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The Changes in Seed Germination Capacity, Seedling Growth, and Leaf Morphology of *Ficus variegata* Blume Influenced by Gamma-Ray Irradiation

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ABSTRACT

Gamma irradiation treatments have commonly been applied to induce variation in plant genetic improvement programs. The improvement of nyawai (*Ficus variegata* Blume) is urgently needed mainly to break the seed dormancy, increase seedling growth, and induce variation for selecting desired traits. This research aimed to assess the effect of gamma irradiation doses on seed germination, seedling growth, and leaf morphology of *Ficus variegate* Blume. This experiment's irradiation doses ranged from 5-240 Gy and were applied to seeds with varying levels of vigor based on the artificial seed aging process. The data displayed a positive relationship between low doses of gamma irradiation and the nyawai seed's endurance. Low irradiation doses, 5 to 30 Gy, are deemed capable of promoting seedling development, leaf retention, and diversity in leaf structure. The higher irradiation doses than 30 Gy decreased seedling growth, leaf number, and seedling survival. In addition, these doses also affect some morphological changes, including seedling height and leaf length and width.

1. Introduction

Nyawai (*Ficus variegata* Blume) falls into a significant flourishing tree category that is naturally distributed in Southeast Asia, India, Japan, China, Taiwan, Australia, and the Pacific Islands region (Zhekun and Gilbert 2003). Nyawai timber is commonly used for wood carpentry, plywood production, face veneer manufacture (Sumarni *et al.* 2009), and as packaging materials and household appliances (Liu 1988). Another benefit of nyawai is that its edible fruit contains high nutrients (Shi *et al.* 2014) and antibacterial and antioxidant compounds (Leonita *et al.* 2015; Rusli *et al.* 2019). The species is also very suitable for reforestation and revegetation of degraded land because it can grow on various soil types and survive in Imperata grassland areas (Wahyuningtyas *et al.* 2022).

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The problem of nyawai cultivation is often related to procuring good seeds/seedlings (Zainudin 2021). Effendi and Mindawati (2015) reported that the storability of nyawai seeds was fewer than 6 months with a lower germination value. This seed can also be categorized as an intermediate seed (Haryjanto and Hadiyan 2014) with a relatively rapid decline in seed physiological quality. The tiny seed of nyawai seeds (23-25 million seeds/kg) has a minimum germination capacity (of 2-3%) (Sriwahyuni 2014; Zainudin 2021). Therefore, pre-treatment is required to promote germination (Garcia *et al.* 2006). Moreover, genetic material from a wide distribution has also become a constraint for improving the quality of the seeds (Haryanto *et al.* 2019; Wicaksono *et al.* 2020). Accordingly, one of the ways to overcome the limitation of improving seed germination and increasing genetic diversity is by using gamma irradiation.

Improving nyawai seed by gamma irradiation in low dose can stimulate seed germination and seedling growth through the acceleration of cell division or direct/indirect stimulation to the auxin-responsive genes (Sudrajat *et al.* 2022; Syamsuwida *et al.* 2020; Zanzibar and Sudrajat 2016). Direct and indirect classifications of the gamma irradiation effect are based on the ionizing radiation dose exposed to the plant tissues (Li *et al.* 2022). Marcu *et al.* (2013) stated that plants have massively different reactions toward gamma irradiation not only based on their physiology, morphology, species, age, size, and genome composition but also on radiation types, time of exposure, and irradiation dose. Biochemical changes due to gamma irradiation affect the process of cellular metabolism that, at some level, can break down chemical inhibitors germination and increase cell division to influence seedling growth (Araújo *et al.* 2016; Kumar *et al.* 2019). Improving seed germination and seedling development was reported on *Fagraea fragrans* (Zanzibar *et al.* 2015), *Magnolia champaca* (Zanzibar and Sudrajat 2016), *Neolamarckia cadamba* (Suhartanto *et al.* 2018), and *Toona sureni* (Zanzibar *et al.* 2021).

Celik and Atak (2017) and Hong *et al.* (2022) mentioned that gamma irradiation induces changes in the mutation rate, cytogenetic biological responses, the structure of the DNA molecule, and its function, which plays a role at the cellular and systemic levels and creates macroscopic phenotypic variations. Thus, potentially inducing morphological changes in plants. The induction of variation using the radiation technique has been widely used in crops to obtain commercial varieties (Ahumada-Flores *et al.* 2020; Perez-Jimenez *et al.* 2019). In forest tree species, some studies have been reported, such as on *Acacia nilotica, Prosopis juliflora* (Goel and Behl 2005), *Terminalia arjuna* (Akshatha *et al.* 2013), *Artocarpus hirsutus, Garcinia xanthochymus, Saraca asoca, Rourea minor, Pterocarpus marsupium, Terminalia chebula, Aporusa lindleyana, Holoptelea integrifolia* and *Oroxylum indicum* (Akshatha and Chandrashekar 2014). By looking at the importance of gamma irradiation on the physiology and genetic improvement of forest tree species, the present study was conducted to evaluate the effect of gamma irradiation doses on seed germination, seedling growth, and leaf morphology variations of nyawai (*Ficus variegata* Blume).

