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Radiosensitivity and Seedling Growth of Several Genotypes of Paddy Rice Mutants Irradiated with Gamma Rays at Different Doses

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***Abstract*— Researchers use mutation induction in rice to create high genetic diversity. The basic population with high genetic diversity will facilitate the selection process for the desired good characters. This study aimed to determine the optimal dose that induces the highest genetic diversity in four lowland rice genotypes. The research materials were four genotypes of lowland rice, namely "G10", "G16", "Baas Selem", and "Inpago Unram-1". Gamma irradiation was carried out at the Center for Isotope and Radiation Application (PAIR) BATAN. Each genotype was irradiated at doses of 200, 300, 400 and 500 Gy. The seeding is done in the glasshouse of the Faculty of Agriculture, University of Mataram. Observations were made on the number of growing seeds, plant height and number of leaves. The LD50 value was determined based on the results of the regression analysis of the number of growing seeds at the four irradiation doses plus control (0 Gy). The results indicated that (1) the numbers of growing seeds decreased as the doses of gamma irradiation increased, (2) the LD50 value of the four rice genotypes ranged from 264 to 518 Gy, (3) the optimal dose of gamma ray irradiation for G10, G16, Baas Selem, and Inpago Unram-1 were 264 Gy, 398 Gy, 316 Gy and 518 Gy, respectively. (4) Among the four rice genotypes teasted, "G10" mutant was the most sensitive to gamma ray irradiation, whereas" Inpago Unram-1" mutant was the least sensitive genotype.*
***Keywords*— Irradiation, paddy rice, gamma ray, sensitivity, LD50.**

I. INTRODUCTION

Rice is one of the main carbohydrate producing crops in the world, especially in Asia, and most of the Asian population consumes rice as a staple food [1]. The Asian continent also produces 90% of the world's rice needs. The increasing number of world population causes rice production to be increased to meet food needs [2]. The United Nations estimates that by 2030 it will grow to reach 8.5 billion people and by 2050 it will reach 10.6 billion people. This will be a problem that will threaten the fulfillment of proper food needs. Therefore, all efforts for increasing rice production worldwide need to be prepared before this food and population problems come.

Various programs have been done to increase rice production. Increased production can be pursued, among others, through the use of new high yielding varieties [3-8], better cultivation techniques [2, 9], increasing planting intensity [10] and opening of new rice planting areas [11]. By invention of new early-maturity, high-yielding varieties, especially IR5 and IR8 through plant breeding programs done by IRRI in the 1960s, world rice production increased dramatically [12].

One of the initial steps of plant breeding programs that can be used to genetically improve productivity and/or quality of crops is induction of mutations in seeds and other planting materials using both physical and chemical mutagenesis [13]. Liu *et al*. [14] and Efendi *et al*. [15] stated that induction of mutation in order to improve the agronomic characters of crops can be done through the application of gamma ray irradiation. Mutations can produce populations with a high degree of genetic diversity as the basis for selection [16]. Most of the

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mutation induction is done by gamma ray irradiation. According to Nunoo *et al.* [17], gamma rays have the advantage that the dosage used is more accurate, the penetration of irradiation into cells is homogeneous and produces new gene combinations with high mutation frequencies, in addition to its ability to improve one character without changing good characters in the plant.

The success of developing mutants is largely determined by the amount of irradiation dose applied. In general, the higher the irradiation dose applied, the chance for the formation of mutants will be higher, along with the higher degree of damage that will occur. Therefore, it is necessary to know the optimum dose that induces the formation of the most mutants. In addition to dosage, the success of mutant formation is also determined by the levels of oxygen and water molecules in the material to be irradiated. Herison *et al*. [18] revealed that the more water and oxygen molecules in the irradiated material, the more free radicals are formed, which causes mutations. Mutants with the desired character were generally acquired at or slightly below the LD50 value [19-21], which is the dose that causes death in as much as 50% of the irradiated population [19, 21].

LD 50 is one of the parameters used to determine the response of plants to gamma ray irradiation [22], in addition to the decreased rate of seedling growth due to gamma ray irradiation [16]. The response of plants to gamma ray irradiation determined by the level of sensitivity of a substance or material (radiosensitivity). The lower the LD50 value, the more sensitive a material is to gamma irradiation, and vice versa [16]. The levels of radiosensitivity vary depending on the species, cultivar, plant organs and physiological conditions [24].

