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Genetic Variability of Lumbu Kuning and Lumbu Hijau Garlic Varieties Induced by ^{60}Co Gamma Ray Irradiation

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ABSTRACT

Garlic has limited genetic variability because the offspring are phenotypically identical to the parent plant. One approach to increase variability is mutation breeding using gamma irradiation. This study aimed to evaluate the effects of different gamma-ray doses on growth characteristics, physiological traits, chromosome number, and to determine the LD_{50} (lethal dose 50%) in Lumbu Kuning and Lumbu Hijau garlic varieties. The experiment was conducted from May to September 2021 in Ngroto Village, Pujon District, Malang Regency, using a single-plot design with observations on individual plants. Garlic bulbs were irradiated with gamma rays at doses of 0, 2, 4, 6, 8, and 10 Gy. The t-test analysis of the M0 generation indicated that gamma irradiation significantly affected growth parameters, including leaf length and width, number of leaves, and plant height, compared to the control. The lowest mean leaf length (7.81 cm), number of leaves (3.22), and plant height (12.32 cm) were observed in the Lumbu Hijau variety at 10 Gy, while the lowest leaf width (0.37 cm) occurred in Lumbu Kuning at 8 Gy, indicating phenotypic reduction at higher doses. The highest chlorophyll content (1,091 mg/g) was recorded in Lumbu Hijau treated with 8 Gy. Gamma irradiation also induced chromosomal abnormalities, including aneuploidy with chromosome numbers such as $2n=2x-1$ and $2n=2x+4$. These results demonstrate that low-dose gamma irradiation can generate useful variability for garlic improvement programs.

1. INTRODUCTION

Garlic (*Allium sativum* L.) is a horticultural plant that has many benefits, including being used as a spice, vegetable pesticide, and medicine. Based on data from the Badan Pusat Statistika (BPS), the production of garlic in Indonesia reached 81.80 thousand tons in 2020. This figure decreased by 7.89% compared to 2019, which reached 88.82 thousand tons, while the area of garlic harvested throughout Indonesia was 4271 ha in 2022. Garlic productivity in Indonesia is far from sufficient to meet domestic demand. The constraints of garlic cultivation in Indonesia include narrow harvestable land, high production costs, low seed quality, and low genetic variability of garlic. Garlic is cultivated vegetatively through bulbs because garlic plants in Indonesia are unable to flower. This results in low genetic variability of garlic. Vegetative propagation will reduce genetic variability because the resulting seedlings will definitely be identical to their parents. Improving the genetic quality of garlic through conventional breeding (crossbreeding) is very difficult. As a result, natural genetic variability within a single variety is very low, which limits the opportunities for selection to obtain new superior traits. Therefore, garlic plant breeding efforts are needed to increase genetic variability. One of the breeding methods that can be done is through mutation breeding.

Mutation is the easiest plant breeding method to obtain variability because it is able to change the genetic material associated with changes in character, and the resulting character can be a new superior trait that is not owned by the parent plant with unexpected results (Aisyah *et al.* 2009). Mutations cause changes in genetic material that are random, unexpected and occur suddenly so that the results obtained are unpredictable. The event of mutation is called mutagenesis, while mutants are living things that experience mutations and factors that cause mutations are called mutagens. Mutations can be induced through physical, chemical, and biological mutagens. One of the physical mutagens is gamma rays. Physical mutagens in the form of gamma rays are obtained from ionizing radiation. Gamma rays are often used as physical mutagens in mutation breeding because they can increase variability so as to produce new mutants. Gamma radiation emitted by the isotope Cobalt has high photon energy, enabling it to penetrate plant tissue and induce changes in DNA structure. The factor that affects the formation of mutants due to gamma irradiation is the radiation dose. The advantages of using gamma rays as a mutagen include being safe for humans and the environment, being widely used on various species of plants, not requiring a detoxification process, being able to be combined with other types of mutagens through in vitro culture, and supporting conventional breeding (Ulukapi & Nasircilar, 2015). Gamma irradiation is reported to be able to induce genetic diversity in black oat plants (Silveira *et al*., 2020). Gamma irradiation in in vivo conditions usually uses a dose of 20–80 Gy and in vitro conditions with a dose of 8–35 Gy (Syukur *et al*., 2018). Based on the results of previous studies, it shows that ionizing radiation causes different effects on plant anatomy, morphology, and biochemistry based on the dose used. Different doses will affect different sensitivity in each plant. Plant sensitivity is influenced by the volume of DNA nuclei, ploidy level and number of chromosomes. As a breeder, it is necessary to pay attention to the dose level of mutagen to be used, because the dose of mutagen is one that determines the formation of mutants.

Gamma rays produce mutant plants such as shallot plants irradiated with 10 Gy gamma rays showing the highest diversity in the character of wet and dry weight of bulbs. Lumbu Hijau irradiated with gamma rays at a dose of 6 Gy produces garlic mutants that can adapt to the lowlands and have a higher chlorophyll content. The mutation frequency due to gamma rays is influenced by the radiosensitivity of plants. The higher the radiosensitivity of irradiated plants, it can be assumed the levels of oxygen and water molecules in genetic material, so that it will indirectly trigger the formation of ROS (Reactive Oxygen Species) (Riviello-Flores *et al*., 2022). Thus, gamma irradiation at relatively low doses has a positive effect on plant growth and tolerance to abiotic stress (Zhang *et al*., 2016).

