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Viability Test of Gamma-irradiated seeds of Jabon Merah (*Neolamarckia macrophylla* (Wall.) Bosser) from Luwu Provenance : Preliminary study

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Abstract. Fast-growing Jabon Merah has been well known as the raw material of plywood and pharmaceuticals. Induction of Gamma-irradiation on Jabon Merah' seeds is expected to increase the productivity of this species to support the availability of superior timber. The objective of this study was to determine the effect of Gamma-irradiation on seeds viability of Jabon Merah from Luwu provenance. Eight doses of Gamma used for irradiating the seeds were 0, 5, 10, 15, 30, 60, 120, and 240 Gy. The irradiated seeds were then evaluated their germination rate using Top of Paper Test for each irradiation dose. Here, we applied a Completely Randomized Design (CRD). 15 Gy presented the highest seed germination rate on the first and the second replications, 96 buds out of 0.2-gram seeds and 117 buds of 0.2-gram seeds, respectively. Overall, 15 Gy increased number of bud up to about 62 – 66% when compared to control which was only able to germinate as many as 59 buds of 0.2-gram seeds on first replication, and 60 of 0.2-gram seeds on the second replication. The results showed that Gamma-irradiation did not significantly affect the viability of the seeds

1. Introduction

Jabon Merah (*Neolamarckia macrophylla* (Wall.) Bosser) is a fast-growing species that is potential to be utilized in plantation forest development. This species is of the straight and cylindrical trunk, high natural shedding, and able to grow in open/marginal area. It is commonly used as the raw material of plywood, board, crate, and chopstick. Its wood processing waste can also be used as the raw material of particleboard and pulp [1].

Jabon Merah has long and thick fibers, high cellulose, and holocellulose (52,47% cellulose and 67,70 % holocellulose) and is suitable for pulp [2]. Its timber has moderate density with moderate dimension, stable, and 0.48 of wood specific gravity which is categorized into Strength Class III; thus it can be used as carpentry wood and lamina board[2,3]. Jabon Merah is resistant to dieback caused by *B.theobromae* due to trichome structure in the epidermis and necrotic resistance [4]. Moreover, the selling price of this species and its wood demand for industrial needs are also high [5]. Mulyana *et al.* (2012) noted that the amount of its wood demand from 22 plywood industries in Central Java reaches



114,000 m³/month with an average of wood price in Indonesia ranged from Rp.670,000/m³ to Rp.750,000/m³.

Procurement of Jabon Merah' superior woods requires superior seeds supply. An alternative for improving seed quality through mutation breeding is by induction of Gamma irradiation. Mutation breeding has been done on various agricultural and forest plants and shows a positive effect on productivity. Induction of Gamma-irradiation with precise doses of farm plants not only had been carried out on cash crops like *Oryza sativa* [6], *Capsicum annuum* [7], *Moringa oleifera* [8] and *Trigonella foenum-graecum* [9], but also on forest trees, such as *Fagraea fragrans* [10], *Toona sureni* [11], *Michelia campaca* and *Falcataria moluccana* [10]. Induction of Gamma irradiation at a specific dose causes positive phenotypic and morphologic changes and germination rate improvement.

Mutation breeding on Jabon Merah using Gamma induction at several irradiation doses needs to be tested for procurement of superior seeds and seedlings. The objective of this study was to determine the effect of Gamma-irradiation on Jabon Merah' seed viability measured by their seed germination rate.

2. Method

2.1. Plant materials and research location

The fruits of Jabon Merah were collected from seed orchard at the class of identified seeds stand in Luwu Regency, South Sulawesi, Indonesia. Induction of Gamma irradiation was carried out at P3TIR BATAN, Jakarta, Indonesia. Seed germination test was performed at BPTH Region II, Makassar, South Sulawesi.

