Early growth of post-gamma irradiated *Indigofera zollingeriana*

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Abstract. This study aims to identify the effectiveness of gamma-ray irradiation to improve Indigofera seed viability. The study employed Randomized Block Design (RBD) consisting of 5 irradiation dosage (treatment) and 4 replication. The treatment consisted of Irradiation Dosage (A) 0Gy; 50Gy; 100Gy; 150Gy; 200Gy. The observed parameter encompassed the germination percentage, mortality percentage, plumule and radicle length, and germination index. The results of ANOVA indicated that gamma-ray irradiated Indigofera seedling had a significant effect (P<0.05) on the parameters of plumule length, germination percentage, and mortality percentage. The significant effect was identified at the dosage range of 0-50 Gy. No significant difference was found at the dosage of 100-200 Gy.

1. Introduction

Plant genetic modification through plant breeding could produce better plant characteristics than the original species [1]. Plant breeding programs can be performed using mutation induction by using physical mutagens, for example, gamma rays [2], [3]. Irradiation method employing gamma-ray enable the spontaneous genetical changes to produce target gene activation that determines productivity [4], [5]. Gamma-ray irradiation aims to increase the genetic variability in a mutant that can be inherited to the next generation. Some studies found that the application of gamma-ray in plants such as soybean [6], [7]; sugar cane [8], [9]; and rice [10] could improve the plant’s tolerance against drought.

A mutation is a genetic alteration on a segment that ranges from the genome, chromosome, DNA, or organism gen that stimulates genetic variability [3]. Mutation can be induced through physical and chemical mutation. Mutation quality had no positive correlation with dosage rate. It has been a common fact that a higher dosage rate does not always contribute better results [12].

One most effective method to produce a high-quality variety is plant breeding through radiation mutation techniques including gamma-ray [10], [13]. In another study with a lower dosage, mutation developed on transferrable minor gen to the following generation without any harmful impacts [14]. On the contrary, a higher radiation dosage caused a lower growth, shortening, and in the end, death.

The breeding of *Indigofera zollingeriana* can be performed through seeds and it will require accurate treatments and media to accelerate the germination of the plants. A number of studies confirmed that Indigofera is tolerant against abiotic stress such as salinity and drought [15], [16]. Dry pasture may
cause plants including rice to insufficiently absorb water and soil nutrients which resulting in stress conditions. There were no further research on the forage seed variety tolerance against drought stress and therefore, promoting tolerance in drought stress condition is necessary. Genetic variability is one important aspect to produce drought tolerance by inducing mutation. One of the most popular methods was the combination of variability induction and selection. The most frequently applied induction that could cause alteration in the morphology, anatomy, and genetic is gamma-ray irradiation [17].

The utilization of gamma-ray irradiation as a seed treatment to improve seedling vigor and quality have been massively performed in the seeds for agriculture. However, it was very rare to be applied in forage seeds. The critical period of plant breeding through seeds is at the first treatment. Therefore, the aim of this study is to identify the effectiveness of the gamma-ray irradiation technique to improve Indigofera seed viability.

2. Materials and method

2.1. Research methods
The study was performed at the Laboratory of Plant Breeding and Seed Science, Biotechnology, Plant Breeding Department, Faculty of Agriculture and National Nuclear and Energy Agency (PAIR_BATAN) Jakarta. The experiment was initially performed by collecting Indigofera zollingeriana seeds for irradiation to produce M0 Each composition of irradiation consisted of 1000 seeds. This study employed a Randomized Block Design (RBD) consisting of 5 treatments and 4 groups. T1 = 50 Gy Gamma irradiation; T2 = 100 Gy Gamma Irradiation; T3 = 150 Gy Gamma irradiation; T4 = 200 Gy Gamma irradiation. Each dosage was applied to 250 seeds. Each seed was planted in a 54 cm x 28 cm tray. The observed parameter encompassed the plumule and radicle length, and germination percentage and mortality percentage.

2.2. Data analysis
The collected data will be analyzed with Analysis of variance (ANOVA) using SPSS v.16.0. If the treatment was revealed to have a significant effect, Duncan Multiple Range Test was run with 95% level of confidence.

3. Results and discussion
The effect of irradiation dosage at different levels on plumule and radicule length, germination percentage, and mortality percentage is presented in table 1. Table 1 demonstrates that the irradiation treatment had a significant effect (P<0.05) on the plumule length, germination percentage, and mortality percentage.

Table 1. The effect of irradiation dosage on the germination parameter.

| Treatment | Plumule Length (cm) | Radicule Length (cm) | Germination (%) | Mortality (%) |
|-----------|---------------------|----------------------|----------------|--------------|
| T0 (0Gy)  | 2.09 ± 0.26b        | 1.83 ± 0.38          | 38.75 ± 3.14a  | 40.69 ± 9.00a |
| T1 (50Gy) | 1.37 ± 0.35a        | 1.98 ± 0.22          | 49.90 ± 8.19b  | 19.82 ± 8.45b |
| T2 (10Gy) | 1.55 ± 0.21a        | 1.80 ± 0.53          | 39.91 ± 7.30a  | 16.07 ± 7.88b |
| T3 (150Gy)| 1.26 ± 0.32a        | 1.76 ± 0.21          | 39.47 ± 4.71a  | 13.00 ± 10.24b|
| T4 (200Gy)| 1.27 ± 0.44a        | 1.79 ± 0.47          | 38.15 ± 8.54a  | 16.67 ± 15.44b|

Description: * = significant; Values represent means ± SD of three replicates; abcd Means in the same column with different letters are significantly different (P<0.05);
mutation technique is to gain a novel characteristic of plants through genetic change and parent plant characteristics after the irradiation of gamma-ray at a certain dosage on parent plant [11].

The lower dosage of gamma irradiation could increase germination percentage and seed growth [2], [18], [19]. The decrease in seed germination ability was caused by the treatment of mutation induction. This was triggered most probably because of damages on basic cell components or the alteration of enzyme activity of the plants. [20] Related to improving the quality of seeds and seedlings, gamma ray irradiation has been widely applied to increase viability and vigor of seeds [21], [22] and to increase genetic diversity in the context of breeding to obtain superior varieties in many types of crops. The use of radiation such as X-rays, Gamma and neutrons as well as chemical mutagens to induce variation in plants has been widely used.

4. Conclusion
The irradiation treatment had a significant effect (P<0.05) on the plumule length, germination percentage, and mortality percentage. This result demonstrated that a significant effect could be identified at 0-50 Gy dosage of irradiation. The dosage range between 100-200 Gy did not cause any significant effect. This study requires further research on irradiation dosage, particularly on Indigofera seed.

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