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CO^{60} dose and dose rate's effects on Edamame Ryoko Jember variety's growth and production

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ABSTRACT

Edamame is a famous type of soybean plant in Japan, edamme is often also referred to as Japanese soybeans. The purpose of this study was to determine the Lethal Dose 20 (LD20) and lethal dose 50 (LD50) radiosensitivity measurements in ryoko varieties originating from Jember which have been irradiated by Cobalt60 gamma rays in BATAN, with 5 dose levels including, 100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy with Gammacell220. Edamame plants of the Jember Local Varieties were treated with a radiation dose of 200 Gy and a dose of 400 Gy. The plant height, number of pods, and weight of 100 seeds were measured. The results of the T Test showed that the number of leaves of the radiated plants has a real difference compared to controls at observations of 28, 42, and 56 HST. The LD20 and LD50 values obtained indicate that genetically edemame plant of local varieties of jember have a high level of radiosensitivity because if plants are treated at doses exceeding 500 Gy, seed germination will be abnormal or damaged.

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INTRODUCTION

Edamame is a famous type of soybean plant in Japan which is often also referred to as Japanese soybeans (Konovsky et al., 2020; Mahoussi et al., 2020; Moinuddin et al., 2023). Edamame, which is generally sold fresh and consumed as boiled soybeans, is favored by the people of Japan, China, and Korea (Gupta & Jeyaseelan, 2022; Nair et al., 2023; Shanmugasundaram & Yan, 2010). The seeds originally came from Japan. This type of soybean is famous for its large seed size. Indonesia is the fourth country in Asia to successfully develop and export edamame to Japan after Taiwan, China and Thailand.

Due to the specific market, edamame has a stable and relatively high price in the market (Ahomondji et al., 2023; Dhakal et al., 2021; Lord et al., 2021). It is expected that edamame can be used as one of the leading agricultural commodities in Indonesia in increasing the level of global business competition (Setyaningsih et al., 2018). According to data from the Central Statistics Agency (2020), the average soybean productivity in 2020 was 15.69 Ku/Ha with the highest average soybean productivity in the West Sulawesi region of 20 Ku/Ha.

This research is one of the efforts to improve the quality of edamame ryoko originating from Jember with mutation breeding methods. The results of this mutation breeding are expected to be able to produce good and stable seed agronomic characters from previous breeding. The desired properties are to increase the number of seeds per pod to above 4 seeds per pod, where previously the number of seeds per pod in the edamame plant of the ryoko variety was below 4 seeds. Mutated breeding plants are also expected to be resistant to disease or abiotic environments so as to increase the amount of production. The expected properties are also seeds have a longer shelf life.

Induction of mutations is one way to increase plant diversity. Mutation induction can be done by treating mutagen materials to reproductive material to be mutated which can change some plant properties and not all that changes. Induction of mutations can occur naturally or artificially. Artificial mutations occur when mutagens are used at a certain dose and time. There are several types of mutagens, namely physical and chemical mutagens. Commonly used physical mutagens are X-ray energy, neutrons, and gamma rays.

Gamma-ray radiation is ionizing radiation. This form of radiation can penetrate cells and tissues easily. Gamma rays are obtained from the decay of radioactive substances emitted from atoms at high speeds due to excess energy. The wavelength of gamma rays is shorter than X-rays, but their energy is greater. Gamma ray radiation can be emitted by 60Co, 137Cs, and others. Gamma-ray plant mutations can cause changes in genetic

traits in positive and negative directions that can be passed on to the next generation, but can also return to normal. Inherited positive changes are desirable mutations in the field of plant breeding.

Radiation is done by determining the radiation dose that suits the plant, because each plant has a different response to radiation exposure. To determine the analysis of radiation results, lethal dose 20 (LD20) and lethal dose 50 (LD50) radiosensitivity measurements were made. Lethal Dose 20 (LD20) and Lethal Dose 50 (LD50) can be known gamma ray doses of 60Co that cause 20% and 50% of deaths in the irradiated population.

The purpose of this study was to determine *Lethal Dose* 20 (LD20) and *Lethal Dose* 50 (LD50) in edamame plants (*Glycin max (L) Merrill*) Jember ryoko varieties that are irradiated by gamma rays and to know the growth of edamame plants (*Glycin max (L) Merrill*) Jember ryoko varieties that are irradiated by gamma rays. The research contribution of this study is to determine the Lethal Dose 20 (LD20) and Lethal Dose 50 (LD50) in edamame plants (Glycin max (L) Merrill) Jember ryoko varieties that are irradiated by gamma rays, as well as to investigate the growth of these plants under such conditions. This study aims to provide scientific data on the effects of gamma radiation on edamame plants, which can be useful for understanding the impact of radiation on plant growth and potentially improving agricultural practices. The results of this study can contribute to the development of more resilient and radiation-tolerant crops, enhancing food security and sustainability.