Various studies have been conducted to obtain plant responses to gamma ray irradiation. The response of plants to gamma ray irradiation has been reported by several researchers, such as on wild tomato [17], banana [20], cassava [22], chili [23], sorghum [19, 25], Wilman lovegrass [26], rice [15, 16, 27, 28], and mungbean [29]. This study aimed to determine the optimal dose of gamma irradiation which induces the highest genetic diversity in four genotypes of lowland rice.

II. MATERIALS AND METHODS

The genetic material used was the seeds of four lowland rice genotypes, namely the G10 line, the G16 line, the “Baas Selem” cultivar, and the superior variety “Inpago Unram-1”. Those rice seeds were packed in brown bags containing 500 seeds per bag and labeled according to the treatment. The seeds were irradiated at the doses of 200, 300, 400, and 500 Gy in the “Gammacell 220” gamma ray radiator belonging to the Center for Isotope and Radiation Application (PAIR)-BATAN. Planting of the irradiated seeds were carried out in the glasshouse of the Faculty of Agriculture, University of Mataram. Mutant M1 seeds are planted in nursery tubs filled with planting media in the form of a mixture of soil, sand and vermicompost fertilizer in a ratio of 1: 1: 1. Non-irradiated seeds of each rice genotype were used as control. Each tub was planted with mutant seeds from one treatment dose with different genotypes to obtain 20 seedbeds.

The median lethal dose (LD50) was calculated based on the number of seeds that survived the different mutagen doses [29] and linear regression analysis [28]. The LD 50 value was determined. Observations of the number of seeds that grew into rice seedlings, plant height and number of leaves were counted on the 14th day after seeding. Data were analyzed using Microsoft Excel for Windows.

III. RESULTS AND DISCUSSION

Gamma ray irradiation is used by researchers to create high genetic diversity. Genetic diversity is very important in plant breeding programs. The first step to initiate a mutant-based plant breeding program is to determine the optimal dose of gamma ray irradiation [26]. According to Human [30], the highest genetic diversity will be obtained at the LD50. This is because the level of damage caused by gamma ray irradiation is proportional to the high rate of mutations induced.

Induced mutation with gamma ray irradiation aims to produce a basic population with a high level of genetic diversity which will then be selected through further breeding programs. The mutants produced after going through a series of selections can be immediately released as superior varieties or as cross parents in the subsequent breeding programs.

G10 and G16 rice lines are cross lines that have weaknesses in one or more characters. The G10 line had a weakness in the number of unfilled grains which was still quite high, while the G16 line had a low number of grains per panicle. “Baas Selem” is a Balinese local cultivar (parent of the G10 line) which has low productivity, while Inpago Unram-1 is a national superior variety whose character changes due to gamma irradiation are wanted to be determined.

From Fig. 1 to Fig. 4, it can be seen that the higher the irradiation doses applied to the seeds, the fewer the number of seeds grew into seedlings. This is because gamma ray irradiation causes damage to DNA due to

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mutation of genes and chromosome structure. The same results were also reported by Nura *et al*. [23] on chili, Suliartini et al. [16] and Kumar *et al*. [28] on rice, and Alvarez-Holguin [26] on Wilman lovegrass (*Eragrostis superba* Peyr.).

The quadratic regression y = -0.0004x2 - 0.789x + 499.39 resulted in an LD50 value of 277 Gy in the G10 rice line (Fig. 1). This value indicates the level of sensitivity of G10 seeds to gamma ray irradiation. The highest genetic diversity in G10 was obtained at a gamma ray irradiation dose of 277 Gy, meaning that the chances of obtaining the desired characters were found at this level of irradiation dose. Astuti *et al*. [25] reported that the optimum dose ranges to induce genetic diversity in two sorghum genotypes ("Konawe Selatan" and "Sorghum Malai Mekar") were 300 to 350 Gy.

Based on the relationship between the gamma ray irradiation doses and the number of seeds that grew into seedlings in the G16 rice line, a lethal dose of 50% was obtained at a dose of 408 Gy (Fig. 2). This value is lower than the LD50 value in the Inpago Unram-1 mutant line (518 Gy) (Fig. 4). Harding *et al*. [31] obtained LD50 values in 13 rice varieties irradiated at doses of 50–800 Gy ranging from 345 to 423 Gy.