Mutation induction using gamma rays applied to garlic bulbs of Lumbu Kuning and Lumbu Hijau varieties in this study was conducted to determine the effect caused by gamma-ray mutagenesis on several garlic characters. Gamma rays are expected to induce changes in various characters for the better. Research on the appropriate radiation dose is crucial, as each variety has a different radiosensitivity. The novelty of this research lies in the LD_{50} value and information on the number of chromosomes resulting from mutation using gamma rays that emerge with broad phenotypic and genotypic diversity as a basis for selecting future garlic varieties that are more productive and adaptive. The purpose of this study was to determine the genetic variability of garlic varieties of Lumbu Hijau and Lumbu Kuning on growth and physiological characters.

2. MATERIALS AND METHODS

2.1. Plant Materials

The research was conducted from May to September 2021. Gamma irradiation was conducted at the Center for Nuclear Materials Technology - BATAN. The research was conducted in Ngroto Village, Pujon District, Malang Regency. This study used ready-to-plant garlic bulbs of Lumbu Hijau and Lumbu Kuning varieties. Garlic bulb seeds were obtained from the East Java Agricultural Instrument Standards Implementation Center.

2.2. Experimental Design

The experimental design used was the single plant method, which involved planting in the same cultivation environment without repetition. The experiment was conducted without repetition because each plant had different genetic potential. There are two varieties of garlic used, namely Lumbu Kuning and Lumbu Hijau. The doses used were 0.2 Gy (D1), 4 Gy (D2), 6 Gy (D3), 8 Gy (D4), and 10 Gy (D5). The weight of the garlic bulbs used was approximately 1.5-3 grams. A total of 40 plants per dose were irradiated, and observations were made on all plants.

2.3. Observation of Growth Characteristic

Some of the growth characters observed in Lumbu Kuning and Lumbu Hijau varieties were: 1) Plant length (cm); 2)

Diameter of stem (cm); 3) Number of leaves (blade); 4) Leaf length (cm); 5) Leaf width (cm). Measurements were taken once a week from 7 days after planting until harvest.

2.4. Stomata Characteristic

The stomatal characteristics observed included: 1) average number of stomata; 2) stomatal density (μm^2); 3) stomatal length; and 4) stomatal width. Stomatal observations were conducted when the plants were 21 days after planting. Stomatal observations were conducted on healthy, green leaves that were free from disease and had opened completely. Preparations were made by isolating the leaf surface with clear nail polish and pulling it using a seed isolation technique. The preparations were then observed under a microscope. The number of stomata, stomatal density, stomatal length, and width were observed using the ImageJ application.

2.5. Chlorophyll Content

The steps carried out were to prepare 2 g of healthy, fresh leaves (3–5 pieces) that were fully open (the top two number 3 leaves that had opened). Next, the sample is ground using a mortar and pestle until it becomes a paste, along with the addition of 10 ml of acetone, and placed in a vial, sealed, and stored in a refrigerator. Then, filter the solution using Whatman 42 filter paper. The extract is collected in a vial. If necessary (still concentrated), take a test tube. Take 1 ml of extract and add 9 ml of acetone, then homogenize. Place in a cuvette and measure the absorbance at wavelengths of 645 nm and 663 nm. The formula for calculating total chlorophyll is as follows (Arnon, 1949):

$$Chlorophyll\ Total\ (Ct, \mu g\ ml^{-1}) = chlorophyll\ a + chlorophyll\ b \quad (1)$$

$$Chlorophyll\ Total\ (Ct, \mu g\ ml^{-1}) = (20.2 \times 645) + (8.02 \times A663) \quad (2)$$

2.6. Chromosome Analysis

The number of chromosomes, which is taking leaves and roots of the treatment results at the age of 4 months, with the criteria of young roots. Observation of the number of chromosomes is done by the squashing method (Syukur & Sastrosumarjo, 2013). The root of the garlic plant is cut at the tip to a length of 0.5–1 cm, then placed in a tube containing 0.002 M 8-Hydroxyquinoline solution and placed in a refrigerator for 24 hours. Next, remove the roots from the tube and place them in a tube containing an HCl solution with acetic acid in a ratio of 3:1 for 2 minutes and heat in a water bath at a temperature of 60 °C for 2 minutes. Transfer the heated roots to a watch glass with the root tips inside the glass, drip 2% acetoorcein for 10 minutes, place the cut root tips 1–2 mm on the object glass, drip 2 drops of 2% acetoorcein and cover with a cover slip, pass the specimen over a bunsen burner 2–3 times to maintain sterility and tap it with a rubber-tipped pencil (Squash), then place the specimen under a light microscope to observe the number of garlic chromosomes.

2.7. Lethal Dose (LD_{50})

Lethal Dose is the dose of gamma rays that can cause death in plants. Determination of LD_{50} which is determined from the ratio of the number of living plants per dose to the number of living plants in the control. The determination of the gamma ray dose that causes a lethal dose 50 (LD_{50}) was performed using a linear regression equation. The LD_{50} value analysis was observed when the plants were 28 days old after planting, which was calculated as a percentage using the following formula (Kurniajati, 2017):

$$Percentage\ of\ plants\ growing = \frac{(number\ of\ living\ plants\ per\ dose)}{(number\ of\ living\ plants\ in\ the\ control\ treatment)} \times 100\% \quad (3)$$

Determination of the best model for each variety by looking at the R^2 value. An R^2 value close to 1 indicates that the model used is better.