METHOD

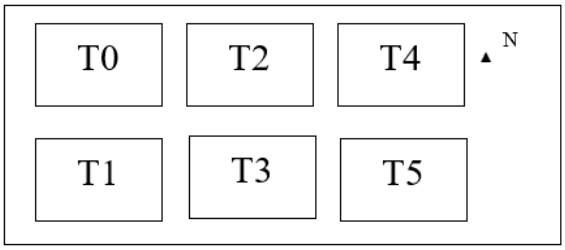
The study was conducted from March 2023 to November 2023. Gamma ray irradiation was carried out at the Central Laboratory of Isotope Application and Irradiation of the National Nuclear Energy Agency (PAIR BATAN) Jakarta. The experiment was conducted in the experimental land of UPT Rice Seed Development Center and Palawija 2 Singosari, Malang Regency located at 7 ºLS and 112 º BT with an altitude of ± 350 meters above sea level.

The material used is ryoko variety edamame seeds originating from Jember which have been irradiated Cobalt60 gamma rays in BATAN, with 5 dose levels including, 100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy with Gammacell220. The planting medium used is soil and manure. The chemical fertilizer used is NPK fertilizer (16:16:16). Pesticides are used according to the attack if needed.

The tools used in this study include gammacell 220 irradiator machine, planterbag, hoe, drill, ruler, meter, trowel, analytical scale, tugal (wood), stationery, camera, computer for data analysis.

This study was conducted using a single plant by planting 210 plants with each dose of 35 plants. One treatment factor is used, namely the dose of 60Co gamma ray irradiation which consists of 6 levels with the following code:

T0 = no irradiation (control)
T1 = irradiation dose 100 Gray
T2 = irradiation dose 200 Gray
T3 = irradiation dose 300 Gray
T4 = irradiation dose 400 Gray
T5 = irradiation dose 500 Gray



Description : T0,1,2,..,5 = 60Co gamma ray irradiation dose

Figure 1. Research Plan of Jember Local Variety Edamame Planting in the Field

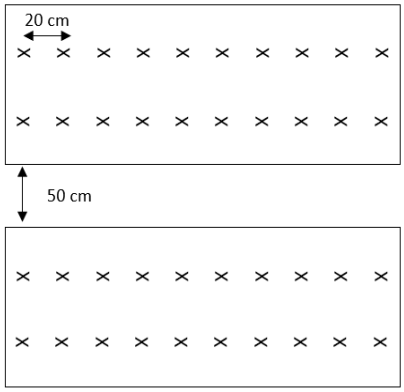


Figure 2. Edamame Plant Population in One Experimental Plot

The experiment was carried out without any repetition because each material irradiated had different genetic potential. Each experimental plot consisted of 35 plants and observations were made on all plant populations (Figure 1).

Research Implementation

Seed preparation

The edamame seed used is a ryoko variety originating from Jember. Before radiation with 60Co gamma rays, edamame seeds are sorted first to produce seeds that have good quality and uniform. The edamame seeds were then irradiated with 60Co gamma rays at a dose of 100 Gy to 500 Gy with a treatment dose interval of 100 Gy. Each irradiation treatment has 35 seeds, which are then put into paper envelopes labeled according to dosage.

Iradiation of Gamma Ray 60Co

Irradiation of ryoko variety edamame seeds was carried out at the Center for Isotope Application and Irradiation of the National Nuclear Energy Agency (PAIR BATAN) Jakarta. Edamame seeds were irradiated at doses of 100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy using a 220 gammacell irradiator machine.

Seedbed and Lethal Dose Value Calculation

Irradiated seed seedbeds are carried out in seedbed media (small polybags) with soil and manure media (2: 1). Seeds were planted as many as 35 seeds from each irradiation treatment. Observation of germination was carried out at the age of 10 days after seedling (HSS). The determination of LD20 and LD50 is done using *Curve Expert* 1.3 software. After the edamame seeds are irradiated, a seedbed medium is prepared for breeding edamame seeds. Observations of germination variables include germination percentage, normal sprout percentage, and abnormal sprout percentage.

Seeding

Seeding is carried out by initial selection of edamame seeds that have successfully grown, the transplanted seeds are seeds that have morphological characteristics that appear different from seeds that are not irradiated or controlled. Then prepare the planting medium, after which the seedlings are transferred to a planterbag measuring 40 x 40 cm at the age of 20 days after sowing. Each planting pit contains 1 edamame seedling.

Preparation of Growing Media

Preparation of planting media is carried out two weeks before planting, planting media is made by mixing soil and manure in a ratio of 2: 1. All planting media materials are mixed and flattened using a hoe, then put into a 35 cm polybag. Each planting hole contains 1 edamame plant seedling.

Planting

Planting edamame is carried out in polybags with a diameter of 35 cm, with one planting hole containing one edamame seedling. The seeds to be planted are seedlings that have been treated with gamma irradiation doses ranging from 0 Gy to 500 Gy. Planting is done in the afternoon to avoid stress on plant seeds. Then watered moderately.

Maintenance

The maintenance of edamame plants includes:

1) Watering: Watering is done every day in the morning and evening, or depending on the weather conditions. Watering is not done if the soil condition is still moist.
2) Fertilization: Fertilization of edamame plants is carried out in three stages, namely the time of giving 7 days before planting, 14 days after planting, 28 days after planting. By using the type of pearl NPK fertilizer (16:16:16). The doses of edamame fertilization are shown in Table 1.