2. Materials and Methods

2.1. Materials

The seed collection of Nyawai was conducted at the seed source stand in P.T. ITCI-Kartika Utama, East Kalimantan Province, in April 2018. Geographically, the location of the seed source stands is at 116°32'2.83" E and 00°51'37.49" S. The soil type under the seed source stands was categorized as red-yellow podzolic soil with a pH of 4.7-5.3. The seed was collected from physiologically mature fruits marked by the orange-reddish fruit color. It was collected from 25 mother trees with superior phenotypic traits, including straight stems with 30-50 cm diameter and 17-24 m height. From each mother tree, 25 to 50 fruits were collected, extracted, dried to a moisture content of 10%, and used as material for this research.

2.2. Accelerated Aging and Gamma Irradiation Treatments

Accelerated aging was used to obtain variations in viability and vigor of nyawai seed lots using an artificial procedure to speed up normal aging. Accelerated aging is a technique to determine seed viability after storage under unfavorable temperature and humidity conditions (Delouche and Baskin 1973). A modification of the accelerated aging technique (Matera *et al.* 2019) was employed in this research by putting the seeds on a shelf in a plastic box filled with water. The plastic box was closed tightly to make a stable condition of 100% relative humidity. The box was incubated at 40°C for two days for the first treatment and four days for the second treatment. Thus, three seed lots with different viability and vigor were obtained, i.e., seeds without accelerated treatment, seeds with 2 days of accelerated aging treatment, and seeds with 4 days of accelerated aging treatment. The three seed lots were then given radiation treatment, which was 5, 10, 15, 30, 60, 120, and 240 Gy, using a gamma cell 220 with ^{60}Co [Cobalt-60] radiation source and a rate of 6645.7 Gy per hour. The doses used in this study refer to the results of research conducted on *Falcataria moluccana* and *Toona sureni*, where the recommended dose is below 240 Gy (Bramasto *et al.* 2016; Zanzibar *et al.* 2021). The number of seeds irradiated for each dose was 4 grams. One gram of seed contains 3000 seeds (Effendi and Mindawati 2015). The application of Cobalt-60 gamma irradiation (Gamma Chamber 4000A-Irpasena, India) was conducted at The Irradiation Center for Isotopes and Radiation Application–National Nuclear Energy Agency (BATAN), Jakarta, Indonesia.

2.3. Seed and Seedling Testing

A completely randomized design (CRD) was used, with a factorial pattern through accelerated aging treatment (seeds without treatment, seeds with accelerated aging treatment for two days, and seeds with accelerated aging treatment for four days) and radiation doses (0, 5, 10, 15, 30, 60, 120, and 240 Gy) as treatments. The germination test was conducted using the top paper test at a room temperature of 20-30°C with 12 hours of supplied light (ISTA 2012). The number of seeds sown was 100 each, with 10 replications for each treatment. The experimental parameters were seed germination capacity and rate (ISTA 2012; Sudrajat 2016) based on normal seedling criteria for the growth of a pair of healthy leaves. Observation and measurement of seed germination capacity and germination rate refers to the formula in ISTA (2012)

The seedling was prepared by sowing seeds that had been given irradiation treatment in a mixed media of sand and topsoil (1:1, v/v) (BSN 2014; Nurhasybi *et al.* 2019). Then one month after sowing, the normal seedling was transplanted to a pot tray containing a mixed media of soil and animal manure compost (1:3, v/v) (BSN 2018; Nurhasybi *et al.* 2019). The number of normal seedlings transplanted into a pot tray was ten seedlings with five replications.

