The Sensitivity of *Leersia hexandra* Sw. to Gamma-Ray Irradiation

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Abstract

Gamma-ray irradiation as a physical mutagen has high penetrating power. Therefore, it is most often used to increase genetic variability or produce new mutant plants. This research was conducted to obtain the lethal dose of gamma-rays in *Leersia hexandra* plants. The used plant part was a single node stolon which had a length of 10 cm with the node in the middle of the stolon. The irradiation doses given were 0, 25, 50, 75, 100 Gy. Stolons were inserted into plastic clips and irradiated using a Cobalt-60 gamma irradiation source at the Center for Irradiation and Radioisotope Applications (PAIR), National Nuclear Energy Agency (BATAN) Jakarta. Gamma-irradiation has a significant effect on inhibiting shoot growth. The growth of irradiated *Leersia hexandra* with the best number of plants and the highest shoots was obtained at a dose of 25 Gy and decreased with increasing irradiation dose. The lethal dose (LD_{50}) was determined eight days after irradiation using CurveExpert 1.4 software. *Leersia hexandra* plants that can sprout and regenerate followed the linear equation y = 1.02 - 7.5x with LD_{50} at 68.85 Gy and LD_{20} at 29.36 Gy.

Keywords: gamma irradiation, genetic variation, *Leersia hexandra*, lethal dose, mutant plant.

INTRODUCTION

Forage is one of the determining factors in the development of the ruminant livestock business. The obstacle faced by breeders is the availability of a fairly good variety of forages in terms of quantity and nutritional quality. One plant that has been used as forage for livestock is the swamp plant *Leersia hexandra*. According to Riswandi [1], plants that grow in swampy areas have the potential to increase the feed diversity for livestock.

The natural swamp plants generally have limitations in their nutritional value, such as their protein content. Natural grass has a scarce protein content which is around 4% [2]. Due to overcome this limited nutritional value, a plant engineering effort is needed.

The engineering to obtain new varieties of plants can be done by mutation induction. In this method, there are two mutagens used, namely chemical mutagens and physical mutagens. Chemical mutagens include EMS (ethyl methanesulfonate), while physical mutagens include infrared and gamma rays.

Physical mutagens are influenced by the frequency and spectrum of irradiation as well as depending on the dose and dose rate used. Physical irradiation is very efficient in producing changes in genetic material [3]. Mutation by physical induction has become a technique for cultivar improvement. It is very effective in increasing natural genetic resources to obtain the desired mutant characteristics [4]. The unit of irradiation dose used in Gray (Gy), which is equivalent to the absorption of one Joule of radiation energy per kilogram of irradiated material [5]. Plants have a certain sensitivity value to irradiation, hereinafter referred to as Lethal Dose (LD). LD is measured at doses that cause death in plant populations [6].

A radiosensitivity assessment is carried out to determine the LD_{50}, which is the safe dose plants can survive to germinate in as much as 50% of the population. LD_{50} can be used as a basis for breeders to increase plant genotypes [7]. Aisyah [8] stated that the LD_{50} was different for each type of plant depending on the stage of growth, plant development, and the part of the plant that was irradiated. Instead of plant species, several factors might respond differently to gamma irradiation, including ploidy level, plant development stage, and physiological factors. Also, oxygen and water molecules (H_2O) contained in the material exposed to irradiation were directly proportional to the free radicals formed, which caused plants to be more sensitive to exposure to gamma irradiation [9].

Information on the optimum dose of irradiation is needed for researchers to produce mutant plants. Mutant plants obtained from gamma irradiation are generally at or slightly below the LD_{50} value [10]. Therefore, this study aims to determine the sensitivity of *Leersia hexandra* plants through analysis of LD_{50} values.

MATERIAL AND METHOD

The research was carried out in the experimental garden of the Beef Cattle Research Station. The gamma irradiation was conducted at

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the Irradiation and Radioisotope Application Center (PAIR), National Nuclear Energy Agency (BATAN), Pasar Minggu, Jakarta. The used plant material was *L. hexandra* stolon obtained from the experimental garden collection of the Beef Cattle Research Station. A stolon with one node was taken from a mature plant. It was chosen from the lower part near the roots of each stem. The stolon was 10 cm long, with the node in the middle of the stolon. The single-node-stolon pieces were stored in a plastic clip [11].