2.8. Data Analysis

Quantitative data were subjected to *t*-test analysis at the 5% confidence level. The *t*-test is one of the tests used to determine whether there is a significant difference between two means (averages) (Payadnya & Jayantika, 2018).

3. RESULTS AND DISCUSSIONS

3.1. Growth Characteristic

The average characters of plant length, stem diameter, number of leaves, and leaf length of M1 potential mutant showed a significant effect based on the results of *t*-test analysis on gamma ray doses of 2 Gy, 4 Gy, 6 Gy, 8 Gy, and 10 Gy on Lumbu Kuning. Furthermore, in the character of leaf width of Lumbu Kuning, doses of 4 Gy, 8 Gy and 10 Gy showed a significant effect based on the results of *t*-test analysis, while doses of 2 Gy and 6 Gy had no significant effect (Table 1). In the Lumbu Hijau, all doses of gamma rays showed a significant effect on the characters of plant length, stem diameter, number of leaves, leaf length, and leaf width (Table 2). Mutagens or materials that cause mutations, new variability can be created in an effort to obtain superior varieties in accordance with the breeding program (Harsanti & Yulidar, 2015).

Table 1. Results of *t*-test of control with gamma rays treated seeds on growth variables of Lumbu Kuning garlic

Variable	Control	D1LK	*t*-test	D2LK	*t*-test	D3LK	*t*-test	D4LK	*t*-test	D5LK	*t*-test
Plant length (cm)	46.98	35.13	9.59*	26.72	17.70*	31.16	10.57*	22.04	20.59*	17.54	35.88*
Stem diameter (cm)	1.32	0.69	10.05*	0.63	12.63*	0.66	10.93*	0.49	16.36*	0.37	17.13*
Number of leaves	6.02	4.95	3.15*	4.22	6.42*	5.00	3.17*	3.52	9.33*	3.47	7.08*
Leaf length (cm)	31.53	25.63	9.59*	21.72	5.50*	24.14	4.21*	18.04	8.59*	11.74	14.86*
Leaf width (cm)	1.11	1.10	0.06ns	0.70	6.74*	1.03	0.98ns	0.37	14.01*	0.70	6.53*

Description: LK = Lumbu Kuning; gamma-ray dose : D1 = 2 Gy; D2 = 4 Gy; D3 = 6 Gy; D4 = 8 Gy; D5 = 10 Gy; *) = significant; ns = not significant at 5% confidence level.

Table 2. Results of *t*-test of control with gamma rays treated seeds on growth variables of Lumbu Hijau garlic

Variable	Control	D1LH	*t*-test	D2LH	*t*-test	D3LH	*t*-test	D4LH	*t*-test	D5LH	*t*-test
Plant length (cm)	37.39	23.05	9.89*	25.61	6.63*	20.41	10.41*	20.93	9.32*	12.32	17.18*
Stem diameter (cm)	0.69	0.55	4.59*	0.54	4.49*	0.36	10.10*	0.34	10.92*	0.31	11.93*
Number of leaves	5.7	3.72	8.08*	3.70	9.18*	3.87	6.32*	3.47	11.07*	3.22	11.85*
Leaf length (cm)	30.39	18.65	8.09*	20.41	5.62*	16.51	8.50*	15.55	9.02*	7.81	15.97*
Leaf width (cm)	1.05	0.81	5.40*	0.69	7.93*	0.67	7.24*	0.46	12.74*	0.50	11.33*

Description: LH = Lumbu Hijau; gamma-ray dose : D1 = 2 Gy; D2 = 4 Gy; D3 = 6 Gy; D4 = 8 Gy; D5 = 10 Gy; *) = significant; ns = not significant at 5% confidence level.

Gamma radiation is often used in plant breeding efforts because it can increase variability, so as to produce new mutants. Gamma rays are physical mutagens used to expand variability and improve plant traits. The benefits of gamma rays as a mutagen are safe for humans and the environment, can be applied to various plant species, do not require post-application processes, and can be combined with other modern mutation techniques, such as in vitro reproduction (Ulukapi & Nasircilar, 2015). Gamma irradiation has been reported to induce genetic variability in chrysanthemum, soybean, and black oat (Silveira *et al*., 2014). Factors that influence the formation of mutants include the amount of radiation dose. The radiation dose applied varies depending on the level of sensitivity of the species and the part of the plant being treated. The level of sensitivity is influenced by the size of the cell nucleus, the number of chromosomes, and the degree of ploidy.

Gamma rays cause damage to plant cells. The decrease in the percentage of plant growth due to gamma radiation is caused by the deterministic effect. The deterministic effect is the effect of cell death caused by radiation exposure. According to Mubarok *et al*. (2011), the higher the radiation dose, the higher the deterministic effect received by plants. Naturally, the chance of mutation in nature is 10–6 for each gene every generation. Previous studies have shown that ionizing radiation has different effects on plant anatomy, morphology, and biochemistry based on the dose used. Gamma irradiation at relatively low doses has a positive effect on plant growth and tolerance to abiotic stress (Zhang *et al*., 2016). Research conducted by Choudry and Dyansagar reported that gamma radiation on garlic produced 16 mutants in MV2 and MV3, while Marchesi obtained garlic mutants from gamma radiation at doses of 1–4 Gy (Kurniajati, 2017).