In early growth, the seedlings were placed in a shaded area (light intensity of 65%), grown for three months, then moved to an open area for one month. The growth seedling parameters were observed for seedling survival, height, and the number of leaves. Seedling height was calculated from the soil plane to the terminal bud in millimeters (mm). The number of leaves was calculated exclusively for completely open leaves with a light green color and an angle of 45° between the petiole and stem (Tjitrosoepomo 2005). The examination was done at 21 days, 42 days, and 63 days after transplanting (DAP). The examination of nyawai leaf morphology was done upon the third leaf upward from the cotyledon. The examination elements were leaf length, color, leaf tip, and leaf shape. The variables of leaf morphology in this study were based on observations during the research and several studies related to changes in leaf shape due to irradiation treatment (Meilawati *et al.* 2016; Riviello-Flores 2022). As for leaf color, the percentage of each color is calculated based on observations during the research. The seed and seedling testing were conducted at the seed testing laboratory of Forest Tree Seed Technology Research and Development Center–Research, Development and Innovation Agency, Ministry of Environment and Forestry, Republic of Indonesia, Bogor.

2.4. Data Analysis

A two-way ANOVA was used for data analysis, followed by a Duncan Multiple Range Test to pinpoint the statistical differences among the treatments. Estimating a 50% seed mortality value (LD_{50}) was observed by regression analysis on seed mortality data, which attributed radiation dose as the independent variable and seed viability percentage as the dependent variable. The programs used for this analysis were Minitab 15 and SPSS 22.0 software (Evans 2009; Landan and Everitt 2004).

3. Results

3.1. Seed Germination

Accelerated aging treatment has resulted in three classes of seed viability, namely seed quality 1 (SQ-1, without aging, initial germination capacity of 16.8%), seed quality 2 (SQ-2, two days accelerated aging, initial germination capacity of 10.4%), and seed quality 3 (SQ-3, four days accelerated aging, initial germination capacity of 7.4%). The analysis of variance showed that the interaction of accelerated seed aging and gamma irradiation treatments significantly affected the germination capacity and germination rate of the three seed quality groups ($p < 0.01$). For SQ-3 seeds, 5 Gy and 120 Gy irradiation treatment could increase germination. SQ-2 seeds, 10 to 120 Gy doses, produced better germination than the control (Table 1).

The germination rate of seeds of SQ-1 is inclined to decrease by gamma irradiation treatment. In seeds of SQ-2, doses of 10, 15, 60, and 120 Gy speed up the germination rate, whereas the dose of 240 Gy slowdown the germination rate. In SQ-3 seeds, 5 and 120 Gy treatment accelerated the germination rate, whereas the dose of 240 Gy slowed germination

Table 1. Germination capacity and germination rate after gamma irradiation treatments on several seed viability levels of nyawai

Doses of gamma irradiation (Gy)	Germination capacity (%)			Germination rate (% etmal^{-1})		
	SQ-1 (p = 0.12)	SQ-2 (p = 0.19)	SQ-3 (p = 0.02)	SQ-1 (p = 0.12)	SQ-2 (p = 0.19)	SQ-3 (p = 0.02)
0	16.80^{a}	10.40^{ij}	7.40^{defghij}	0.81^{a}	0.35^{jkl}	0.51^{defghij}
5	13.50^{abcd}	9.80^{efghij}	11.50^{cdefg}	0.66^{abcd}	0.46^{fghijkl}	0.54^{cdefghi}
10	10.90^{defgh}	11.70^{cdefg}	8.80^{ghij}	0.55^{cdefgh}	0.56^{cdefgh}	0.41^{hijkl}
15	12.50^{cdef}	12.10^{cdefg}	8.00^{hij}	0.53^{cdefghi}	0.59^{cdefg}	0.37^{ijkl}
30	14.30^{abc}	10.20^{defghij}	9.40^{fghij}	0.69^{abc}	0.50^{efghijk}	0.46^{fghijkl}
60	16.10^{ab}	12.10^{cdefg}	9.40^{fghij}	0.77^{ab}	0.60^{cdefg}	0.44^{ghijkl}
120	13.00^{bcde}	10.70^{defghi}	13.30^{bcd}	0.61^{bcdef}	0.60^{cdefg}	0.63^{bcde}
240	11.00^{cdefgh}	7.30^{j}	7.70^{hij}	0.48^{efghijkl}	0.34^{kl}	0.31^{l}

SQ-1 = seed quality 1 resulted from seeds without aging with a germination capacity of 16.8%, SQ-2 = seed quality 2 resulted from accelerated aging for 2 days with a germination capacity of 10.4%, SQ-3 = seed quality 3 resulted from accelerated aging for 4 days with germination capacity of 7.4%. (Values followed by the same letter are not significantly different at the 95% confidence level for each response)

(Table 1). Our results indicate that the viability of nyawai seeds, which had been worsening, could be re-improved through low-dose gamma irradiation. For the seeds that had undergone accelerated aging for two days and four days, the dose of 5 Gy was the dose that could accelerate germination capacity. In general, a high dose could demote the germination capacity and rate.

