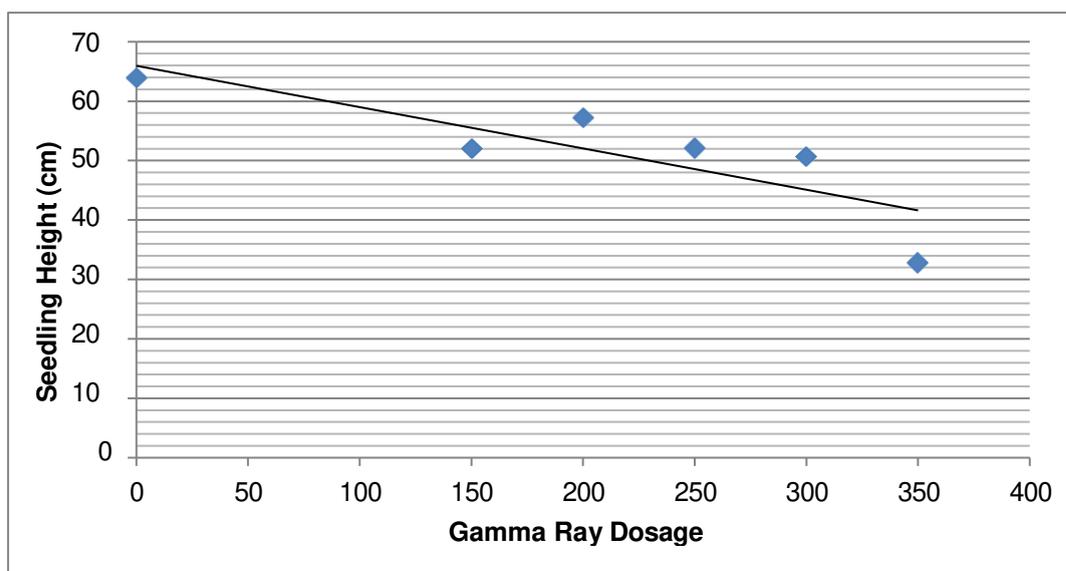
## 2. Seedling Height (Cm)

Seedling height serves as an indicator to assess the genetic response to mutagens. In this particular study, the impact of gamma-ray irradiation on seedling height was examined when the seedlings reached 21 days after planting (DAP). Among all the gamma-ray

irradiation treatments, the tallest seedlings were observed in the control group, which received no treatment and had a height of 13.40 cm. Following that, the treatment with a dose of 150 Gy resulted in seedlings with a height of 13.01 cm. The seed height for the 200 Gy treatment was measured at 11.45 cm, while the 250 Gy treatment yielded

seedlings with a height of 10.41 cm. The lowest seed height was recorded in the 350 Gy treatment, with a value of 6.56 cm (Table 1). Figure 3 visually demonstrates the decline in seedling

growth as the doses of gamma irradiation increase in the Singgam Putih genotype. The varying radiation tolerance levels indicate the response to the reduction in rice growth.



