Radiosensitivity of rice varieties of Mira-1 and Bestari mutants using gamma rays irradiation

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Abstract. New rice varieties could be released by various plant breeding methods including mutation induction using gamma rays irradiation. Radiosensitivity and LD_{50} values (Lethal Dose, 50%) can be determined from the morphological response of plants to irradiation treatment in M_1 generation. The research aims to determine the value of LD_{50} and the performance of rice mutant traits of Mira-1 and Bestari. The experiment was conducted using the Randomized Complete Block Design (RCBD) with two factors (rice varieties and gamma irradiation doses) and three replications. The plant traits observed were the percentage of germination, seedling height, and root length in the seedling phase. The results showed that LD_{50} values in the Mira-1 and Bestari varieties differed in all observed characters. The optimum dose to induce rice mutation of the varieties under investigation is within the range of 521.40 – 663.68 Gy.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in several countries. In Indonesia, rice is the main food for domestic consumption and always grows up every year. In the past half-decade, rice production and consumption have risen in Indonesia islands [16]. Increasing and optimization of rice production and undesired characters need to be focused on in the future. Most of the objective of plant breeding to save food welfare is to create a new variety that can adapt to various environmental factors. One efficient method for producing a high diversity of plants is the induction mutation technique. Many scientists have made several attempts to determine the most successful mutagenic treatment for the induction of desirable characteristics in rice [3]; [5]; [6]; [8]; [10]; [11]; [13]; [14]; [15].

The mutation breeding method needs preliminary data of optimum dose to result in high diversity using radiosensitivity. Radiosensitivity is a measurement tool to describe the irradiation effect for an irradiated object [9]. Radiosensitivity was used to know the dose that caused lethal dose or inhibited growth of 50% of plant population irradiated (LD_{50}) or 20% (LD_{20}). Determination of lethal dose is the main factor to gain a suitable mutant because the theory on interval dose can reach high diversity. The optimum irradiation dose that can increase plants’ genetic diversity is between LD_{20} and LD_{50} [12]. Each variety or commodity of the plant has a different dose of irradiation to raise high diversity. Research by [3] reported that the interval dose of LD_{50} value for rice is 345 to 423 Gy.
The main role of nuclear technology in plant breeding is to induce mutation on the genetic materials. Nuclear technology has a good source of energy to create many changes in the structure of a plant's genetic matter composition [2]. The shifting can be made rapidly, randomly, and primarily to the next generation, but can conserve the desired character on plant or wild type [7]. Therefore, this research aims to determine the LD<sub>50</sub> value of several growth characters on the rice seedling phase for Mira-1 and Bestari varieties in the M<sub>1</sub> generation.

2. Materials and Methods

The field experiment was conducted from January to August 2019 at a plant breeding greenhouse, Center for the Isotopes and Radiation Application, The National Nuclear Energy Agency of Indonesia (BATAN). The experiment was designed using the Randomized Complete Block Design (RCBD) with two factors. They are rice varieties and doses of gamma rays irradiation. The genetic material used is Mira-1 and Bestari, whether gamma irradiation doses are 0 Gy, 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy, 800 Gy, 900 Gy, and 1000 Gy. There were three replicates which each dose has used 30 seeds. It was exposed to gamma irradiation at Gamma Cell 220 (IGC 220). Mira-1 and Bestari planted at substrate contain soil and manure with a 2:1 ratio. The maintenance was carried out manually based on rice cultivation procedures such as washing, irrigating, weeding, fertilizing, and controlling pest and plant disease.

Observational variables consist of:
1. The percentage of germination: counted the number of seeds germinated at each dose of irradiation manually. The experiments were observed for 7, 14, and 21 days after sowing, respectively. The percentage of germination was calculated using the formula:

\[
\text{% germination} = \frac{\text{amount of germinial seeds}}{\text{amount of sowing seeds}} \times 100\%
\]

2. The seedling height was measured from the root base to the highest leaf tip, which was investigated at 7, 14, and 21 days after sowing, successively.
3. The root length was counted from the root base to the root tip, observed 21 days after sowing.

LD<sub>50</sub> determined using Curve Expert Software. Meanwhile, the seedling and vegetative phase data were analyzed using ANOVA and Tukey's HSD (Honestly Significant Difference) test at P < 0.05.

3. Results and discussion

3.1 The percentage of germination

According to the ANOVA test, gamma rays irradiation doses at the germination of the rice of Mira-1 and Bestari showed highly significant differences. Otherwise, the interaction between varieties and irradiation doses was not significant difference (Table 1). The LD<sub>50</sub> of Mira-1 and Bestari at germination rate was showed in figures 1 and 2.

