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Germination and seedling growth of Kipas Putih soybean (*Glycine max* [L.] Merrill) in various dosage of gamma rays irradiation

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Abstract. Gamma irradiation is the widely used mutagen in assembling plant genetic diversity. The important thing to do is looking for the most appropriate dose that gives rise of diversity without turning off irradiated plants. This study aims to determine the initial growth response of Kipas Putih soybean seeds due to gamma ray irradiation treatment. Kipas Putih soybean seeds measure their water content up to 11%. Furthermore, gamma ray irradiation was carried out at doses 0, 200, 400, 600, 800 and 1000 Gray. The results showed that there was decrease percentage of germination in Kipas Putih soybean seeds at 200 Gray reaching 50% at 14th day after planting. Irradiation doses of 400 Gray and 600 Gray reduce percentage of germination until 20% and 30%, respectively. While at doses of 800 Gray and 1000 Gray, all seeds died at 21st day after planting. Giving gamma ray irradiation also showed a decrease in seedling height along with increasing irradiation dose.

1. Introduction

Soybean (*Glycine max* [L.] Merrill) is a very important and miraculous plant in the 21st century. Soybean is in great demand because it has benefits in fulfilling human foods and animal feed. According to Malek, et al., [1] soybean contains vegetable protein which is rich in essential amino acids. Soybean oil also fulfills 30% of the world's vegetable oil which is friendly for people with heart disease. Furthermore, soybeans contain lactose free fatty acids, antioxidants and folic acids, vitamin B complex and isoflavones.

The increasing need of soybeans must be followed by increased production. Various efforts can be taken to achieve this goal. One of them is by assembling new high yielding varieties. A superior variety assembly program can be carried out using plant breeding techniques. The genetic diversity of population is the basis for starting a plant breeding program by plant breeder. Broad genetic diversity can be the basis for successful genetic improvement through selection in plant breeding. There will be no improvement in heritable plant character without genetic diversity. This happens



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because if a character has narrow genetic diversity or variability, then each individual in the population is almost the same, making it difficult to improve character through selection.

Mutation breeding is one of technology in increasing diversity to improve the desired plant characteristics. Manjaya et al., [2] stated that mutation breeding is a way to increase the genetic variability of plant characters, both in quantitative and in qualitative manners. The important benefit of mutation breeding is that mutants with certain characters can be created without changing the essential character of the existing variety. Therefore, the mutants can be easily cultivated as a normal type.

Gamma rays are the most energetic form of electromagnetic radiation. Their energy level is from ten to several hundred kilo electron volts. Gamma rays are considered as the most penetrating compared to other radiations [3]. Gamma rays can cause changes at both the gene and chromosome level. Changes in genes and chromosomes will result in changes in plant characters. It is expected to increase genetic diversity in the Kipas Putih variety.

Hanafiah et al., [4] reported gamma ray irradiation treatment to Argomulyo soybeans at 200 Gray, 400 Gray, 600 Gray, 800 Gray and 1000 Gray. The results showed that the lethal dose (LD 50) was at a dose of 457.136 Gray. The IAEA (International Atomic Energy Agency) recommended gamma ray irradiation dosage for soybean plants is 200 Gray, which is useful for improving the quantitative character of plants. The irradiation dose is different for each cultivar and soybean varieties [5]. Therefore, it is necessary to conduct research to determine irradiation dose for the Kipas Putih variety which can induce the highest genetic diversity without causing plant mortality. This is an early stage research which aims to find irradiation doses that will be used to obtain mutant lines of soybean plants which can later be released as high yielding and early maturing varieties.

2. Materials and methods

Soybean seed irradiation is carried out at the Center for Isotope and Radiation Technology Research and Development, National Nuclear Energy Agency (BATAN), Jakarta. The irradiation doses used were 0 Gray, 200 Gray, 400 Gray, 600 Gray, 800 Gray and 1000 Gray, and the dose rate was 0.96481 kgray hour⁻¹ (96.481 krad hour⁻¹). A dose of 0 Gy was carried out as a control treatment. This research used Kipas Putih soybean seeds (water content 11%).

After being irradiated with a dose according to each treatment, soybean seeds were planted in polybags for seed germination. The medium for the germination of polybags was soil, sand and manure with 1: 1: 1 ratio. Each treatment contained 30 seeds so that the total number of seeds was 180 seeds. Watering was done in the morning or afternoon in accordance with the conditions to keep the soil moist.

The observation was made as percentage of germination in 2 weeks after planting and seedling height. The calculation is done by dividing the total number of seeds that grow after being irradiated by all the seeds irradiated with gamma rays then multiplying by 100%. The seedling height is measured from the base of the stem to the tip of the seed. Seedling height measurements were carried out at 7th, 14th and 21st days after germination.

3. Results and discussion

The results showed the decrease in the percentage of germination by giving gamma ray irradiation to soybean seeds compared to the control. The germination percentage ranges from 60% (200 Gray) to 96.6% (0 Gray). The treatment of gamma irradiation at 200-1000 Gray showed 60 -76.6% germination percentage at 14th day after planting (Table 1). The damage due to mutagenic substances in the early stages of cell division at high irradiation doses affects seed germination. The embryos in the seeds were subjected to mutagen treatment.