Giving a high dose of irradiation to garlic bulbs results in slow growth due to metabolic changes and results in disruption of protein synthesis that plays a role in plant growth (Winarni *et al*., 2022). Gamma radiation affects the length of garlic plants. In the Lumbu Kuning, the 10 Gy gamma ray treatment (17.54 cm) has the lowest plant length compared to the control (46.98 cm) and other doses. Similarly, in the Lumbu Hijau, the 10 Gy gamma ray treatment (12.32 cm) has the lowest plant length compared to the control (37.39 cm) and other doses. These results are in accordance with the results of research Nurhasanah (2019) on garlic varieties of Lumbu Kuning and Lumbu Hijau which showed that the higher the dose of gamma rays has the lower the plant length. Plants appear shorter because of the inhibition of the development and growth of shoot meristem cells due to high radiation energy. In the characters of stem diameter, number of leaves, leaf length and leaf width, gamma ray treatment decreased the observation results. The higher the dose of gamma rays given, the lower the stem diameter, number of leaves, leaf length and leaf width. Gamma-ray treatment is thought to cause physiological damage that affects the ability to grow and stimulate plant organs. This is in accordance with the statement of Anshori *et al*. (2014) that gamma- ray irradiation inhibits the growth of plant height. Gamma irradiation also reduced plant height, number of leaves, fresh weight and resulted in explant death in rose plants (Moharrami *et al*., 2015).

Table 3. Results of *t*-test of control with gamma rays treated seeds on the stomatal characteristic of Lumbu Kuning garlic

Variable	Control	D1LK	*t*-test	D2LK	*t*-test	D3LK	*t*-test	D4LK	*t*-test	D5LK	*t*-test
Number of stomata	54.33	49.00	2.43ns	62.67	-9.45ns	52.67	0.56ns	68.67	-2.01ns	64.33	-3.97ns
Stomatal density (mm^{-2})	276.84	249.68	2.43ns	319.32	-9.45ns	268.37	0.56ns	349.89	-2.01ns	327.81	-3.97ns
Stomatal length (µm)	21.57	22.54	-0.52ns	15.44	7.06*	21.40	0.43ns	18.54	5.65*	20.48	0.76ns
Stomatal width (µm)	7.77	8.29	-0.73ns	6.26	4.03*	9.82	-18.52ns	7.90	-0.20ns	7.70	0.12ns

Description: LK = Lumbu Kuning; gamma-ray dose : D1 = 2 Gy; D2 = 4 Gy; D3 = 6 Gy; D4 = 8 Gy; D5 = 10 Gy; *) = significant; ns = not significant at 5% confidence level.

Table 4. T-test of control treatment with D1LH, D2LH, D3LH, D4LH, D5LH on stomata characteristic

Variable	Control	D1LH	*t*-test	D2LH	*t*-test	D3LH	*t*-test	D4LH	*t*-test	D5LH	*t*-test
Number of stomata	43.33	48.33	-1.23ns	48.33	-1.32ns	52.00	-1.01ns	55.33	-2.61ns	46.33	-1.00ns
Stomatal density (mm^{-2})	220.81	246.28	-1.23ns	246.28	-1.32ns	264.97	-1.01ns	281.95	-2.61ns	236.09	-1.00ns
Stomatal length (µm)	20.11	21.40	-0.65ns	20.38	0.37ns	15.59	2.08ns	18.98	0.59ns	25.74	-31.34ns
Stomatal width (µm)	7.50	6.37	1.01ns	7.96	-1.01ns	6.59	0.59ns	6.83	0.47ns	7.98	-0.35ns

Description: LH = Lumbu Hijau; gamma-ray dose : D1 = 2 Gy; D2 = 4 Gy; D3 = 6 Gy; D4 = 8 Gy; D5 = 10 Gy; *) = significant; ns = not significant at 5% confidence level.

3.2. Stomata Characteristic

Based on the results of the t-test analysis, it shows that gamma irradiation significantly affects the length of stomata on Lumbu Hijau with doses of 4 Gy and 8 Gy, then gamma irradiation significantly affects the width of stomata on Lumbu Hijau with a dose of 4 Gy (Table 3). Gamma irradiation did not show a real effect on all observation characters on Lumbu Hijau (Table 4). Comparison of stomata in the control treatment and gamma irradiation is presented in Figure 1.

In general, gamma irradiation increases the number of stomata and stomatal density but decreases stomatal length and stomatal width in garlic plants of Lumbu Kuning and Lumbu Hijau. Stomata on the leaves have a function to control the gas exchange process (CO_2 and water vapor) that occurs between the atmosphere and the leaves. According to Pangesti & Ratnawati (2022), the decrease in the number and density of stomata is due to an increase in the dose of gamma irradiation, which occurs due to damage to stomatal formation in cells that are sensitive to high irradiation exposure. Generally, low-level irradiation produces higher observation values than the control (Rosmala *et al*., 2016). This is in accordance with the results of the study which showed that doses of 2 Gy, 4 Gy, 6 Gy, 8 Gy and 10 Gy had a higher number of stomata than the control. Based on the statement of Meliala *et al.* (2016) stated that genetic changes caused by mutation are unexpected, so that in one dose of treatment can appear diverse genetic variations. Irradiation negatively affects plant tissue, damaging roots, stems, and growing shoots, which causes morphological changes. These changes create an opportunity to obtain characters superior to their parents, resulting from gamma radiation that inhibiting auxin production required for DNA formation (Qosim, 2006). Furthermore, the formation and distribution of stomata are influenced by auxin regulatory pathways Balcerowicz & Hoecker (2014). Auxin has the ability to influence the division and differentiation process of cells involved in stomatal formation.