Based on analysis of LD_{50}, nyawai seed was generally found at gamma irradiation doses greater than 120 Gy. Seeds with SQ-1 showing seed demise due to ionization have the following model: $Y = 42.3 - 0.00000013 X_4 + 0.000053 X_3 - 0.00588 X_2 + 0.181 X_1$. Based on this equation, LD_{50} occurred at an irradiation treatment of 155 Gy. Seeds with SQ-3 possessed LD_{50} at a dose of 169 Gy, which was based on the equation of $Y = 45.464 - 0.0000004 X_4 + 0.0000148 X_3 - 0.000816 X_2 - 0.0462 X_1$. LD_{50} in seeds with SQ-2 occurred at dose of 166.5 Gy based on equation of $Y = 44.4571 - 0.0000001 X_4 + 0.00004087 X_3 - 0.004748 X_2 + 0.14101 X_1$. As a result of the ionization process of the three groups of seed conditions, LD_{50} for nyawai seeds could be suggested between 155 to 169 Gy.

3.2. Growth of Seedlings

Seed irradiated with a dose of 240 Gy did not succeed in growing until the seedling phase, so this treatment was excluded. Gamma irradiation significantly affected the seedling height growth ($p<0.01$). Seedlings at 21 days after transplanting (DAP-21), seedlings at 42 days after transplanting (DAP-42), and seedlings at 63 days after transplanting (DAP-63), and all of them treated with low doses (5-30 Gy) performed seedlings with better growth than the control (without irradiation treatment). On the DAP-42 and DAP-63, enhancement in the doses of ionization was followed by an acceleration in seedling height up to an irradiation dose of 30 Gy. In contrast, at further higher doses, there was a decline in the seedling growth performance (Figure 1B).

There is a difference between the number of leaves of DAP-42 and DAP-63 ($p<0.01$) among treatments. Increasing doses (ranging from 5 to 30 Gy) at DAP-42 causes an increase in the number of leaves. On the DAP-63, the highest number of leaves was shown by the 15 Gy treatment. Furthermore, in 30 Gy ionization, the performance of seedlings did not show a difference from that of the control, whereas at higher doses (>60 Gy), there was a decline in the number of leaves (Figure 1D). The nyawai seedlings in DAP-63 experienced a progressive decline in survival percentage, especially after irradiation treatment at a dose of 60 Gy. The use of doses of 5 to 30 Gy relatively increased the seedling survival (Figure 1A). The low-dose gamma irradiation could increase seedling survival and growth, but it negatively affected seedling growth at high doses.

The 60 Gy treatment led to an increase in the diversity of seedling height, indicated by the high value of its coefficient of variance. The older the age of the seedling, the higher the variety of seedling sizes. Irradiation treatment below and up 60 Gy did not increase the diversity compared to the control. Instead, it showed a tendency to decrease the diversity of seedling height, especially at the age of DAP-42 and DAP-63 (Table 2). Table 2 shows a wide diversity of seedling height growth at the three seedling ages based on the coefficient of variance (CV), which is more than 25%.

The diversity of seedling growth at the age of 21 days after planting (DAP-21) with a dose of 0 Gy (control) until 30 Gy was not different, indicated by the CV value in the range of 30.6-38.7% (Table 2). This shows that the induction of mutations up to 30 Gy has not shown an increased diversity in seedling height at DAP-21. However, in DAP-42 and DAP-63, the CV