Table 1. Follow-up Fertilization Dosage on Edamame

Timing of Giving	Types and Doses of Fertilizer Per Plant	How to Give
Before the last hoeing (7 days before planting)	NPK (16:16:16) 4.8 grams/plant Compost	In tugal and covered with soil
First follow-up (14 HST)	NPK (16:16:16) 4.8 grams/plant	In tugal and covered with earth
Second follow-up (28 HST)	NPK (16:16:16) 4.8 grams/plant	In tugal and covered with earth

3) Weeding: Weeding weeds is done in treatment at least once a week using a sickle or manually by hand.
4) Pest and Disease Control: Pest and disease control is carried out by spraying fungicides made from mancozeb, spraying according to the dose of attack on plants, or depending on the needs and attacks that occur.

Harvest

Edamame is harvested in the age range of 105 – 115 HST for seed production. The harvesting process is carried out by uprooting the plant from the base of the stem. Generally, edamame plants that are ready to harvest are characterized by brown bean pods, each pod has 3-4 fruit seeds per pod, soybean leaves have turned yellow, and seeds have passed physiological ripening. Collection of soybean seeds based on the dose of treatment to facilitate observation (labeling). Edamame seeds are air-hardened or up to a weight shrinkage of 24 – 25%.

Observation Parameters

The characteristics of edamame plants observed in this study include:

1) Germination Percentage (%): The calculation of germination percentage is done at the age of 14 HST. Calculating the germination percentage is done because germination is the ability of seeds to grow normally at optimum conditions expressed in percent. The calculation of germination on irradiated edamame is carried out at 14 days after sowing. The formula for calculating the percentage of seed germination of each dose is calculated by the formula:

$$\text{Germination percentage} = \text{x } 100\% \frac{number\ of\ seeds\ germinated}{number\ of\ seeds\ planted}$$

The germination percentage can be calculated by counting the number of seeds germinated at each dose divided by 35 seeds planted multiplied by one hundred percent.

2) Lethal Dose 20 (LD20) and Lethal Dose 50 (LD50): Analysis of LD20 and LD50 values can be calculated by live plant data at 7 – 14 HST which is used as a percentage with the formula:

$$\text{Live plants (\%)} = \text{x } 100\% \frac{number\ of\ live\ plants\ per\ dose}{number\ of\ seeds\ planted}$$

Counting live plants in each dose was used for data analysis using *Curve expert* 1.3 or RStudio software by calculating the number of live seeds per dose divided by 35 seeds planted multiplied by one hundred percent. Determination of the best model for each variety by looking at the R2 value. An R2 value close to 1 indicates that the model used is getting better.

3) Plant Height (cm): Plant height is measured using a ruler from ground level to the highest plant growing point. Plant height measurement is carried out at 2 weeks after planting. Observations were continued once every 2 weeks until harvest or at 2, 4, 6, 8, 10, 12, 14 (weeks after plants) MST.
4) Number of Leaves (strands): Observation of the number of leaves is done by counting the number of leaves on the whole plant, carried out at 2 weeks after planting. Observations were continued once every 2 weeks until harvest or on 2, 4, 6, 8, 10, 12, 14 MST.
5) Leaf Shape: Leaf shape is observed by looking at the shape of irradiated plant leaves compared to control plants or those that are not irradiated treatment. Observation of the shape of the leaves is carried out at 3 weeks after planting.

6) Number of Seeds Per Pod (seeds): Observation of the number of seeds per pod is done by counting the number of seeds per pod in the whole plant. The pods are opened and the seeds are taken and then counted many seeds per pod.
7) Number of Pods Per Plant (pods): Observation of the number of pods per plant is done by counting the number of pods on the whole plant. The pods are plucked from the plant stalk and then counted many pods per plant.
8) Weight of 100 Seeds (g): Air dry bean weight weighing is carried out after the crop has been harvested. Seeds are cleaned of adhering dirt. Seeds are dried for approximately 10-14 days, then the total dry weight of air is weighed with the help of analytical scales.

Data Analysis

LD (Lethal Dose) Value Test

Observation of Lethal dose value is done by calculating the percentage of plants that live up to 28 HST. Calculate the LD value using Curve expert 1.3 software by entering the germination percentage value that has been observed for 28 HST into the software.

T Test

A t-test was performed to compare each treatment dose of 60Co gamma ray irradiation (100 Gy – 500 Gy) with a control (0 Gy). The t-test analysis is performed by comparing the calculated t values and the table t. How to find t count is as follows:

$$\text{t count} = \frac{|\underline{A} - \underline{B}|}{S_{(\underline{A}-\underline{B})}}$$

$$S_{(\underline{A}-\underline{B})} = \sqrt{\frac{S^2A + S^2B}{n}} \text{ in which } S_A^2 = \frac{1}{n}\sum_{i=1}^{n} \left(A_i - \underline{A} \right)^2 \text{ and } S_B^2 = \frac{1}{n}\sum_{i=1}^{n} \left(B_i - \underline{B} \right)^2$$

Information:

$\underline{A}$ = average value of sample A
$\underline{B}$ = average value of sample B
$S_{(\underline{A}-\underline{B})}$ = standard error
S_A^2 = variant value (variety) A
S_B^2 = variant value (variety) B
A_i = sample value A to i
B_i = sample value B to i
n = sample size

Standard Deviation

The calculation of standard deviation values is used to measure the spread of data values. The standard deviation formula is as follows:

$$s = \sqrt{\frac{n\sum Xi^2 - (\sum Xi)^2}{n(n-1)}}$$

Information:

s = standard deviation value
n = number of samples
Xi = sample value X to i

RESULTS AND DISCUSSION

Growth Variables

Germination Percentage (%), LD20, and LD50

Germination percentage is obtained from the number of seeds germinated from the total seeds planted during the study. Germinated seeds are categorized into 2, namely abnormal sprouts and normal sprouts. Abnormal sprouts are seeds that do not sprout and normal sprouts are seeds that grow. The percentage of germination of irradiated Jember Local varieties of edamame is presented in table 2.