**Figure 3.** Plant Height for All Treatments

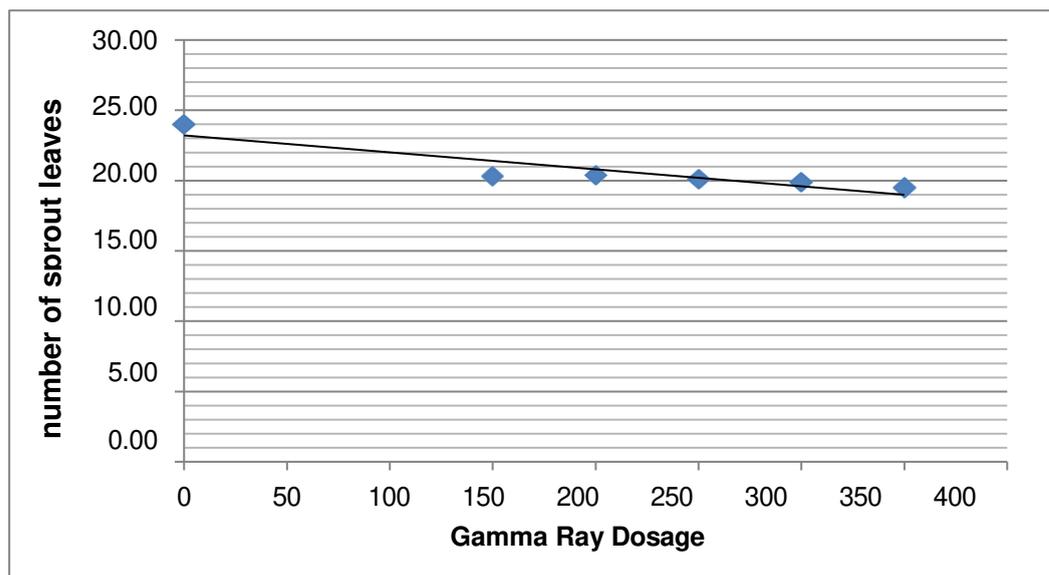
Seedling height decreases as the gamma-ray irradiation increases, as Ramchander *et al.* (2015) and Tabasum *et al.* (2011) state. Other studies have shown that gamma ray treatment leads to a gradual reduction in plant height across all treatments compared to the control group. However, the relationship between the treatments and plant height is not linear, and there is no significant difference in plant height between the control group and all treatments except for the highest dose of 500 Gy, as El-Dgwy (2013) reported. Sari *et al.* found that the seedling height of the Madang Pulau, Putih Papanai, and Banang Kuniang M0 generation genotypes decreased with increasing irradiation dose.

Another perspective, presented by Shu *et al.* (2012), suggests that measuring seed height is widely used as an indicator to determine the biological effects on the M0 generation caused by various physical mutagens, which can impact the plant's embryonic level.

### 3. Number of Leaves (Pieces)

Based on the study's findings, it was

determined that the gamma-ray dose treatment did not impact the quantity of leaves on rice seedlings. However, it did have an effect on the shape of the leaves. The shape of the leaves of the rice seedlings observed on the 21st day of growth did not align with the development of the seedlings, indicating that the increase in leaf longevity did not correspond with higher doses of gamma-ray irradiation. Rohaeni *et al.* (2019) suggested that phenotypic diversity reflects genotypic diversity, which is influenced by environmental diversity and escalating doses. The average number of leaves during the seedling phase was observed to be 4 leaves for all treatments. The most effective treatment was observed in the 0 Gy treatment with 4.80 strands, followed by the 200 Gy treatment with 4.08 strands 150 Gy with 4.06 strands, while the 250 Gy treatment had 4.02 strands. The lowest leaf count was recorded in the 350 Gy treatment with 3.90 leaves, which was not significantly different from the 300 Gy treatment with 3.98 leaves. According to research (Suspidayanti *et al.*, 2021), the identification of rice growth phases.



**Figure 5.** Effect of irradiation on the number of sprout leaves in the local variety Kuantan Singingi Regency generation M<sub>0</sub>

#### 4. Root Length (cm)

According to the study's findings, an increase in the dosage of gamma-ray radiation leads to a gradual decrease in the length of the roots. Gamma-ray radiation can affect the development of roots, causing them to grow less optimally compared to those without radiation exposure. The measurement of root length was conducted on the 21st day after planting. The highest recorded root length was observed in the treatment without radiation exposure (0 Gy), measuring 6.06 cm. This was followed by the 150 Gy treatment with a root length of 5.32 cm and the 200 Gy treatment with a root length of 4.46 cm. The root length in the 0 Gy treatment significantly differed from the 150 Gy, 250 Gy, 300 Gy, and 350 Gy treatments. The treatment with the lowest root length value was the 350 Gy treatment, measuring 3.00 cm (Figure 4). A study conducted by Gowthami *et al.* (2017) supports these findings, stating that an increase in the dosage of gamma radiation can lead to a decrease in the root length of rice seedlings.