The ability of seeds of Mira-1 and Bestari rice in M<sub>1</sub> generation for seven days after sowing have shown the highest percentage of germination of Mira-1 rice at the dose of 200 Gy with 98.89% value, while at Bestari rice at doses of 100, 300, and 400 Gy, respectively with the same value. In fact, [18] showed that doses of 200 Gy and 300 Gy influenced the number of seedlings of Bestari and Mira-1, successively [18].

The experiment with gamma irradiation doses of 0-600 Gy at two varieties has significant data than the others (700-1000 Gy). The high dose irradiation caused severe damage to the cell which the percentage of germination decreased (figure 3). A negative correlation was observed between the percentage survival and gamma radiation doses, which is in agreement with the results of [17]. [13] also reported that the treating of seeds with high doses of gamma irradiation showed reducing germination with a corresponding decline in seedlings' growth. The increasing of chromosome damage
frequency was caused by the treatment of gamma irradiation with high doses, which the ability of seed has not a good performance.

Table 1. ANOVA test for percentage of germination of rice mutant of Mira-1 and Bestari

| Source of variation | df  | Sum of Squares (SS) | Mean Squares (MS) | F-value | F-critical |
|---------------------|-----|---------------------|-------------------|---------|------------|
| Group               | 2   | 52,8620             | 26,4310           | 0.70    | 0.5035     |
| Varieties           | 1   | 67,3401             | 67,3401           | 1.78    | 0.1897     |
| Doses               | 10  | 127828,9562         | 12782,8956        | 337.33  | 0.0000     |
| Varieties*Dose      | 10  | 369,6970            | 36,9697           | 0.98    | 0.4785     |
| Standard error      | 42  | 1591,5825           | 37,8948           |         |            |
| Total               | 65  | 129910,4377         |                   |         |            |

Figure 1. The percentage of germination of Mira-1 at seven days after sowing (Using Quadratic Fit with the equation of \( y = 96.8 + 0.03x - 0.0015x^2 \) and \( r = 0.93 \))

Figure 2. The percentage of germination of Bestari at seven days after sowing (Using Quadratic Fit with the equation of \( y = 100.18 + 0.03x - 0.0015x^2 \) and \( r = 0.92 \)
Figure 3. Effect of irradiation on the percentage of rice germination of Mira-1 and Bestari in M1 generation

Table 2. Effect of gamma rays irradiation toward morphology characters in M1 generation

| Varieties | Doses (Gy) | Percentage of germination (%) | Seedling height (cm) | Root length (cm) |
|-----------|------------|------------------------------|----------------------|------------------|
| Mira-1    | 0          | 91.11 ab                     | 24.01 a              | 11.87 a          |
|           | 100        | 95.56 a                      | 22.31 ab             | 10.67 ab         |
|           | 200        | 98.89 a                      | 19.87 bc             | 10.33 ab         |
|           | 300        | 95.56 a                      | 19.99 bc             | 11.03 ab         |
|           | 400        | 96.67 a                      | 18.31 cd             | 9.50 abc         |
|           | 500        | 96.67 a                      | 14.57 d              | 8.70 bc          |
|           | 600        | 72.22 b                      | 9.10 e               | 6.93 c           |
|           | 700        | 13.33 c                      | 3.24 f               | 2.50 d           |
|           | 800        | 0.00 c                       | 0.00 f               | 0.00 d           |
|           | 900        | 0.00 c                       | 0.00 f               | 0.00 d           |
|           | 1000       | 0.00 c                       | 0.00 f               | 0.00 d           |
| Bestari   | 0          | 96.67 a                      | 20.31 a              | 12.57 a          |
|           | 100        | 98.89 a                      | 18.68 ab             | 12.87 a          |
|           | 200        | 96.67 a                      | 18.53 ab             | 12.57 a          |
|           | 300        | 98.89 a                      | 17.58 ab             | 8.97 b           |
|           | 400        | 98.89 a                      | 15.25 bc             | 10.43 ab         |
|           | 500        | 92.22 a                      | 11.92 cd             | 8.47 b           |
|           | 600        | 86.67 a                      | 8.94 d               | 7.90 b           |
|           | 700        | 13.33 b                      | 3.15 e               | 2.67 c           |
|           | 800        | 0.00 c                       | 0.00 e               | 0.00 c           |
|           | 900        | 0.00 c                       | 0.00 e               | 0.00 c           |
|           | 1000       | 0.00 c                       | 0.00 e               | 0.00 c           |