Table 2. Germination Percentage of Edamame Local Varieties of Jember with 60Co Gamma Ray irradiation

Dosage Treatment (Gy)	Germination			
	Total Seeds	***Abnormal sprouts***	***Normal sprouts***	***Germination ability***
T_0	35	4	31	88%

T_1	35	0	35	100%
T_2	35	13	22	63%
T_3	35	16	19	54%
T_4	35	9	26	74%
T_5	35	16	19	54%

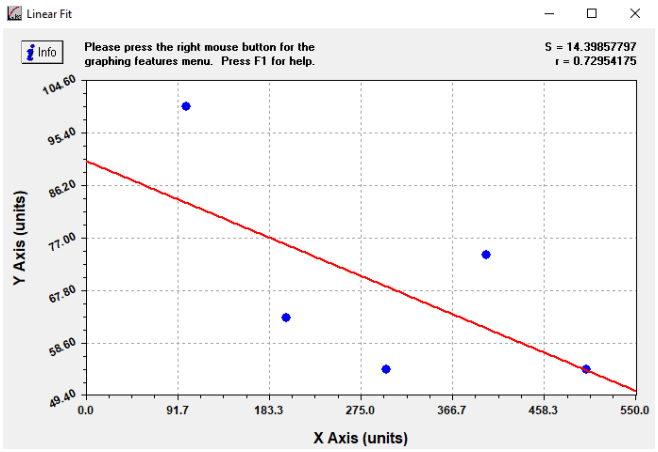
Information: T0 = control, T1 = 100 Gy, T2 = 200 Gy, T3 = 300 Gy, T4 = 400 Gy, T5 = 500 Gy.

Table 2 shows that edamame of Jember Local varieties at doses T0 – T5 (0 Gy – 500 Gy) has a good or normal germination percentage. It is known from the table that the higher the radiation dose, the smaller the germination. Some edamame plants experience abnormal germination. 60Co gamma ray radiation can cause changes in plant growth by stimulating seed growth or damaging seed growth.



Figure 3. Germination of Each Gamma Ray Irradiation Dose 60Co Edamame Plant Local Variety Jember Age 7 hst

The percentage of soybean germination with 60Co gamma-ray irradiation can be seen by the linear germination curve in Figure 4. Using the Curve Expert application and then the germination curve is analyzed to obtain a simple regression equation.



Description : = Irradiation Dose (Gy), Y = Germination Percentage (%) Regression Equation : y = 9.73 + (-4.05x)

Figure 4. Germination Response Curve of Edamame Plant Local Variety Jember Irradiation Results of Gamma Ray 60Co

Figure 4 shows the direction of the curve downward towards the right. This showed that seed growth from the control treatment (0 Gy) to the 500 Gy treatment decreased. The curve above can show a simple regression equation for germination of irradiated edamame plants.

Determination of gamma ray doses that cause lethal dose 20 (LD20) and lethal dose 50 (LD50) can be found using the curve expert application program 1.3 (Appendix..) or can be done using a simple regression equation y = a + bx. Based on the response curve in Figure 4, the linear egression equation y = 90.52 + (-0.07x) is obtained. The Y value is the percentage of living plants and the X value is the irradiation dose that causes death in plants (lethal dose). On the expert curve whose y value is filled with 80 which means 80% of plants live with 20% of plants die, then it can be known the value of x which is a lethal dose of 20 (LD20) which causes plants to die as much as 20%, which is 143.32, when the value of y is filled with 50 which means 50% of plants live with 50% of plants die, then it can be known the value of x which is the lethal dose value 50 (LD50) which causes plants to die as much as 50% which is 551. 881.