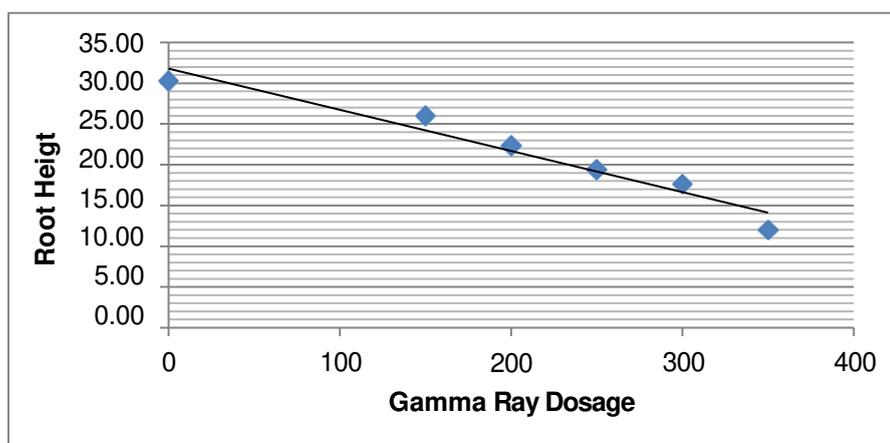
Applying mutagenic treatment in rice plants leads to the inhibition of root length growth. This is primarily caused by suppressing cell mitotic activity in the meristematic tissue, especially when exposed to high treatment doses

(Gowthami *et al.*, 2017). According to the viewpoint presented by Jankowics-Cieslak *et al.* (2017), the development of plant roots is hindered due to the presence of reactive oxygen species, which can negatively impact the transcriptional regulation of the gene responsible for encoding the amylase enzyme. Amylase, an enzyme, plays a crucial role as a catalyst in the hydrolysis of sodium into maltose and glucose. During the phase of root formation, the energy requirements are fulfilled by using maltose and glucose, which the plant extensively utilizes.

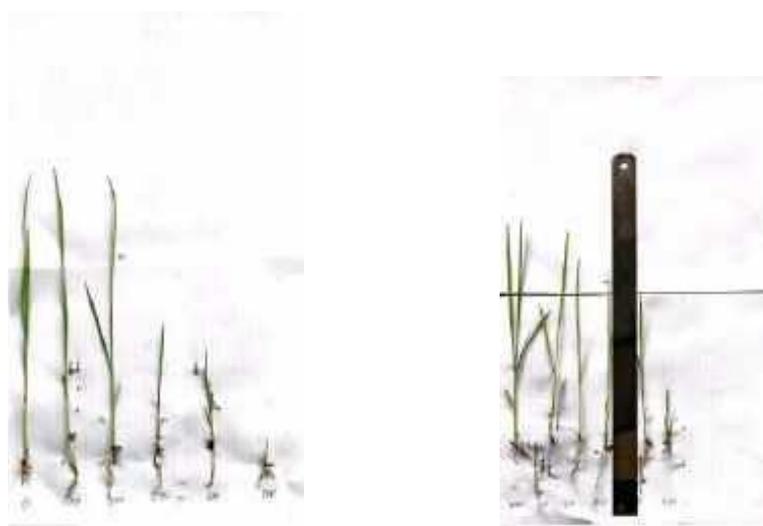
Lethal Dose 50 (LD50) Value for Local Rice in Kuantan Singingi Regency

Different genotypes exhibit varying responses to gamma radiation based on their radiosensitivity, reflecting the sensitivity level to radiation dose. One method to assess radiation sensitivity is by determining the LD50 value. Establishing the LD50 value in the M<sub>0</sub> generation is crucial for identifying the optimal dose to induce significant diversity in the desired trait. A local rice variety from Kuantan Singingi Regency, characterized by the Singgam Putih genotype, underwent irradiation ranging from 0 to 350 Gy with a 50 Gy interval using Cobalt 60 (C60) gamma rays. The LD50 value, representing the radiation dose causing 50% plant mortality in the

Singgam Putih genotype, is calculated through a linear regression equation.



**Figure 6.** Effect of irradiation on germination percentage in local varieties of Kuantan Singingi Regency generation  $M_0$