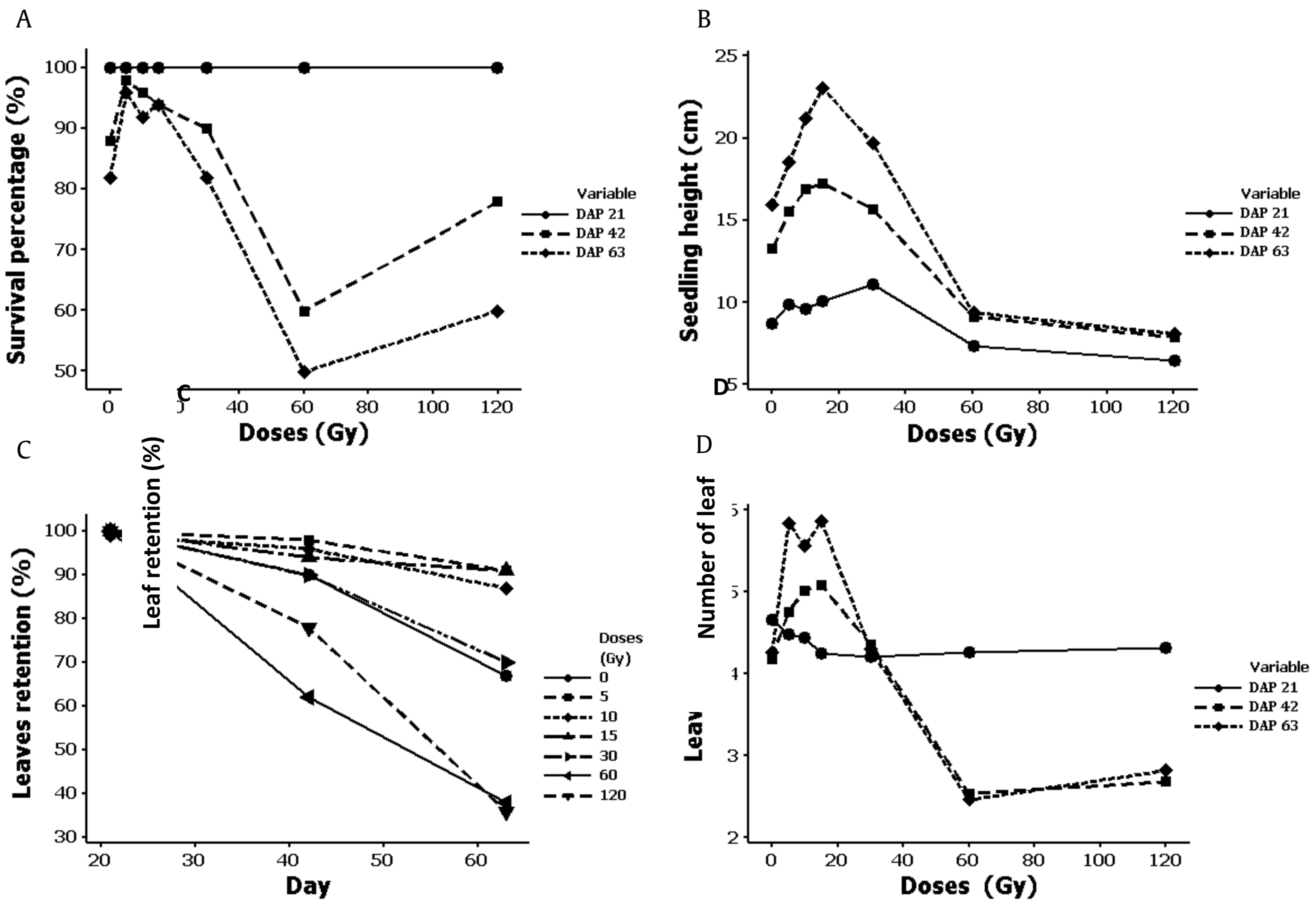

Figure 1. Effect of gamma irradiation on (A) seedling survival (B) seedling height (b), (C) leaf retention, and (D) number of leaves

Table 2. Coefficient of variance of nyawai seedling height (%) at three seedling ages

Doses of Gamma Irradiation (Gy)	Coefficient of Variance (%)		
	DAP-21	DAP-42	DAP-63
0	32.1	46.3	51.1
5	32.2	27.4	29.7
10	38.3	41.2	43.5
15	38.7	39.4	38.0
30	30.6	42.5	50.3
60	50.9	106.0	118.9
120	22.4	71.1	106.5

DAP-21 = seedling at 21 days after planting; DAP-42 = seedling at 42 days after planting, DAP-63 = seedling at 63 days after planting

of irradiation treatment of 5 to 30 Gy was lower than the control. It was suspected that mutation induction did not occur in nyawai seedlings with irradiation treatment. However, the CV was still more than 25 %, indicating the existence of seedling growth variation (Table 2).

3.3. Leaf morphology

Changes in leaf color can indicate the development of the age of the leaf, starting from bright green (young leaves), dark green, and yellowish green to brownish-green, and the leaves will fall off. On the DAP-21, the leaves were hegemony by light green and dark green colors. On the DAP-42, the number of leaves with dark green color escalated, and leaves with yellowish-green color started to emerge. On the DAP-63, leaves with brownish-green color started to arise. On the DAP-63, at the dose of 0 Gy, as many as 33% of the leaves were fallen out (Table 3). Small-dose ionization caused an escalation in leaf sturdiness, especially in handlings of 5 Gy, 10 Gy, and 15 Gy, with shedding levels at DAP-63, respectively 9%, 13%, and 9%. After 30 Gy, a dose increase leads the leaves to become senescent and fall faster.