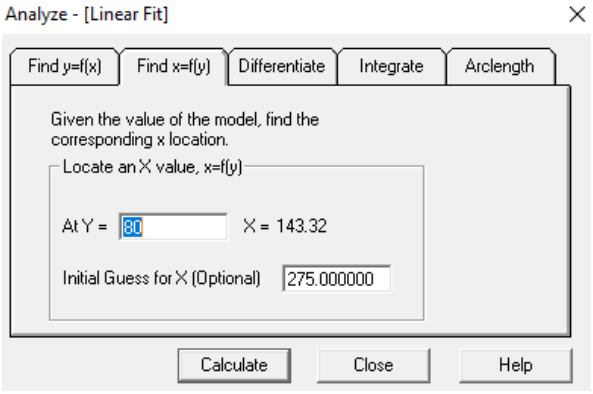


Figure 5. LD50 Edamame Plant Results of 60Co Gamma Ray Radiation

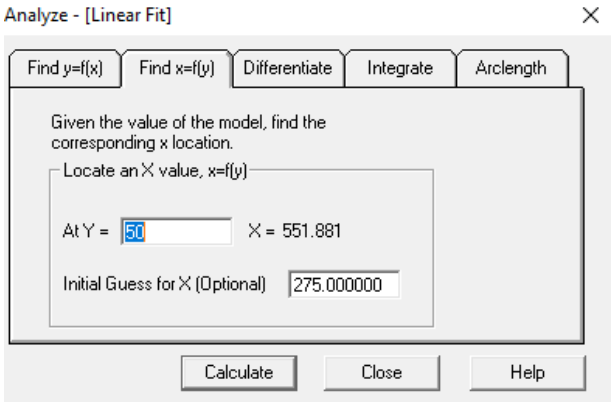


Figure 6. LD20 Value of Edamame Plant Results of 60Co Gamma Ray Radiation

Plant Height (cm)

The results of the T Test showed that the height of the plants resulting from edamame radiation had a very noticeable difference in the treatment of T4 (400 Gy). The T test was performed by comparing the levels of 1% and 5% with the control treatment. Plant height was observed from 7 HST to 84 HST or until the plant showed no growth. The average height value of edamame plants of Jember Local varieties resulting from 60Co gamma ray irradiation is in table 3.

Table 3. Average Plant Height of Edamame Local Varieties of Jember Results of 60CO Gamma Ray Irradiation

Dosage Treatment (Gy)	Plant Height(cm) HST					
	14	***28***	***42***	***56***	***70***	***84***
T0	17.07	20.6	22.47	26.33	32.6	32.8
T1	16.07	18.73	23.47	28.4	32.6	32.47
T2	17.53	23.47*	26.07*	29.73*	36.87	36.87
T3	14.8	19.67	21.6	25.8	31.67	31.67
T4	18.93	25.33**	27.93**	29.13	31.93	32.33
T5	13.6*	18.07	20.93	24.8	27.87	28.47

Information: T0 = control, T1 = 100 Gy, T2 = 200 Gy, T3 = 300 Gy, T4 = 400 Gy, T5 = 500 Gy.

Table 4.2 shows that the height of irradiated edamame plants has a marked difference in 28, 42, and 56 HST treated with 200 Gy. Very distinct results were shown at doses of 400 Gy when plants were 28 and 42 HST old. Different results were also shown at a dose of 500 Gy when the plants were 14 HST old. The average height of the highest plants is indicated at a dose of 400 Gy. And on average, the lowest plants are shown at a dose of 500 Gy. This is shown by the presence of very real different data on edamame plants irradiated at a dose of 400 Gy. Plant growth at a dose of 400 Gy had better results compared to controls.

Number of Leaves (strands)

The results of the T Test show that the number of leaves of the radiated edamame plant has a real difference and a very real difference in certain treatments. The T test was performed by comparing the levels of 1% and 5% with the control treatment. The number of leaves was observed from 14 HST to 84 HST or until the plant showed no growth. The average value of the number of leaves of the Jember Local variety edamame plant resulting from 60Co gamma ray irradiation is in Table 4.

Table 4. Average Number of Leaves and Number of Edamame Pods Local Varieties of Jember Results of 60CO Gamma Ray Irradiation

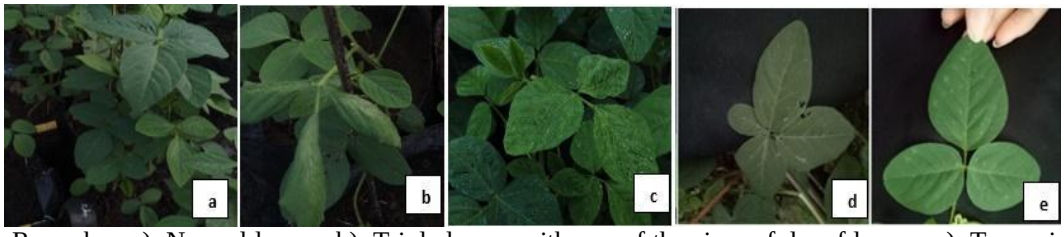
Dosage Treatment (Gy)	Number of Leaves (Strands) HST					
	14	***28***	***42***	***56***	***70***	***84***
T0	4,33	6,33	8,33	9,27	12,40	12,53
T1	4,53	7,20	10,73*	12,27*	15,40	14,67
T2	4,80	9,60**	10,13	10,53	12,80	11,73
T3	4,80	8,60*	10,40	10,80	11,93	11,87
T4	3,67	13,87**	16,33**	18,20**	14,33	13,93
T5	3,60	6,27	7,53	9,60	12,87	10,47

Information: T0 = control, T1 = 100 Gy, T2 = 200 Gy, T3 = 300 Gy, T4 = 400 Gy, T5 = 500 Gy

The data in Table 4 showed that plants with radiation doses of 100 Gy had marked differences at observations of 42 and 56 HST. At radiation doses of 200 Gy and 300 Gt when the plants were 28 HST were very markedly different and markedly different compared to controls. Edamame plants irradiated at doses of 400 Gy had markedly different mean values compared to controls at observations of 28, 42, and 56 HST. The mean value of the number of leaves of plants from radiation at all doses had a higher value compared to the control, except in plants with a dose of 500 Gy which had a smaller average value of leaf count compared to the control.