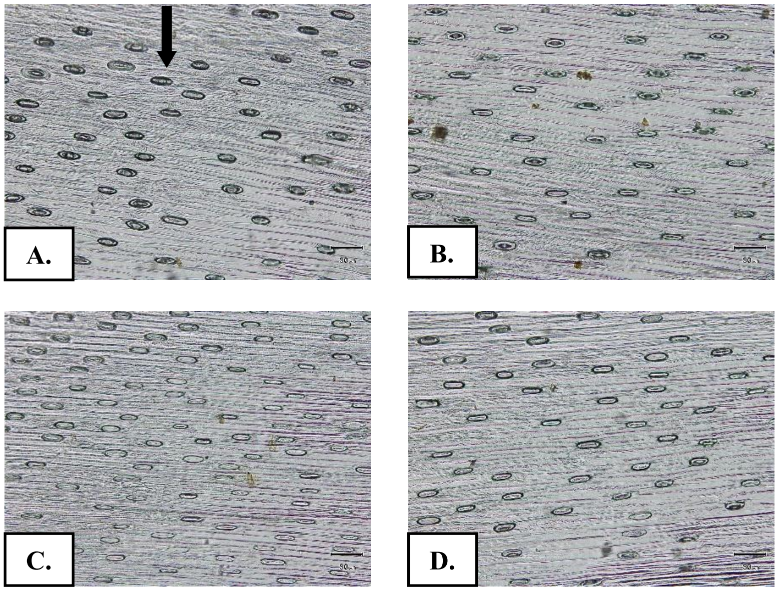


Figure1. Gamma-irradiated stomata; Description: A). Control Lumbu kuning; B). Control Lumbu Hijau; C). Lumbu kuning + 8 Gy gamma ray dose; and D). Lumbu Hijau + 8 Gy gamma-ray dose.

3.3. Chlorophyll Content

Based on the analysis, it can be seen that the Lumbu Hijau responds better than the Lumbu Kuning when given gamma irradiation. This can be seen in Table 5, which shows that the total chlorophyll content of Lumbu Hijau is higher than Lumbu Kuning. In the control treatment, the Lumbu Hijau also had a higher chlorophyll content. The highest total chlorophyll content was the Lumbu Hijau treatment with 8 Gy gamma irradiation dose, while the lowest chlorophyll content was the control treatment of Lumbu Kuning. This shows that gamma irradiation can increase the chlorophyll content in garlic plants.

Table 5. Chlorophyll content of Lumbu Hijau and Lumbu Hijau due to gamma irradiation treatment

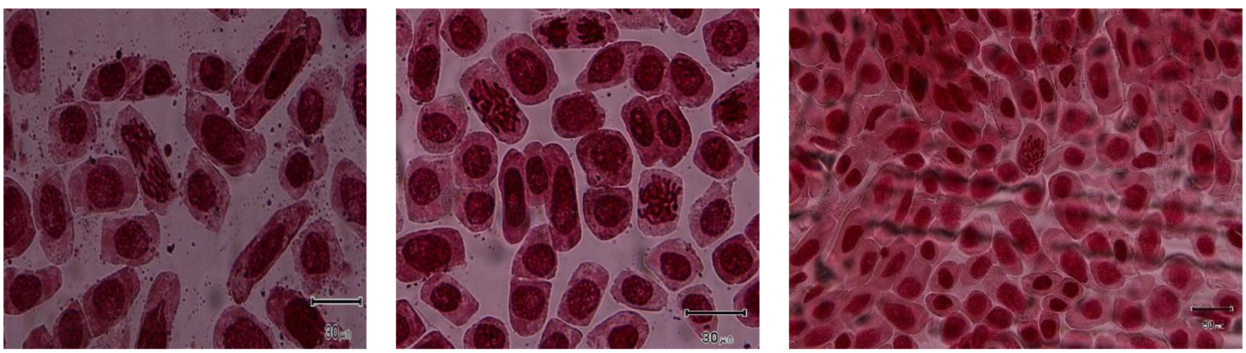
Treatment	Chlorophyll a (mg/g DW)	Chlorophyll b (mg/g DW)	Total Dry Weight (mg/g)
Control LK	0.524	0.130	0.654
Control LH	0.684	0.188	0.871
D1LK	0.659	0.190	0.849
D2LK	0.648	0.176	0.823
D3LK	0.727	0.208	0.935
D4LK	0.622	0.168	0.790
D5LK	0.662	0.173	0.841
D1LH	0.782	0.231	1.013
D2LH	0.760	0.217	0.977
D3LH	0.772	0.239	1.011
D4LH	0.819	0.272	1.091
D5LH	0.725	0.268	0.993

Description: LK = Lumbu kuning LH = Lumbu Hijau; gamma-ray dose : D1 = 2 Gy; D2 = 4 Gy; D3 = 6 Gy; D4 = 8 Gy; D5 = 10 Gy; *) = significant; ns = not significant at 5% confidence level.