Gamma irradiation was conducted at doses of 0 (control), 25, 50, 75, 100 Gy using a gamma irradiation source Co-60, and temperature in the chamber 35-40°C. Each treatment dose consisted of 100 replications. The stolons as a control that were not irradiated by gamma rays remained in the plastic clip to keep the stolons moist until implanted. Immediately after irradiation, the stolons were implanted [11].

The used planting medium was soil, husk charcoal, and compost with a volume ratio (1: 1: 1). Stolons were planted in the planting medium in seedling polybags of 15x10 (cm) size then the medium was maintained to be moist. The air humidity also was maintained by providing a plastic cover after the stolons were planted in seedling polybag seedling; the used lighting was natural sunlight [12].

Observations were made on the sprouted stolons eight days after irradiation. The preliminary research results showed that the number of shoots growing from 100 stolons without irradiation was 69 shoots (control). Therefore, the percentage of the lethal dose was calculated based on the number of these living individuals. The lethal doses of 20 (LD_{20}) and 50 (LD_{50}) were determined by the CurveExpert 1.4 analysis program to find the best model equation. Further, plant shoot height was measured from the soil surface to the tip of the plant growth point. Plant height data were grouped into 4 clusters, including a height range of 0.5-4.5; 4.6-8.5; 8.6-12.5; 12.6-16.5. Individuals with height in each of these clusters were counted [13].

RESULT AND DISCUSSION

Shoot growth

L. hexandra plants reproduce vegetatively using stolons. Stolons are extensions of plant stems that spread horizontally above the soil surface and have a role as vegetative propagation organs for plants [14].

As explained in the materials and methods section, the preliminary study showed that *L. hexandra,* which did not obtain the irradiation dose, was able to grow as many as 69 individuals per 100 stolon samples. Therefore, the calculation of the percentage of shoot growth in subsequent treatments was based on this preliminary study. The percentage of shoot growth from *L. hexandra* stolons observed at the age of 8 days after irradiation obtained results as shown in Table 1.

Table 1. Percentage of Growing Shoots from Stolons

No	Irradiation Dose (Gy)	Percentage (%)
1	0	100*
2	25	84
3	50	62.5
4	75	50.7
5	100	21.7

Note: * 69 individuals living of 100 replicates

Based on table 1, the 25 Gy irradiation dose produced the survived stolons with a growth percentage of 84%. Similar to studies using the grass stolon Augustine genotype FA-243, Floratam, and the green mutant, at a dose of 3000 rads or 30 Gy irradiation, they produced the greatest number of lives [15].

At different irradiation doses, the response of survived stolons was different. Also, the higher the irradiation dose, the number of stolons that grown shoots decrease. The same results were shown by Zanzibar and Dede's research, that the highest irradiation dose of 100 Gy resulted in plants growing at the lowest as about 5.33% on *Magnolia champaca* [16]. It was due to the greater the dose of irradiation given, the more severe cell damage occurred, then plants could not survive and die [17]. Moreover, according to Al-Safadi *et al.*, the use of low-dose gamma-ray irradiation potentially stimulated plant growth in vivo [18].

The decrease in the number of plants that could sprout at higher mutagen doses might be due to cellular, physiological or physical disturbances. A study using Bermuda grass stolon showed that the higher the irradiation dose used, the percentage of shoot growth also decreased [11]. The same thing happened to chickpea seeds that received gamma irradiation, the higher the irradiation dose, the lower the percentage of germination [19].