2.2. Extraction of jabon Merah seeds

The collected Jabon Merah' fruits were from physiological ripe fruits which were of dark brown to blackish color. Fruits were collected from a plus-tree which had better phenotype than other trees in the research site. The fruit collection was carried out by picking the fallen fruits on the ground due to the high tree height.

Fruits were then extracted using dry extraction with the following steps: endosperms of the fruits were crushed and separated from their fruits' knop and then dried for three days. They were then sifted with several sieve filters until obtaining the seeds.

2.3. Gamma irradiation and germination

Induction of Gamma irradiation was performed using several doses: 0 Gy, 5 Gy, 10 Gy, 15 Gy, 30 Gy, 60 Gy, 120 Gy, and 240 Gy by irradiation Gamma Chamber 4000 A. The irradiated seeds were then germinated using Top of Paper Test. The seeds on each dose treatment were spread as many as 0, 2 gram on merang paper which was placed on Petri dishes.

2.4. Experimental design

The study was set in Completely Randomized Design (CRD) using one factor which was irradiation doses consisted of eight levels. Observation on seed germination was performed for four weeks. The first observation was done at ten days after planting. Second and third observations were conducted one week after the previous view. The inspections were performed by counting some germinated seed.

2.5. Data analysis

Data were analyzed using SPSS 16 software to determine the effect of irradiation doses on seed viability. Analysis of variance (ANOVA) was performed to evaluate the impact of the treatment on the parameters being assessed.

3. Results and discussion

Mutation in the plants is the genetical change that occurs in gene, genome, or chromosome [12]. It happens in all parts of the plants, but it frequently occurs in actively differentiate plants' elements, such as shoot and seed. Induction of Gamma-irradiation on Jabon Merah' seeds is expected to promote positive response on its productivity. [13] had stated that mutation could be induced by mutagen with a specific dose and time. Therefore, we evaluated several Gamma irradiation doses on Jabon Merah' seeds for elucidating the response of seed germination on the evaluated irradiation doses. Seedling number of Jabon Merah on each irradiation dose is presented in table 1.

Table 1. Seedling number of Gamma-irradiated jabon Merah at different irradiation doses

| No | Level of Irradiation (Gy) | Number of seedling at the final observation (seedling/0,2-gram of seed) | | The increase of Percentage of seedling number compared to Control (%) | |
|----|---------------------------|---|---------------|---|---------------|
| | | Replication 1 | Replication 2 | Replication 1 | Replication 2 |
| 1 | 0 | 59 | 60 | | |
| 2 | 5 | 61 | 83 | 50.83 | 58.04 |
| 3 | 10 | 74 | 91 | 55.64 | 60.26 |
| 4 | 15 | 96 | 117 | 61.94 | 66.10 |
| 5 | 30 | 69 | 63 | 53.91 | 51.22 |
| 6 | 60 | 81 | 108 | 57.86 | 64.29 |
| 7 | 120 | 90 | 79 | 60.40 | 56.83 |
| 8 | 240 | 81 | 65 | 57.86 | 52.00 |

Table 1 shows that the induction of Gamma-irradiation gave a positive response on the seedling number for each dose. Increasing on several seed germination was varied for each treatment. An increase on seed viability was started at 5 Gy, which was 50.83% to control (first replication) and 58.04% (second replication). The number of germinated seed continually rose at 10 Gy and 15 Gy. The highest seed viability was observed on 15 Gy that compared to control on first and second replications, 61.94 % and 66.10%, respectively. Seed viability at 30 Gy, 60 Gy, 120 Gy, and 240 Gy were varied and higher than control. 240 Gy could still induce seed germination that better than power, 57.86% (first replication) and 52.00% (second replication) (Figure 1).