Chlorophyll content characterizes the chlorophyll content in the leaves that will be used for the photosynthesis process. Chlorophyll is the green color of leaves. Chlorophyll is synthesized in chloroplasts. Chloroplasts are organelles that are very sensitive to irradiation with high doses of gamma rays, while irradiation at low rates does not cause changes in chloroplast structure (Wi *et al*., 2007). In dates (*Phoenix dactilyfera*) chlorophyll-a and carotenoids are more sensitive to irradiation than chlorophyll-b (Al-Enezi *et al*., 2012). This is in accordance with the results of research showing that chlorophyll a is more sensitive than chlorophyll b due to gamma irradiation. In this study, gamma-ray irradiation had a positive effect on chlorophyll content presumably because the dose given was low. Research conducted by Kim *et al*. (2009) showed that gamma irradiation with a rate of 50 Gy increased the content of chlorophyll and carotenoids in Arabidopsis leaves when compared to the control because the transcription process of genes involved in chlorophyll and anthocyanin anabolism increased. In another study, it was mentioned that soybean plants that had been irradiated at high doses (50–100 Gy) experienced a decrease in chlorophyll content (Alikamanoglu *et al*., 2011). Thus, it can be concluded that low-dose gamma irradiation can increase chlorophyll content due to the stress of gamma rays.

3.4. Chromosome Analysis

The results of chromosome analysis show that gamma irradiation affects the number of chromosomes in Lumbu Kuning and Lumbu Hijau. In Lumbu Kuning, the doses of 2 Gy ($2n = 2x+1$), 4 Gy ($2n = 2x+4$), 6 Gy ($2n = 2x+1$), and 8 Gy ($2n = 2x+4$) produced aneuploid plants. In the Lumbu Hijau, doses of 2 Gy ($2n = 2x+4$) and 4 Gy ($2n = 2x+1$) produced aneuploid plants. Aneuploidy is a genetic disorder in which there is an extra copy or loss of one chromosome. The basic chromosome number of garlic is $2n = 2x = 16$. Chromosome analysis is done to determine the number of chromosomes due to gamma irradiation. Physical mutagens with gamma rays will cause large-scale DNA deletions and changes in chromosome structure (Parry *et al*., 2009). Aneuploidy almost always hurts fertility due to genomic imbalance. However, garlic is propagated vegetatively, and the impact on generative fertility is less of a major constraint in breeding. The implications of aneuploidy on plant vigor can have both positive and negative effects. Negative effects of aneuploidy include stunted growth, narrow leaves, and abnormal root development. Positive effects include an increase in the size of certain cells and thicker leaves. However, these positive effects are very rare. Gamma rays produce Reactive Oxygen Species (ROS), which interact with DNA and cause base substitutions as well as genome rearrangements (Kebeish *et al*., 2015).



(a) (b) (c)

Figure 2. Chromosomes resulting from gamma irradiation treatment: (A). Lumbu Kuning gamma rays 2 Gy B); Lumbu Hijau gamma rays 2 Gy; C.) Control.

3.5. LD_{50}

LD_{50} value of Lumbu Kuning variety is 8 Gy with R^2 value = 0.4897. LD_{50} value of Lumbu Hijau variety is 10 Gy with R^2 value = 0.9249. The R^2 value that is getting closer to 1 indicates that the equation model used is getting better so that it can represent the concentration and dose limits that can cause death in plant populations. LD_{50} can be used to determine the concentration and dose used for consideration in determining the treatment with the aim of obtaining high diversity with a high percentage of mutants as well. The following are the percentages of plants surviving at each dose of gamma radiation (Table 6).

Plant radiosensitivity is a measure of success to increase diversity through mutation that can be measured through the lethal dose (LD_{50}) (Yelni *et al*., 2019). The optimum dose to produce mutants is generally at or slightly below LD_{50}. Exposure to LD_{50} can produce maximum mutant variants and minimize unwanted mutants. The LD_{50} in this study was used to determine the most effective irradiation dose, which is the dose that causes 50% of the planting material (tubers) to die or fail to grow. This dose is considered optimal because it is high enough to produce genetic

Table 6. Percentage of living plants

No	Dose	Lumbu Kuning	Lumbu Hijau
1	Control	100	100
2	2 Gy	92.5	67.5
3	4 Gy	87.5	60
4	6 Gy	92.5	50
5	8 Gy	50	70
6	10 Gy	97.5	67.5

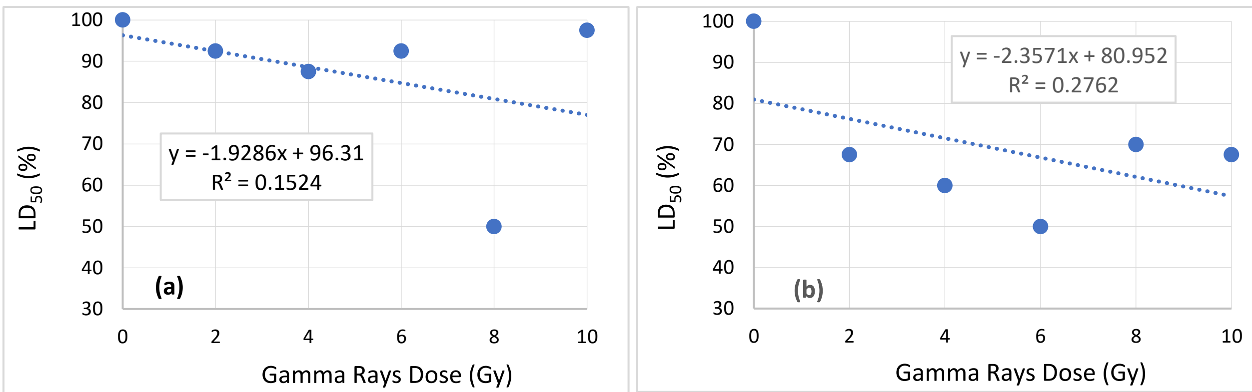


Figure 3. Effect of gamma rays dose on the percentage of abnormal seed growth (%) in the determination of LD_{50}.

diversity and not too high so that there are still living individuals left for selection. The smaller the LD_{50} value, the greater the level of sensitivity to radiation (Azizah, 2015). Lumbu Kuning showed more tolerance to gamma rays compared with Lumbu Hijau. This is suspected because Lumbu Kuning has greater genomic stability. High sensitivity to radiation can result in the emergence of deadly mutants. Gamma rays in doses around LD_{50} cause damage to physiological functions and result in the death of half of the plant, but it is proportional to the genetic changes obtained (Zanzibar & Witjaksono, 2011).