Leaf shapes of nyawai seedlings were categorized into shapes of the heart (acute tip (b1), acuminate tip (b2), obtuse tip (b3)), cordate (c1), ovate or egg-shaped (c2), and oval (c3) (Figure 2). The form of nyawai leaves at doses of 0 to 15 Gy was mastered by heart shape and oval shape.

Ionization by gamma-ray at 30 and 60 Gy steps up the number of seedlings with ovate (egg-shaped) leaves. Alteration in leaf size (leaf length and width) occurred at doses of 60 and 120 Gy. Leaf size became smaller in the nyawai seedlings treated with 60 Gy and 120 Gy of gamma irradiation (Table 4).

Similar to seedling size (height), 60 Gy gamma irradiation also resulted in the highest increase in

Table 3. Change in leaf color of nyawai seedlings from gamma-irradiated seeds at 21, 42, and 63 days after transplanting (DAP)

Gamma irradiation doses (Gy)	DAP	Leaf color				Leaf-shedding (%)
		Light green (%)	Dark green (%)	Yellowish green (%)	Brownish green (%)	
0	21	49	50	0	0	0
	42	0	72	18	0	10
	63	0	38	16	13	33
5	21	50	50	0	0	0
	42	0	92	6	0	2
	63	0	45	37	9	9
10	21	47	52	0	0	1
	42	0	88	8	0	4
	63	0	31	39	17	13
15	21	47	53	0	0	0
	42	0	88	6	0	6
	63	0	32	42	17	9
30	21	47	53	0	0	0
	42	0	90	0	0	10
	63	0	22	42	6	30
60	21	40	60	0	0	0
	42	0	53	9	0	38
	63	0	14	19	5	62
120	21	44	54	0	0	0
	42	0	78	0	0	22
	63	0	36	0	0	64

DAP = days after transplanting

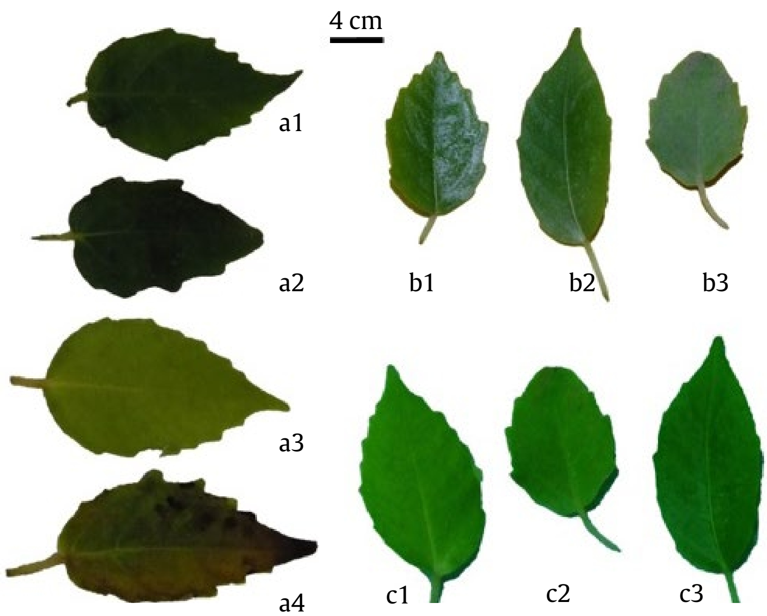

Figure 2. Variation in color of nyawai leaves (a1 = light green, a2 = dark green, a3 = yellowish green, a4 = brownish green) and shape of nyawai leaves (heart shape were three categorized such as b1 = acute tip, b2 = acuminate tip, b3 = obtuse tip, and others categorize were c1 = cordate, c2 = ovate, c3 = oval)

Table 4. Average of leaf size after gamma irradiation treatments on the 21, 42, and 63 days after transplanting of nyawai seedlings