Leaf shape

60Co gamma ray radiation can affect the structure and function of plant cells, including changes in leaf shape. Gamma radiation sourced from coblat-60 can cause morphological changes in leaves, such as changes in leaf size, shape, or color. The impact of 60Co gamma ray radiation on plants can vary depending on the level of exposure and exposure time. Changes in leaf shape that occur in edamame plants are shown in Figure 7.



Remarks : a). Normal leaves, b). Triple leaves with one of the sizes of dwarf leaves, c). Two primary leaves with one abnormal shape, d). One leaf crown totaling 4 leaves, e). Triple leaf with white spots

Figure 7. Normal Leaf Shape and Abnormal Leaves of Edamame Plant Local Variety Jember Results of 60Co Gamma Ray Irradiation

60Co gamma-ray irradiation also affects the shape of edamame leaves. Shown in Figure 4.5. b. triple leaf shape where one of the leaves is stunted, the leaves are found in plants with radiation treatment of 200 Gy and 400 Gy. In figure 4.5. c. there are abnormal leaves in the form of two simple primary leaves that are not round or oval in shape, these leaves are found in plants with radiation treatment of 100 Gy and 500 Gy. Figure 4.5. d. shows 1 leaf canopy totaling 4 leaves found in plants with a radiation dose of 300 Gy. And leaves that have whitish spots in Figure 7 e. found in plants with a radiation dose of 100 Gy.

Production Variables

Weight of 100 Seeds (g) and Number of Pods (pods)

60Co gamma-ray irradiation also affects the weight of the seeds, the number of pods, and the amount of pod content of the edamame plant. The average value of the weight of 100 seeds and the number of pods obtained at the time of observation, then tested using the T Test by comparing the levels of 1% and 5% with the control treatment. The average weight of 100 seeds of edamame plants showed no noticeable difference in each treatment. While on the parameter of the number of pods, the average value of the number of pods in edamame plants also showed no noticeable difference in each treatment except for the 200 Gy treatment. The average value of weight of 100 seeds and the number of pods of edamame plants of the Jember Local variety are in Table 5.

Table 5. Average Weight of 100 Seeds and Number of Pods of Edamame Plant Irradiated by 60Co Gamma Rays

60Co Gamma Ray Irradiation Dose	Weight 100 Seeds (gr)	Number of pods
T_0	40,67	17,89
T_1	41	15,15
T_2	39,33	21,12
T_3	40,33	18,27
T_4	41	20,97
T_5	39	17,01

Information: T0 = control, T1 = 100 Gy, T2 = 200 Gy, T3 = 300 Gy, T4 = 400 Gy, T5 = 500 Gy.

Table 5 shows the average weight values of 100 seeds of each edamame plant showing no noticeable difference. The average treatment value of 100 Gy and 400 Gy was the highest average value compared to the control, which was 41 gr. The smallest average compared to the control at 500 Gy treatment was 39 gr. The average value of the number of pods also showed no noticeable difference except at a radiation dose of 200 Gy, which is 21.12. The radiation dose of 100 Gy had the lowest average number of pods compared to the control.

Number of Seeds per Pod (Seeds)

Exposure to 60Co gamma ray radiation can potentially affect plant growth and development, including the number of seeds per pod. The exact effect of gamma radiation on the number of seeds per pod can vary depending on a number of factors, including the level of radiation exposure, the duration of exposure, and the type of plant involved. The average value of the number of seeds per pod of edamame plants is shown in Table 4.5.

Table 6. Average Number of Seeds per Pod of Edamame Plants Local Varieties of Jember Results of 60Co Gamma Ray Irradiation

60CO Gamma Ray Irradiation Dose	Pods Filled with 1 Seed	Pods Filled with 2 Seeds	Pods Filled with 3 Seeds
T_0	9,19	12,81	1,31
T_1	13,54	8,17	1
T_2	8,59	13,55	1,31
T_3	9,63	12,63	1,55
T_4	9,77	12,23	1,45
T_5	6,11	14,05	1,79

Information: T0 = control, T1 = 100 Gy, T2 = 200 Gy, T3 = 300 Gy, T4 = 400 Gy, T5 = 500 Gy.

The variable number of seeds per pod produced by edamame plants shows the average yield of each treatment having pods containing 2 seeds. In edamame plants with treatment doses, the average number of pods contains 1 seed more than pods containing 2 seeds and 3 seeds. The average value of pods containing 2 seeds in each treatment had higher values compared to controls, except at treatment doses of 100 Gy, 300 Gy, and 400 Gy. The pods containing 1 seed and 3 seeds also showed higher average values compared to the control.

Standard Deviation Value

Standard deviation values in plant diversity is a statistical measure used to evaluate how far values in a data set vary from their mean. In the context of plant diversity, standard deviation is used to measure the extent of genetic or phenotypic variation among individual plants in a population. Standard deviation helps

researchers and breeders to understand and select plants that have relevant genetic diversity. The variable standard deviation values of plant height, number of leaves, number of pods, and weight of 100 seeds of edamame plants of Jember Local varieties are shown in Table 7.