The LD50 of "Baas Selem" rice mutant was obtained at the irradiation dose of 316 Gy (Fig. 3). The mutant growing ability of the local cultivar "Baas Selem" was higher than that of the G10 rice line but lower than that of the G16 rice line. This can be seen from the number of seeds that grew into seedlings and the LD50 values. At a dose of 500 Gy, no seeds survived until 14 days of germination, in contrast to the results of the study by Harding *et al*. [31] with seeds survive up to a dose of 600 Gy. This could be due to the different sensitivity of different genotypes to gamma ray irradiation

Mutation inductions are expected result in high diversity in the traits to be selected while maintaining the original good characters in the plants. The success rate of gamma ray irradiation in increasing the genetic diversity of a population is determined by the radiosensitivity of the irradiated genotype [32]. The sensitivity of Inpago Unram-1 was the lowest compared to the other three genotypes tested, which is indicated by the highest LD50 value of Inpago Unram-1 rice mutants (518 Gy).

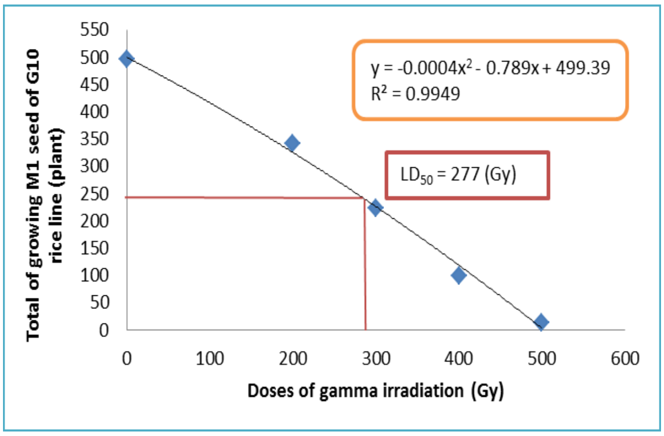

Fig. 1. LD 50 of the "G10" rice line, based on the number of growing M1 seeds of the G10 mutants irradiated with different doses of gamma rays

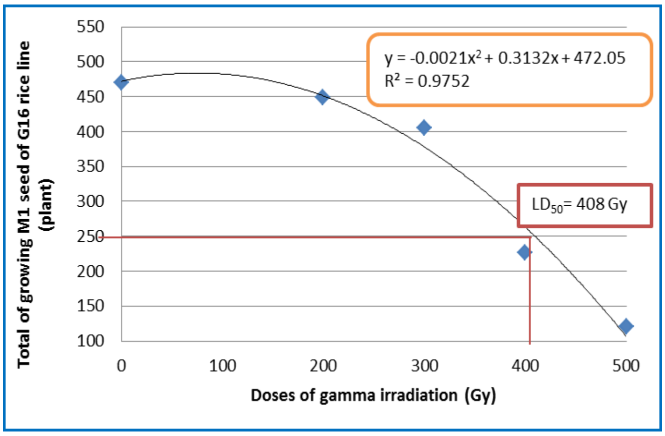

Fig. 2. LD 50 of the "G16" rice line, based on the number of growing M1 seeds of the G16 mutants irradiated with different doses of gamma rays

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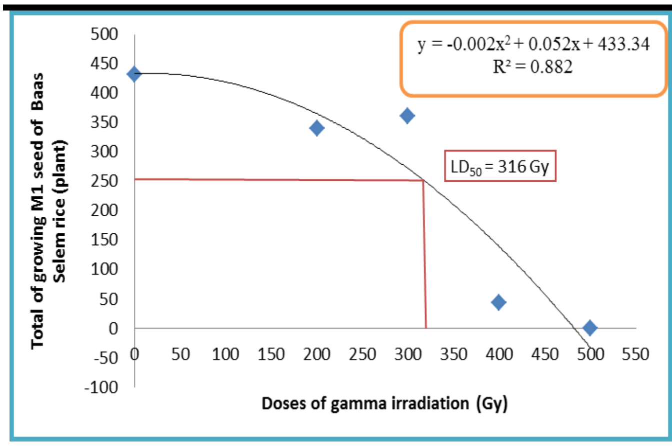