The treatment of gamma rays irradiation doses with morphology character at the seedling phase will be continued with Tukey’s HSD test at P < 0.05. Mean within a column followed by the same letter are not significantly different (p<0.05)
[11] reported that increasing frequency of chromosome damage was caused by increasing doses of gamma irradiation which atomic or molecule ionization has been caused DNA changes simultaneously on the genetic matter which has irradiated. The genetic materials containing water irradiated will react with the nitrogen base of DNA or change genetic matter such as amylase enzymes. Changes in DNA caused the increasing base of ionization and base deletion, which can inhibit seed germination. Free radicals can be formed as long as radiation processing and changed plant cell components such as morphology, biochemistry, and plant physiology [4].

3.2 Seedling height
This research investigated that the dose of 0 Gy (control) showed the highest seedling in M1 generation of Mira-1 and Bestari, which are 24.1 cm and 20.31 cm, respectively (Table 2). The findings indicated that both varieties had shown significant differences in the control treatment. [14] reported that the unirradiated rice seeds exhibited the highest shoot and root length of rice seedlings. Based on variance analysis, one interesting finding is the varieties and doses of irradiation influenced the seedling height of rice mutant. Meanwhile, the variable interaction is not impacted (Table 3).

Table 3. Variance analysis of the seedling height of rice mutant of Mira-1 and Bestari at 21 days after sowing

| Source of variation          | df | Sum of Squares (SS) | Mean Squares (MS) | F-value | F-critical |
|-----------------------------|----|---------------------|-------------------|---------|------------|
| Groups                      | 2  | 5,1597              | 2,5799            | 0,96    | 0,3930     |
| Varieties                   | 1  | 39,5638             | 39,5638           | 179,63  | 0,0004     |
| Doses of Gamma Irradiation  | 10 | 4851,8928           | 485,1893          | 14,65   | 0,0000     |
| Varieties*Doses             | 10 | 36,7567             | 3,6757            | 1,36    | 0,2316     |
| Standar Error               | 42 | 113,4430            | 2,7010            |         |            |
| Total                       | 65 | 5046,8160           |                   |         |            |

Figure 4. Effect of gamma rays irradiation to seedling height of Mira-1 and Bestari mutants in M1 generation. (A) Mira-1; (B) Bestari
The decreasing of the seedling height of Mira-1 and Bestari observed in contrast with gamma rays irradiation doses (figure 4). Similarly, [6] reported the same findings at rice varieties of CO 47 and white ponni. [10] explained that increasing seedling height caused by gamma irradiation could change the mitotic process in the cell. The seed which has irradiated will disrupt DNA and protein synthesis and hormone stability. According to [4], gamma irradiation treatment also can block auxins hormone synthesis, which has a main role to plant growth.

Figure 5. The LD₅₀ value of the seedling height of Mira-1 at 21 days after sowing. (Using Quadratic Fit with equation of \( y = 104.55 - 0.09x - 0.00005x^2 \) dan \( r = 0.97 \))

Figure 6. The LD₅₀ value of the seedling height of Bestari at 21 days after sowing. (Using Quadratic Fit with equation of \( y = 105.51 - 0.08x - 0.00005x^2 \) dan \( r = 0.97 \))

This study found that the LD₅₀ value in the seedling height of Mira-1 and Bestari are 521.40 and 539.68 Gy, respectively (figure 5 and 6). These varieties result in high diversity in the M₁ generation due to the LD₅₀ effect. However, it will be able to change many genes, which will affect the diversity of plants in the following phase and next generation. [8] explained that increasing doses above 400 Gy caused severe morphological damages to the rice plants. Increased doses of gamma irradiation negatively correlated with germination and other plant growth parameters. [3] reported that physiological damage at above 500 Gy on seedling height became more severe as none of the varieties
survived. Moreover, doses above 800 Gy will cause physiological damage to the seedling of plant height and root length, while none of the seedlings were survived [14]. Therefore, this study will be a recommendation for gamma rays irradiation doses for rice mutation breeding.

### 3.3 Root length

This research found that the Mira -1 mutant had a root length of almost 12 cm with a dose of 0 Gy (control), which is the highest in root length variable (Table 2). Meanwhile, at Bestari mutant showed 12.87 cm using 100 Gy of irradiation dose. Another critical finding is at 1000 Gy can physiologically inhibit plant growth, including root length.

The ANOVA test showed significant differences in irradiation dose to root length character (Table 4). The height dose of irradiation can retardant the growth and development of root plants at the varieties (Figure 7). Similar studies on rice have been documented by [10] revealed similar findings.