Table 7. Standard Deviation Value of Plant Height, Number of Leaves, and Number of Pods of Edamame Plants Local Varieties of Jember Results of 60CO Gamma Ray Irradiation

Dosage Treatment (Gy)	Standard Deviation			
	Plant Height	***Number of leaves***	***Number of pods***	***Weight 100 seeds***
T0	6,46	3,27	9,02	1,53
T1	7,01	4,26	12,69	2
T2	7,66	2,77	8,70	1,53
T3	6,78	2,71	8,80	1,15
T4	4,98	5,05	10,67	1
T5	5,76	3,24	8,92	1

Information: T0 = control, T1 = 100 Gy, T2 = 200 Gy, T3 = 300 Gy, T4 = 400 Gy, T5 = 500 Gy.

Table 7 shows the standard deviation value in plant height parameters, the treatment that has the highest standard deviation value is the treatment with a dose of 200 Gy with a value of 7.66. In the leaf count parameter, the highest standard deviation value is in the treatment of 400 Gy with a value of 5.05. The parameter of the number of pods, the highest standard deviation value occurs at a radiation dose of 100 Gy with a value of 12.69. While in the weight parameter of 100 seeds, the highest standard deviation value is at a dose of 100 Gy with a value of 2. Varying standard deviation values indicate that 60Co gamma-ray irradiation exerts different effects on each plant treatment. This is indicated by a high standard deviation value that does not only occur at one radiation dose, but occurs at different doses.

Effect of 60Co Gamma Ray Irradiation on Plant Growth Variables

The results of germination tests conducted on irradiated edamame shown in Table above showed that soybeans had different germination percentages compared to controls. Based on the table it can be known that the higher the radiation dose, the less seeds are able to germinate. This is indicated by a linear germination curve that decreases to the right. High levels of mutagen doses given at the time of radiation will affect the plant's response. The higher the dose given, the response produced will be more different from plants given low doses (Belz et al., 2018; Belz & Sinkkonen, 2019; Caplin & Willey, 2018).

Table 2 shows that edamame plants with a radiation dose of 300 Gy have the lowest germination percentage, which is 53%. While the highest germination percentage with a dose of 100 Gy, which is 100%. The difference in germination percentage is related to the general effects of radiation that are often shown with damage to plant physiology. At each dose there are abnormal seeds so that the seeds are not able to germinate and do not grow into plants, this is in line with the research of Sanipar J et al (2013) whose results show that the initial effect of mutations is shown by seed germination, indicated by the presence of abnormal sprouts.

The use of the CurveExpert application is carried out based on seed germination percentage data that can obtain a linear regression equation $y = a + bx$ with a value of $y = 90.52 + (-0.07x)$, which means that every addition of one dose to x, the percent of live plants y increases by 90.52. Where y is the percentage of living plants and value is the irradiation dose that causes death in plants. Lethal dose 20 (LD20) and lethal dose 50 (LD50) are used as a reference for further breeding methods. According to Soeranto (2003), the highest mutant variability is found in gamma-ray irradiated mutants with values between LD20 and LD50. The lethal dose 20 (LD20) value obtained was 143.32 Gy and the lethal dose 50 (LD50) value obtained was 551.881 Gy. This shows that the edamame plant of the Jember Local Variety with a radiation dose of 500 Gy has a small germination percentage, which is 53%. LD20 and LD50 values obtained indicate that genetically edamame plants of local varieties of jember have a high level of radiosensitivity because if plants are irradiated at doses exceeding 500 Gy, seed germination will be abnormal or damaged. This is in line with Soeranto's research (2003) which says that doses above LD50 will be able to cause fatal plant physiological damage which is often characterized by high mortality, sterility, and abnormality rates.

Astuti et al. (2019) said that lethal dose can be determined through measurements of seed germination, shoot length, and root length of the seedling phase as well as the ability to germinate in field conditions in the M1 generation. The success rate in inducing genetic diversity is determined by plant radiosensitivity measured through LD50 values. This value can kill 50% of the plant population and is considered the optimum dose because it is capable of producing mutants or the highest genetic diversity. Low doses of gamma ray irradiation are also able to stimulate cell division, growth, and development as organisms (Kurniajati et al., 2020).

60Co gamma ray irradiation can affect plant physiological characters such as plant height and number of leaves on plants. On plant height parameters, plant growth decreases with each dose. there is a marked difference in radiation doses of 200 Gy at 28 HST to 56 HST. The radiation dose of 400 Gy showed a very noticeable difference at 28 HST and 42 HST and the 500 Gy dose showed a marked difference at 14 HST. Based on the overall data, the radiation dose of 500 Gy has the smallest average compared to other radiation doses.

The parameters of the number of leaves in edamame plants at radiation doses of 100 Gy, 200 Gy, 300 Gy, and 400 Gy have differences compared to control plants. The mean value of the number of leaves of radiation dose of 100 Gy at the observation of 42 HST showed a noticeable difference with a value of 10.73. The average number of leaves radiation dose of 200 Gy and 300 Gy at 28 HST observations showed a very noticeable difference and a noticeable difference with values of 9.60 and 8.60. The highest average value was found in plants with a radiation dose of 400 Gy which had a very noticeable difference at the age of plants 28, 42, and 56 HST.