Lethal dose

The success of mutations using gamma-ray irradiation depended on the irradiation dose used. The mutagenic effectiveness was decreased with the increase in the mutagen dose used. It indicates that there was a negative relationship between both of them. The results of data analysis using CurveExpert 1.4 were presented in Figure 1. The LD_{50} value was obtained at a dose of 68.85 Gy while LD_{20} was at a dose of 29.36 Gy, with the linear equation y = 1.02-7.56x

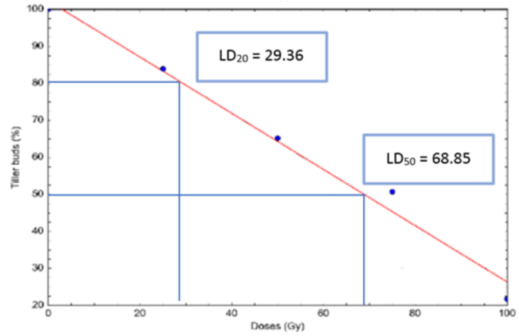

Figure 1. Determination of LD_{20} and LD_{50} in *L. hexandra*

The sensitivity of plants to gamma-ray irradiation varies, for example, the LD_{50} in Bermuda grass studies showed the irradiation that caused 50% plant mortality was at a dose of 85.45 Gy [11], whereas on Augustine grass ranged from 30 - 48.5 Gy [15], on *Anthurium andreanum* plants at dose 22.37 Gy [20], on *M. champaca plant* at dose 30 Gy [14]. It indicated that the LD_{50} yield is different for each species and also for varieties.

The level of plant sensitivity to gamma irradiation is affected by physical and biological factors. Physical factors, including plant morphology, may affect the physical resistance of cells in receiving gamma irradiation. Meanwhile, biological factors include genetic factors and environmental factors (oxygen, the water content of irradiated material, storage treatment after irradiation, and temperature) [17].

Plant height

Gamma-ray irradiation in small doses gave a positive response to plant height. The number of plants in each group/plant height range was presented in Table 2. The results of the 25 Gy irradiation produced plants that were higher than the control plants.

The irradiation dose of 100 Gy resulted in the shortest plant height, range 0.5 – 4.5 cm. The results of this study were the same as the research results shown by Zanzibar and Sudrajat in *M. champaca* plants at a low dose of irradiation, produced higher mutant plants compared to control plants, and at a dose of 100 Gy produced the shortest mutant plants [16]. Similar to Wahyuni's research, the 100 Gy dose on cassava plants produced the shortest mutants [21].

Table 2. Number of tiller bud growing from stolon under several irradiation doses on 10 DAI

Dose (Gy)	Number of plants*			
	0.5 - 4.5	4.6 - 8.5	8.6 - 12.5	12.6-16.5
0	41	13	2	1
25	20	14	2	2
50	31	7	0	0
75	14	4	0	0
100	8	0	0	0

Note: *the number of plants in the range of tiller bud height in centimeter units

The irradiated rice plants by gamma-rays also showed that the higher the irradiation dose, the lower the plant height [22]. The direct effect that arises as a result of giving gamma irradiation mutagens was the cell damage. Also, the decrease in plant height by the increasing dose of irradiation happened due to the damage or change of chromosomes. Further, it was expressed by mutant plants through changes in plant morphology, physiology, and biochemical content [23]. Disruption in DNA synthesis and physiological and biochemical changes after gamma-ray irradiation could cause a decrease in plant height. The variation of shoot growth of *L. hexandra* on 10 DAI was presented in Figure 2.

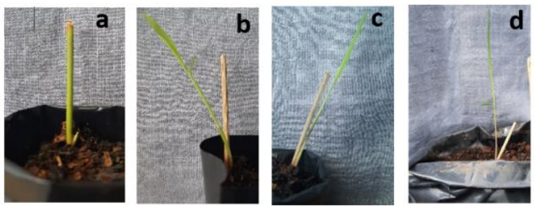

Figure 2. Variation of shoot growth of *L. hexandra* on 10 DAI. a. 0.5 cm b. 7 cm c. 9 cm d. 14 cm

CONCLUSION

Gamma-ray irradiation to the stolon has the potential to induce variations in *L. hexandra*. The sensitivity of plants to gamma rays is indicated by the value of LD50 at a dose of 68.85 Gy and LD20 at a dose of 29.36 Gy, with the linear equation y = 1.02-7.56x. The gamma irradiation dose of 25 Gy is the best dose to produce higher plants than the control (wild type).

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