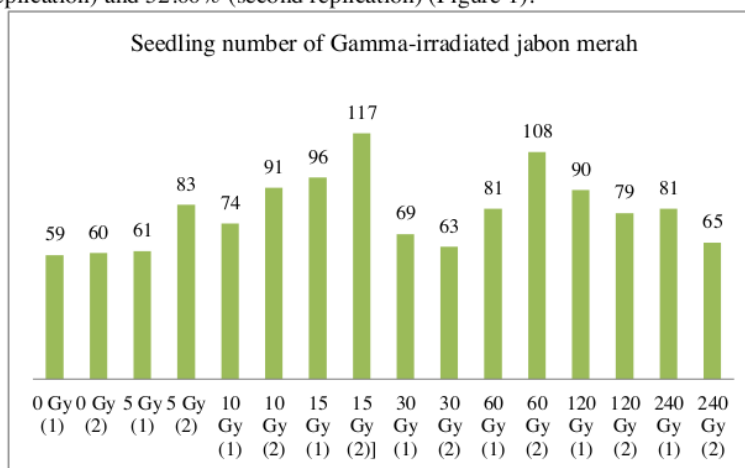


Figure 1. Seedling number of Gamma irradiated Jabon Merah at different irradiation doses

High level of Gamma-irradiation generally inhibits the seed germination [14] [15]. In contrast to the high irradiation level, the low level of irradiation stimulates seed germination through increasing enzyme activity, repairing the respiratory cells, improving the reproduction structure. Induction of Gamma-irradiation on Jabon Merah' seeds up to 240 Gy could still induce a positive effect on seedling number. 240 Gy of Gamma irradiation in this study has not yet inhibited the seed growth. The limits of the highest Gamma irradiation dose on the species are different. Bramasto and his colleagues (2016) proved the induction of gamma-irradiation which range from 5 Gy to 90Gy could stimulate seeds germination and growth, and inhibit the seeds germination or growth above 90Gy [10]. In Suhartanto *et al.* (2018) study, the Gamma irradiated seeds of Jabon Putih with 10 to 50 Gy tended to improve seed germination and eventually decreased at 100 Gy. The changes caused by Gamma irradiation are random and consequently, generate variation among individuals in a species [16]. The sensitivity of seeds to Gamma irradiation doses is different due to different initial seed viability and genetic of seed origin [16].

F value and p-value are presented in Table 3. Before analyzing the ANOVA, we tested the data normality using the Kolmogorov Smirnov test to determine the regularity of the data. The result is displayed in Table 2.

Table 2. Kolmogorov Smirnov Normality Test

| One-Sample Kolmogorov-Smirnov Test | | |
|------------------------------------|----------------|-----------------|
| | | Seedling number |
| N | | 16 |
| Normal Parameters ^a | Mean | 79.8125 |
| | Std. Deviation | 17.34827 |
| Most Extreme Differences | Absolute | .116 |
| | Positive | .116 |
| | Negative | -.115 |
| Kolmogorov-Smirnov Z | | .464 |
| Asymp. Sig. (2-tailed) | | .983 |

3.1. Test distribution is Normal

Kolmogorov-Smirnov normality test was 0,464, which indicated the data was normally distributed, thus the analysis could be followed by ANOVA test.

Table 3. ANOVA of Kolmogorov Smirnov

| ANOVA | | | | | |
|---------------------|----------------|----|-------------|-------|------|
| Number of seedlings | Sum of Squares | df | Mean Square | F | Sig. |
| Between Groups | 3335.938 | 7 | 476.562 | 3.235 | .061 |
| Within Groups | 1178.500 | 8 | 147.312 | | |
| Total | 4514.438 | 15 | | | |

ANOVA showed p-value was 0.061 that indicated the irradiation doses did not significantly affect the seed germination. The irradiation dose did not significantly influence the increase in seedling number. It means seed viability in control (0 Gy) was not different from those on the irradiation treatments.

4. Conclusion

The 15 Gy performed the best increasing in several seedlings compared to control; 61,94 % (the first replication) and 66,10 % (the second replication). Overall, the irradiation treatment presented an insignificant effect to control. The further research is needed on genetic variation of the irradiated seeds of Jabon Merah.

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