DAP	Gamma irradiation doses (Gy)	Leaf length (cm)	Leaf width (cm)	Leaf L/W
21	0	14.64±4.379[c]	10.46±2.866[d]	1.63±0.951[a]
	5	13.98±3.761[c]	9.52±2.764[cd]	1.50±0.544[a]
	10	15.02±4.335[c]	10.46±3.315[d]	1.52±0.505[a]
	15	13.28±4.870[c]	8.58±3.150[c]	1.58±0.499[a]
	30	14.36±4.402[c]	9.10±3.352[c]	1.76±0.744[a]
	60	9.84±5.762[b]	6.68±3.744[b]	1.82±1.167[a]
	120	8.04±3.557[a]	5.40±2.330[a]	1.50±0.505[a]
42	0	15.91±3.549[c]	11.02±2.628[c]	1.47±0.226[a]
	5	15.02±3.750[c]	10.41±2.541[c]	1.46±0.258[a]
	10	16.06±4.029[c]	10.81±2.951[c]	1.52±0.243[a]
	15	15.15±4.782[c]	10.13±3.417[bc]	1.53±0.311[a]
	30	15.78±3.747[c]	10.20±2.651[bc]	1.59±0.373[a]
	60	12.70±6.374[b]	8.93±4.571[bc]	1.45±0.236[a]
	120	8.59±3.972[a]	5.87±2.618[b]	1.50±0.313[a]
63	0	16.42±3.961[b]	11.85±3.053[b]	1.41±0.241[a]
	5	15.93±3.707[b]	10.98±2.387[b]	1.49±0.230[ab]
	10	16.93±4.200[b]	11.21±2.807[b]	1.52±0.229[ab]
	15	15.36±5.179[b]	10.09±3.444[b]	1.56±0.318[ab]
	30	16.65±3.634[b]	10.91±2.539[b]	1.54±0.210[ab]
	60	15.06±6.620[b]	10.33±4.472[b]	1.47±0.193[ab]
	120	12.33±6.275[a]	7.22±3.246[a]	1.59±0.283[b]

DAP = days after transplanting, Gy = dose of gamma irradiation, values followed by the same letter are not significantly different at the 95% confidence level for each response

leaf shape diversity. In the observations of DAP-21, DAP-42, and DAP-63, the coefficient of variance in leaf length was 59%, 50%, and 44%, respectively, compared to the control, namely 30%, 22%, and 24%. The variation in leaf width was 56%, 51%, and 43, respectively, compared to the control 27%, 24%, and 26% (Table 5). The diversity of leaf sizes in the 60 Gy irradiation treatment increased about twice that of the control.

There was variation in the leaf tip shape of nyawai, namely acute, acuminate, and obtuse tip (Table 5). Seedlings from low-dose irradiated and non-irradiated seeds show mostly acuminate leaf tip form. Seedlings with irradiation treatment between 15 to 120 Gy possessed more acute leaf tip form than the control (around 10% difference). About 1–4% of the leaf tip shape of 30 to 60 Gy treatment is obtuse. From Table 5, we can conclude that the leaf morphological changes are observed more at higher doses, 30 and 60 Gy.

4. Discussion

The positive impact of a low dose of gamma irradiation (below 100 Gy) and the negative impact of high dose irradiation (above 100 Gy) on seed viability have been reported on *Carica papaya* (Chan and Lam 2002), *Pterocarpus santalinus* (Akshatha and Chandrashekar 2013), *Cajanus cajan* (Desai and Rao 2014), *Fagraea fragrans* (Zanzibar *et al.* 2015), and *Toona sureni* (Zanzibar *et al.* 2021). Higher seed viability caused by irradiation was possibly due to the activation of RNA/protein synthesis or growth inhibition of the population of some bacteria or fungi in the seeds (Majeed *et al.* 2009).

The effect of high-dose gamma irradiation (above 100 Gy) was in line with studies for seeds of *Jatropha curcas* (200 Gy to 400 Gy), *Oryza sativa* (50 Gy to 800 Gy), *Amaranthus cruentus* (150 to 250 G), *Arachis hypogaea* (700 Gy to 1900 Gy), *Sesamum indicum* (100 Gy to 1000 Gy), *Coriandrum sativumdava* (100 Gy to

Table 5. Variation of morphological characters of nyawai leaves after seed irradiation at 21, 42, and 63 days after transplanting

Gamma irradiation doses (Gy)	DAP	Leaf shape			Leaf tip shape			Coefficient of variation	
		cordate shaped (%)	ovate (%)	oval (%)	acute (%)	acuminate (%)	obtuse (%)	Leaf length (%)	Leaf width (%)
0	21	53	2	45	10	90	0	30	27
	42	51	2	37	8	82	0	22	24
	63	39	2	26	9	58	0	24	26
5	21	80	0	20	9	91	0	27	29
	42	78	0	20	9	89	0	25	24
	63	75	0	18	6	85	0	23	22
10	21	77	4	18	90	91	0	29	32
	42	74	4	18	87	89	0	25	27
	63	68	3	16	81	85	0	25	25
15	21	23	4	73	16	84	0	37	37
	42	23	4	67	16	78	0	32	34
	63	23	4	64	16	75	0	34	34
30	21	28	10	62	15	84	1	31	37
	42	26	10	54	14	75	1	24	26
	63	19	10	41	11	58	1	22	23
60	21	31	18	51	29	67	4	59	56
	42	18	16	28	19	37	4	50	51
	63	15	6	17	13	22	3	44	43
120	21	13	4	83	38	62	0	44	43
	42	11	4	63	29	49	0	46	45
	63	2	1	26	8	28	0	51	45