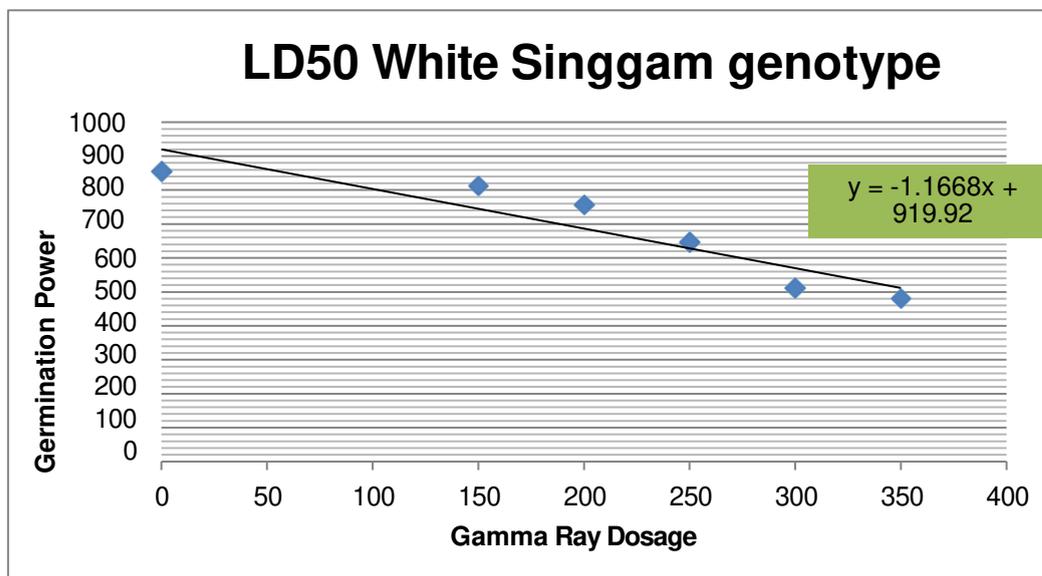
**Figure 7.** Root length of the Singgam Putih genotype

Based on the analysis of germination percentage, it was found that the LD50 value for the white lion genotype was 313.63 Gy. It is important to note that the highest growth ability does not exceed 50%. This implies that if the dose is further increased, the seeds cannot germinate. Gamma-ray irradiation can affect each genotype differently, resulting in varying sensitivity levels. This process of inducing mutations can lead to a significant increase in genetic diversity for the selected traits. However, it is crucial to maintain the original characteristics of the plant. The success in enhancing genetic diversity within a population depends on the radiosensitivity of the irradiated genotype.

The Singgam Putih genotype exhibited a considerable level of sensitivity, as indicated by its LD50 of

313.63 Gy. According to Ramchander et al. (2015), the LD50 values for India's White Ponni and BPT 5204 genotypes were 354.80 Gy and 288.40 Gy, respectively. The White Ponni variety was found to be more susceptible to mutagens compared to BPT 5204. Another perspective provided by Warman et al. (2015) suggests that the LD50 value for local West Sumatra black rice ranges from 300-340 Gy, determined through seedling height and root length analysis. Suliartini et al. (2020) reported LD50 values based on the number of seeds grown in four rice genotypes: G10 (264 Gy), G16 (398 Gy), Baas Selem (316 Gy), and Inpago Unram-1 (518 Gy). In a separate study by Sari et al. (2023), the LD50 values for the Madang Pulau, Putiah Papanai, and Banang Kuniang

genotypes were determined as 333.58 Gy, 377.62 Gy, and 291.14 Gy, respectively.



**Figure 8.** LD50 Value of Gamma Radiation in Three Local Rice Genotypes in Kuantan Singingi Regency

#### 4. CONCLUSION

The control treatment (0 Gy) exhibited the highest germination percentage at 85.50%, followed by the 150 Gy treatment at 81.20%, 200 Gy at 75.60%, 250 Gy at 64.60%, 300 Gy at 51.20%, and 350 Gy at 48.30%. A notable decrease in sprout percentage was observed for the Singgam Putih genotype. The tallest seedlings were observed in the control treatment (0 Gy) at 13.40 cm, while the shortest was in the 350 Gy treatment at 6.56 cm. The height of seedlings in the M0 generation of the Singgam Putih genotype decreased as the irradiation dose increased. The Singgam Putih genotype displayed irregular leaf shapes with increasing irradiation doses but no significant impact on the number of leaves. The heightened gamma radiation dose negatively affected root development, resulting in suboptimal growth compared to controls. The most extended root length was recorded at the control dose (0 Gy) at 6.06 cm, which was not significantly different from the 150 Gy and 200 Gy doses but significantly different from the 250 Gy, 300 Gy, and 350 Gy doses. The shortest root length was observed at the 350 Gy

dose. The LD50 value for the Singgam Putih genotype was determined to be 313.63 Gy based on germination percentage. The growth potential of the local Kuantan Singingi rice mutant was found to be relatively high.

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