4. CONCLUSION

Doses of gamma rays 2 Gy, 4 Gy, 6 Gy, 8 Gy, and 10 Gy can affect the appearance of garlic plants of Lumbu Kuning and Lumbu Hijau varieties on plant length, leaf length, number of leaves, and leaf width. Doses of 2 Gy, 4 Gy, 6 Gy, and 8 Gy produce aneuploid plants in the Lumbu Kuning. LD_{50} value of Lumbu Kuning is 8 Gy, and Lumbu Hijau is 10 Gy. Based on the results obtained, further research needs to be done to test the M1 results of gamma-ray mutation.

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
AN	✓	✓			✓	✓	✓		✓	✓	✓		✓	
ALA			✓					✓						
LS				✓								✓		✓

C: Conceptualization	Fo: Formal Analysis	O: Writing - Original Draft	Fu: Funding Acquisition
M: Methodology	I: Investigation	E: Writing - Review & Editing	P: Project Administration
So: Software	D: Data Curation	Vi: Visualization	
Va: Validation	R: Resources	Su: Supervision	

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REFERENCES

Aisyah, S.I., Aswidinnoor, H., Saefuddin, A., Marwoto, B., & Sastrosumarjo, S. (2009). Induksi mutasi pada stek pucuk anyelir (*Dianthus caryophyllus* Linn.) melalui iradiasi sinar gamma. *Jurnal Agronomi Indonesia*, ***37***(1), 62–70.

Al-Enezi, N.A., Al-Bahrany, A.M., & Al-Khayri, J.M. (2012). Effect of X-irradiation on date palm seed germination and seedling growth. *Emirates Journal of Food and Agriculture*, ***24***(5), 415–424.

Alikamanoglu, S., Yaycili, O., & Sen, A. (2011). Effect of gamma radiation on growth factors, biochemical parameters, and accumulation of trace elements in soybean plants (*Glycine max* L. Merrill). *Biological Trace Element Research*, ***141***(1–3), 283–293. https://doi.org/10.1007/s12011-010-8709-y

Anshori, Y.R., Aisyah, S.I., & Darusman, L.K. (2014). Induksi mutasi fisik dengan iradiasi sinar gamma pada kunyit (*Curcuma domestica* Val.). *Jurnal Hortikultura Indonesia*, ***5***(2), 84–94. https://doi.org/10.29244/jhi.5.2.84-94

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiology*, ***24***(1), 1–15. https://doi.org/10.1104/pp.24.1.1

Azizah, N. (2015). Penentuan Lethal Dose 50 (LD_{50}) Iradiasi Sinar Gamma pada Beberapa Kultivar *Heliconia* spp. [*Undergraduated Thesis*]. Institut Pertanian Bogor. http://repository.ipb.ac.id/handle/123456789/74611

Balcerowicz, M., & Hoecker, U. (2014). Auxin - a novel regulator of stomata differentiation. *Trends in Plant Science*, ***19***(12), 747–749. https://doi.org/10.1016/j.tplants.2014.10.006

BPS (Badan Pusat Statistika). (2020). *Garlic production in Indonesia*. Website: Badan Pusat Statistik Indonesia

Harsanti, L., & Yulidar. (2015). Pengaruh iradiasi sinar gamma terhadap pertumbuhan awal tanaman kedelai (*Glycine max* (L.) Merrill) varietas Denna 1. *Prosiding Pertemuan dan Presentasi Ilmiah – Penelitian Dasar Ilmu Pengetahuan dan Teknologi Nuklir*, 59–63.

Kebeish, R., Deef, H.E., & El-Bialy, N. (2015). Effect of gamma radiation on growth, oxidative stress, antioxidant system, and alliin producing gene transcripts in *Allium sativum*. *International Journal of Research Studies in Biosciences*, ***3***(3), 161–174.

Kim, J.H., Lee, M.H., Moon, Y.R., Kim, J.S., Wi, S.G., Kim, T.H., & Chung, B.Y. (2009). Characterization of metabolic disturbances closely linked to the delayed senescence of Arabidopsis leaves after γ irradiation. *Environmental and Experimental Botany*, ***67***(2), 363–371. https://doi.org/10.1016/j.envexpbot.2009.07.001

Kurniajati, W.S. (2017). Perakitan keragaman bawang merah (*Allium cepa* L. kelompok Aggregatum) dengan induksi mutasi sinar gamma. [*Undergraduated Thesis*]. Institut Pertanian Bogor

Meliala, J.H.S., Basuki, N., & Seogianto, A. (2016). Pengaruh iradiasi sinar gamma terhadap perubahan fenotipik tanaman padi gogo (*Oryza sativa* L.). *Jurnal Produksi Tanaman*, ***4***(7), 585–594.