DAP = days after transplanting

400 Gy) and *Hordeum vulgare* (0.4 kGy) (Anbarasan *et al.* 2013; Aparna *et al.* 2013; Aynehb and Afsharinafar 2012; Harding *et al.* 2012; Salve and More 2014; Rozman 2014; Songsri *et al.* 2011). Meanwhile, Lokesha *et al.* (1994) suggested that the inability of seeds to germinate due to high-dose irradiation might be caused to the changes in histology and cytology, interference and intrusion of the seed layer, and mitosis disruption or cell division removal in the meristematic zone during germination.

Our results showed that the low-dose gamma irradiation (below 100 Gy) could improve the survival and growth of the seedling. This result was in line with seed treatments for Lactuca sativa (2 Gy to 30 Gy), Zea mays (1 Gy to 8 Gy), Datura innoxia (5 Gy to 20 Gy) and Magnolia champaca (10 Gy) (Aref *et al.* 2015; Emrani *et al.* 2013; Marcu *et al.* 2013; Zanzibar and Sudrajat 2016). At low doses, optimum gas exchange and carbon and nitrogen metabolism can promote growth quality. (Ahuja *et al.* 2014). At higher doses, the carbon expenditure might be inhibited and resulting in metabolic disorders in plants and causing disturbed growth (Hadley and Woodwell 2014).

The slow aging process at low doses is thought to cause an increase in leaf chlorophyll content, which can be seen from the slow process of changing the color from green to yellowish. In this study, it was seen that leaves owned by seedlings that had been irradiated at doses of 5, 10, 15, and 30 Gy had green leaf values of 92%, 88%, 88%, and 90%, respectively, up to DAP-42 (Table 3). These values were higher than the control or the value of the 60 and 120 Gy irradiation treatments. (Table 3). Our research did not analyze the chlorophyll content, but we assumed that the difference in chlorophyll content caused the difference in leaf retention. An increase in the content of chlorophyll in the leaves affects the physiological process, which plays a role in effective photosynthetic activities. This condition is a beneficial impact of irradiation on seeds, especially at low doses of irradiation (Aref *et al.* 2015; Akshatha and Chandrashekar 2013; Marcu *et al.* 2013). However, the opposite condition occurs in high-dose irradiation treatment, which is caused a decrease in chlorophyll content (Aref *et al.* 2015; Azigwe *et al.* 2020; Desai and Rao 2014; Hadley and Woodwell 2014; Marcu *et al.* 2013).

This experiment presents that gamma irradiation might change the seedling morphological structure. Genetically, the low dosage of irradiation might alter the size of the chromosome, whereas, in high dose, there were chromosome failure and abnormal mitosis division (Bevilacqua and Vidakovic

1963). However, the explant's physical shape and morphology can affect cells' physical resistance when receiving gamma irradiation (Roux *et al.* 2004), as well as explant moisture content, storage periods, post-irradiation, and temperature during incubation (Soeranto 2003). It can be seen that varied *Brachiaria decumbens* have different sensitivities to gamma irradiation (Hussin 2003). Kodym *et al.* (2014) also mentioned that most effects investigated in the first generation of mutant (M1) were changes in some physiological and morphological characters. The changes can be quantitatively determined as the impact level of mutagen in the M1 generation.

Furthermore, in plant genetic improvement by irradiation, Mba (2013) stated that the most crucial aspect is determining the effective dose that LD_{50} can determine. The effective dose, expressed as LD_{50}, induced a scaling down by 50% of the good seed viability. In addition to the LD_{50}, there is the RD_{50}, a dose that reduces the viability of seeds of the M1 population by 50% (Layek *et al.* 2022). Calculating the lethal dose of 50% is a standard method for obtaining mutants; it is based on the fact that there is a balance between physiological damage and acquired genetic changes.

Based on our research, we conclude that low-dose of gamma irradiation, ranging from 5-30 Gy, could promote the development of seedlings and leaf retention and morphological changes. Higher irradiation doses result in a decrease in seedling growth and survival as well as the leaf number. Finally, low-dose irradiation treatment tested on the seed in this research could be used as a reference for seed pre-treatment before germinating to obtain a good growth of forest tree seedlings.

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