Fig. 3. LD 50 of the "Baas Selem" rice variety, based on the number of growing M1 seeds of the Baas Selem mutants irradiated with different doses of gamma rays

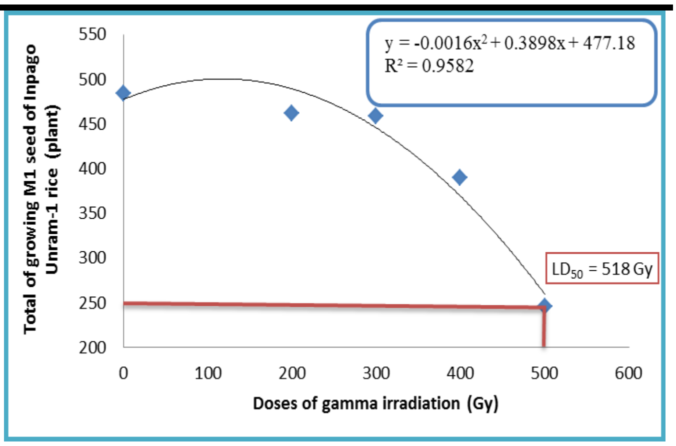

Fig. 4. LD 50 of the "Inpago Unram-1" rice variety, based on the number of growing M1 seeds of the Inpago Unram-1 mutants irradiated with different doses of gamma rays

Table 1. Seedling height and leaf number on the seedlings of rice mutants of several genotypes irradiated at different doses of gamma rays

Doses of gamma ray irradiation (Gy)	Seedling height (cm)				Leaf number			
	G10	G16	Baas Selem	Inpago Unram-1	G10	G16	Baas Selem	Inpago Unram-1
0	24.93	25.19	23.52	22.04	4.10	3.50	4.02	3.14
200	19.55	24.07	20.73	21.11	3.22	3.16	3.26	3.04
300	16.11	16.20	19.13	19.25	3.48	3.52	3.30	3.42
400	12.86	14.53	16.89	17.06	3.30	3.44	3.84	4.10
500	12.94	16.40	0.00	23.65	2.93	4.18	0.00	4.02

The sensitivity of the four genotypes tested was higher than the two upland rice genotypes tested by Suliartini *et al*. [16], i.e. 195 Gy on red rice and 202 Gy on black rice. Fuji and Matsumura found that the LD50 ranged from 200 to 500Gy for the japonica variety and from 200 to 650 Gy for the indica variety [33].

In terms of seedling growth, seedling height decreased as irradiation doses increased, except for Inpago Unram-1 whose height was highest at 500 Gy (Table 1). This is due to different plant sensitivity and random mutations that give different responses. Harding *et al*. [31] also reported a decrease in M1 mutant seedling height and M1 survival percentage under field conditions. A significant reduction in seedling height due to increases in mutagen dose also occurred in Wilman lovegrass [26], roselle [34] and pigeon pea [35].

The number of leaves showed a different response. The "G10" and "Baas Selem" mutants had lower leaf number than the control (0 Gy). On the other hand, the "G16" and "Inpago Unram-1" mutants increased leaf numbers at random irradiated doses (Table 1). This is because gamma irradiation causes random mutations in genes and plant chromosomes.

The number of rice mutant leaves decreased at 200 Gy, then increased at 300 and 400 Gy (Table 1). At a dose of 500 Gy there was a variation, in which leaf number of G10 and Inpago Unram 1 decreased, while that of G16 increased. It is suspected that this was due to the different sensitivity of the genotypes to gamma ray irradiation.

IV. CONCLUSION

It can be concluded that the number of growing seeds decreased as the doses of gamma irradiation increased; the LD50 value of the four rice genotypes ranged from 264 to 518 Gy; and the optimal dose of gamma irradiation for G10, G16, Baas Selem, and Inpago Unram-1 mutants were 264, 398, 316, and 518 Gy, respectively. Among the genotypes tested, "G10 rice line" mutant was most sensitive whereas "Inpago Unram-1" mutant was least sensitive to gamma ray irradiation.

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