Present result are in accordance with the finding of Ramesh et al., [6] in rice, [7] in cowpea, [8] in mungbean, [9] in bhendi, [10] in rosella and [11] in soybean. [12] also reported a decrease in germination of soybean seeds given gamma ray mutagen on Denna 1 soybean. They irradiated soybean seeds with 60 Co gamma rays at doses of 0, 100, 200, 300, and 400 Gray. The results showed that the highest percentage of seed germination at 3 days after planting was found at 100 Gray (74%),

while the lowest was at 200 Gray and 400 Gray, namely 67.7%. Similar results were also found by [13] in soybean. He found the different doses of gamma rays treatment showed gradual reduction of seed germination (10th day), seedling survival (30th day) and plant height (30th day) than control in soybean seed var. Co-1. The effects of gamma rays were different doses on survival percentage, mutation frequency and mutagenic effectiveness. The survival percentage and mean value of M_1 generation were decreased with increase the dose of treatments.

Table 1. The percentage of germination due to various dosage of gamma ray irradiation in Kipas Putih soybean seed.

Doses (Gray)	Germination (%)			Average (%)
	1	2	3	
0	100	100	90	96.66
200	50	60	70	60
400	80	50	70	66.66
600	70	70	60	66.66
800	70	70	60	66.66
1000	80	90	60	76.66

The decline number of survival plants can be seen clearly in this study. The plants still survive even though only 30 -50 % at 200 - 400 Gray doses. In the mutagen treatment at a dose of 600-1000 Gray, most plants were only able to germinate until they released cotyledon leaves, no growth occurred at the next stage. One month after planting, all plants in this treatment died (Figure 1).



Figure 1. Effects of gamma rays on the seedling growth of Kipas Putih Soybean at 14th day after germination

The maximum reduction was recorded in higher doses of gamma rays (1000 Gray). This was in collaboration with the earlier reports [4]. They found the increased of irradiation dose up to 800 Gray, there was a decrease in germination. Whereas, the germination continued at 1000 Gray, but the sprouts growth stagnation and have abnormalities. The stems and cotyledon were thickened, and the plant height remained (not increased) until the end of observation (two weeks after planting). This was in confirmation with the finding of [14] in bhendi. The results showed that there was a decrease in the number of survival plants treated with gamma irradiation compared to control plants. This was also reported by Satpute et al., [15] The result indicated that lower doses of the mutagen are more effective for induction and recovery of mutations for improvement of soybean.

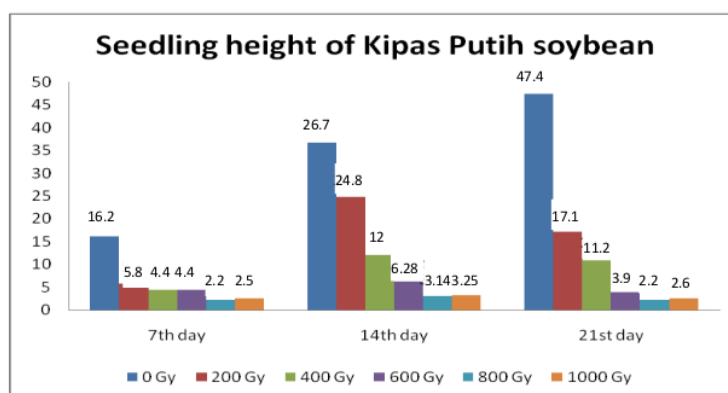


Figure 2. Seedling height of Kipas Putih soybean due to gamma ray irradiation at 7th, 14th and 21st day after germination

The present study revealed that the seedling height decreased progressively as the doses of mutagens treatment increased at 7th, 14th and 21st days after planting (Figure 2). Seedling height was maximum in control than the mutagenic treatment. The reduction in seedling height on 21st day varied from 47.4 cm (0 Gray) to 2.2 and 2.6 cm (800 Gray, 1000 Gray). The highest seedling height reduction was observed at 600, 800 and 1000 Gray treatment. There are many early reports on dose depended reduction on seedling height in soybean given by [16,17]. A reduction in seedling height can be attributed to the inhibition of growth due to low rate of cell division, decreased amylase activity and increased peroxide activity.



Figure 3. Effects of gamma rays on the seedling growth of Soybean (*Glycine max* L.) cv. Kipas Putih A. at 7th day after planting; B. at the 14th day after planting

The control plants (0 Gray) showed the growth of cotyledons and plumula leaves which had opened perfectly (Figure 3). The 200 Gray irradiation dose treatment showed the growth of cotyledon leaves which had opened completely but the plumule leaves had not appeared. Plants with a dose of 400-1000 Gray showed that many cotyledon leaves had not opened completely and the plumule leaves had not yet appeared. The higher the irradiation dose, the lower the seedling height. At a dose of 600-1000 Gray, it was also seen that the plants could only germinate until they sprouted the cotyledon leaves. Furthermore, the longer it does not show any further growth.

Jagajanantham et al. [14] noted that the biological effect of gamma ray was determined by the interaction among atoms or molecules in the cell. These radicals could damage or modify the important components of plant cells, and changed morphology, anatomy, biochemistry and physiology

of plants depending on the radiation dose. The irradiation of seeds with high doses of gamma ray disturbs the synthesis of protein, hormone balance, leaf gas exchange, water exchange and enzyme activity [18]. Girija and Dhanavel [19] stated the ability of mutagens to enter the cells of living organisms for interacting with the DNA produces toxic that associate with their mutagenic properties. Thus, mutagens can cause physiological damages mainly showed by growth retardation and death in M₁ generation.

4. Conclusion

Gamma ray irradiation in various dosage affects percentage of germination and seedling height of Kipas Putih soybean seed. The dosage under 400 Gray can be used to induce genetic diversity in Kipas Putih soybean.

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