**Table 4. Variance analysis of root length character of Mira-1 and Bestari rice mutants**

| Sources of variation | df  | Sum of Squares (SS) | Mean Squares (MS) | F-value | F-critical |
|----------------------|-----|---------------------|-------------------|---------|------------|
| Groups               | 2   | 1,9876              | 0,9938            | 0,94    | 0,3986     |
| Varieties            | 1   | 3,2741              | 3,2741            | 3,10    | 0,0857     |
| Doses                | 10  | 1535,3912           | 153,5391          | 145,26  | 0,0000     |
| Varieties*Doses      | 10  | 21,4409             | 2,1441            | 2,03    | 0,0543     |
| Standard Error       | 42  | 44,3924             | 1,0570            |         |            |
| Total                | 65  | 1606,4862           |                   |         |            |

**Figure 7.** Effect of irradiation dose to root length at Mira-1 and Bestari in M1 generation

[10] stated that growth inhibition of root length was caused by a high dose of irradiation which can block cell mitotic activity at meristematic tissue. On the other hand, the inhibition of root growth was also caused by free radicals, damaging the amylase enzyme's promoter gene. This enzyme has the
main role as a catalyst to change starch into maltose and glucose. The maltose and glucose supply energy for the germination process and root form at the seedling phase.

![Figure 8. LD50 value for root length of Mira-1 in M1 generation (Using Quadratic Fit with the equation of y=100-0.03x-0.00005x2 and r=0.96)](image)

We investigated that LD50 value for root length of Mira-1 and Bestari was 604.22 and 581.37 Gy, respectively (Figure 8 and 9). One interesting finding is that these values are higher than other studies of LD50, which has been reported. Based on these findings, some variable tests were genetic materials of Mira-1 and Bestari, irradiated from wild type. Then water content of the seed also becomes the main factor to the high level of LD50.

![Figure 9. LD50 value for root length character of Bestari in M1 generation (Using Quadratic Fit with the equation of y=104.02-0.05x-0.00005x2 and r=0.96)](image)

Root length of Mira-1 and Bestari increase at 300 Gy and 400 Gy, successively. [1] reported that a low dose of gamma-ray could create a plant growth that affects plant growth hormone in a plant cell or increased antioxidant capacity in a cell. [4] also explained that the increased capacity of antioxidants
in the cell was caused by a low dose of irradiation treatment that can survive from abiotic stress and light and thermal change.

We reported that the interval of LD$_{50}$ value of Mira-1 and Bestari in M$_1$ generation is 521.40 - 683.68 Gy. Interestingly, the interval of dose in this research is higher than LD$_{50}$ in other rice experiments. [3] reported that LD$_{50}$ for 13 varieties of Sierra Leone rice was 345-423 Gy, which was analyzed based on the germination percentage. Besides, [15] also reported that the LD$_{50}$ value of local black rice from West Sumatera was 300-340 Gy, calculated based on seedling height and root length of the plant. LD$_{50}$ value can determine the percentage of germination, seedling height, and root length [15].

LD$_{50}$ value reached Mira-1 and Bestari showed that the varieties have lower irradiation treatment sensitivity than the others. The sensitivity of irradiation was affected by the water content in the rice kernel. We investigated that Mira-1 and Bestari in M$_1$ generation result in the difference of LD$_{50}$ value (Table 1). The findings showed that the LD$_{50}$ value of each plant is different among genetic plants. [1] stated that each plant variety has unique responses toward variation doses of gamma-ray. On the other hand, radiosensitivity also showed a variation on the plant, based on object size and irradiation treatment to object before and after mutagenic treatment [12]; [9]. The most efficient and optimum dose to induce rice mutation of Mira-1 and Bestari in M$_1$ generation under investigation is within the range of 521.40 – 663.68 Gy. Besides LD$_{50}$, plants' irradiated sensitivity can be observed from the percentage of germination, seedling height, and root length in M$_1$ generation [15].

4. Conclusion
The LD$_{50}$ value of Mira-1 and Bestari in the M$_1$ generation was 521.40 – 683.68 Gy. LD$_{50}$ value at two varieties is higher than the other researches of rice mutant. This experiment was assumed that the irradiated plant's sensitivity level is one of the factors that affect LD$_{50}$ value. Besides, water content and technique factors are also implicated in determining the optimum dose of high diversity. LD$_{50}$ value this experiment results in severe damage. However, another alternative way to create high diversity is with LD$_{20}$ with an interval dose of 200-300 Gy on varieties or genetic materials.

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