Moharrami, M., Vadadi, S., & Ebrahimi, M.A. (2015). Effect of various levels of gamma irradiation on some characteristics of rose seedling. *International Journal of Sciences: Basic and Applied Research*, ***9***(9), 1460–1464.

Mubarok, S., Suminar, E., & Murgayanti. (2011). Uji efektivitas sinar gama terhadap karakter pertumbuhan sedap malam. *Jurnal Agrivigor*, ***11***(1), 25–33.

Nurhasanah, A. (2019). Pengaruh radiasi sinar gamma terhadap pertumbuhan dua varietas lokal bawang putih (*Allium sativum* L.) [*Undergraduated Thesis*]. Universitas Brawijaya.

Pangesti, M.H., & Ratnawati, R. (2022). Pengaruh iridiasi sinar Gamma Co-60 terhadap karakteristik morfologis dan tanaman marigold (*Tagetes erecta* L.) *Kingdom*: *The Journal of Biological Studies*, ***8***(2), 94-108.

Parry, M.A.J., Madgwick, P.J., Bayon, C., Tearall, K., Hernandez-Lopez, A., Baudo, M., Rakszegi, M., Hamada, W., Al-Yassin, A., Ouabbou, H., Labhilili, M., & Phillips, A.L. (2009). Mutation discovery for crop improvement. *Journal of Experimental Botany*, ***60***(10), 2817–2825. https://doi.org/10.1093/jxb/erp189

Payadnya, I.P.A.A., & Jayantika, I.G.A.N.T. (2018). *Panduan penelitian eksperimen beserta analisis statistik dengan SPSS*. Yogyakarta, Indonesia: Deepublish.

Qosim, W.A. (2006). Studies on gamma ray irradiation of mangosteen nodular callus for improvement genetical and morphological variability of the regenerants [*PhD Dissertation*]. Institut Pertanian Bogor.

Riviello-Flores, M.d.I.L., Cadena-Iñiguez, J., Ruiz-Posadas, L.d.M., Arévalo-Galarza, M.d.L., Castillo-Juárez, I., Soto Hernández, M., & Castillo-Martínez, C.R. (2022). Use of gamma radiation for the genetic improvement of underutilized plant varieties. *Plants*, ***11***(9), 1161. https://doi.org/10.3390/plants11091161

Rosmala, A., Khumaida, N., & Sukma, D. (2016). Alteration of leaf anatomy of handeuleum (*Graptophyllum pictum* L. Griff) due to gamma irradiation. *HAYATI Journal of Biosciences*, ***23***(3), 138–142. https://doi.org/10.1016/j.hjb.2016.12.003

Silveira, G., Moliterno, E., Ribeiro, G., Costa, P.M.A., Woyann, L.G., Tessmann, E.W., Oliveira, A.C., & Cruz, C.D. (2014). Increasing genetic variability in black oats using gamma irradiation. *Genetics and Molecular Research*, ***13***(4), 10332–10340. https://doi.org/10.4238/2014.December.4.28

Silveira, S.F.d.S., Oliveira, D.C.S., Maltzhan, L.E., Corazza, T., Oliveira, V.F.d., Stulp, C., Carbonari, H.P., Rother, V., Haverroth, M.C., Gutkoski, L.C., Maia, L.C.d., & Oliveira, A.C.d. (2020). Associations between agronomic performance and grain chemical traits in oat. *Communications in Plant Sciences*, ***10***, 001–007. https://doi.org/10.26814/cps2020001

Syukur, M., & Sastrosumarjo, S. (Eds.). (2013). *Sitogenetika tanaman*. IPB Press.

Syukur, M., Sujiprihati, S., & Yuniarti, R. (2015). *Teknik Pemuliaan Tanaman*. Penebar Swadaya, Jakarta.

Ulukapi, K., & Nasircilar, A.G. (2015). Developments of gamma ray application on mutation breeding studies in recent years. *Proceedings of the International Conference on Advances in Agricultural, Biological & Environmental Sciences*, 31–34. https://doi.org/10.15242/IICBE.C0715044

Wi, S.G., Chung, B.Y., Kim, J.S., Kim, J.H., Baek, M.H., Lee, J.W., & Kim, Y.S. (2007). Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron*, ***38***(6), 553–564. https://doi.org/10.1016/j.micron.2006.11.002

Winarni, U., Dinarti, D., & Aisyah, S.I. (2022). Evaluasi metabolomik mutan putatif bawang putih (MV3) hasil iradiasi gamma LD_{50}. *Jurnal Agronomi Indonesia*, ***50***(1), 49–56. https://doi.org/10.24831/jai.v50i1.38086

Yelni, G., Syarif, Z., Kasim, M., & Hayati, P.K.D. (2019). Meningkatkan keragaman genetik bawang putih (*Allium sativum* L.) melalui mutasi iradiasi gamma. *Jurnal Sains Agro*, ***4***(2), 1–13.

Zanzibar, M., & Witjaksono, W. (2011). Pengaruh penuaan dan iradiasi benih dengan sinar gamma (^{60}Co) terhadap pertumbuhan bibit suren (*Toona sureni* Blume Merr). *Jurnal Penelitian Hutan Tanaman*, ***8***(2), 89–96.

Zhang, L., Zheng, F., Qi, W., Wang, T., Ma, L., Qiu, Z., & Li, J. (2016). Irradiation with low-dose gamma ray enhances tolerance to heat stress in *Arabidopsis* seedlings. *Ecotoxicology and Environmental Safety*, ***128***, 181–188. https://doi.org/10.1016/j.ecoenv.2016.02.025