Edamame plants with a radiation dose of 400 Gy had very markedly different growth compared to controls. This shows that 60Co gamma ray radiation affects the growth of edamame plants of the Jember Local variety. The difference in average plant height and higher leaf count indicates the effect of radiation dose. Similarly, with a radiation dose of 500 Gy, plants irradiated at a dose of 500 Gy showed slow growth. This was indicated by the low average value of plant height and number of leaves compared to controls. This is in line with the opinion of Gunckel & Sparrow (1967) who state that gamma rays can affect plant growth and development by inducing cytological, genetic, biochemical, and physiological changes in tissue cells. Amilin et al. (2015) also said that increasing the dose of irradiation will further inhibit the growth power of the plant due to the influence of determinicic effects due to gamma rays.

Plant diversity caused by 60Co gamma ray radiation also has an impact on the shape of the leaves of soybean plants. Soybean leaves are divided into four types, namely: (1) cotyledons or seed leaves, (2) two simple primary leaves, (3) three leaves, and (4) profila (Adie & Krisnawati, 2007). The shape of the leaves that changed after 60Co gamma ray radiation at each radiation dose treatment had abnormal or incomplete leaf blade shapes. The shape of the leaf shown is a leaf with two simple primary leaves, one of the leaves has an incomplete or incomplete shape. At a radiation dose of 300 Gy has the number of leaves in one clump totaling 4 leaves. And at radiation doses of 200 Gy and 400 Gy, the changes that occur show the shape of three leaves, where one of the leaves has a dwarf size. In figure 4.5. It is also seen that edamame leaves have spots that Changes that occur in the physical leaves of the edamame plant indicate that mutai can cause changes in plant DNA which are then expressed in the physical form of the plant. But the changes that have occurred have not been stable. The traits that arise due to the radiation process will begin to stabilize and will be inherited in the next offspring, namely in the M2 generation and so on.

The effects of 60Co gamma ray radiation on plants can lead to positive or negative depending on the purpose of breeding. Mutations in a negative direction may cause lethality, abnormality, sterility, or other physiological damage. The effect of mutations leading positively and being passed on to the next generation is an expected mutation (Monroe et al., 2022). So that the response of each plant to 60Co gamma ray radiation has different results. The response given by edamame plants also showed that edamame plants with a radiation dose of 400 Gy had better growth compared to controls.

Effect of 60Co Gamma Ray Irradiation on Plant Production Variables

The number of pods produced by edamame plants has mixed results, indicating genetic diversity caused by 60Co gamma ray radiation . Based on the data, the radiation dose of 200 Gy has the highest average number of pods at 21.12. Plants irradiated at a dose of 100 Gy had the lowest average value of 15.15. The number of pods produced by plants has a pattern of relationship with the number of flowers produced. If the two have a positive relationship then it will be beneficial. But if the relationship between the two is negative, it will have a negative impact on crop production. The number of flowers produced is not proportional to the number of pods produced. This can be caused by the possibility of flower loss when plant growth takes place. Decreasing the number of flowers produced by plants can affect the number of pods that can be harvested. This is evidenced in research conducted by Anggraito & Pukan (2015) where the results of the study showed a decrease in flowers produced by koro bean plants related to the number of filled pods that can be harvested. The abundance of fallen flowers will lead to low pod formation efficiency.

The weight of 100 seeds of edamame plants has an important effect on crop production. This relates to the yield of the amount of crop production at a certain area. Based on the data, the weight of 100 seeds of edamame plants showed variations in each dose of treatment given, but the results of the T test did not cause a noticeable difference in each treatment. The weight of 100 seeds in the irradiated plant weighed below the control plant. Except plants with treatment doses of 100 Gy and 400 Gy weighing 100 seeds 41 g. The lowest weight value of 100 seeds occurred in plants with a treatment dose of 500 Gy (39 g). Sadashiv et al. (2012) found that the effects of radiation have different responses. The effects of radiation can stimulate but can also

hinder the filling of the pods running in full. It also shows that sensitivity in each plant has a different response. The effect of radiation on grains is also greatly influenced by type, genotype, water content, age of seed harvest, and so on. Talebi et al. (2012) also said that the variation in weight data of 100 seeds of black soybean plants is thought to be due to gene changes in plants.

Table above shows the number of seeds per pod of edamame plants has differences at each treatment dose. Each plant (including controls) had more 2-seed pods than 1-seed and 3-seed pods. In plants, a radiation dose of 100 Gy has a greater number of pods containing 1 seed compared to pods containing 2 seeds and 3 seeds. The absence of empty or unfilled pods indicates that pod filling in edamame plants is running optimally. This is in accordance with the statement of Anggraito & Pukan (2015) who said that with a smaller number of pods, the filling of pods will be more optimal because there are fewer sinks (seed stockpiles), on the contrary, plants with excess pods will divide the pile evenly so that the number of seeds that can be harvested is less. Kuswantoro et al. (2016) also stated that differences in plant diversity and seed yield are influenced by genotype traits that have different adaptability even though they grow in the same conditions.

Changes that occur in 3 characters (number of pods, weight of 100 seeds, and number of seeds per pod) in edamame plants of the Jember Local variety are the result of genetic diversity caused by 60Co gamma ray radiation. Changes in growth factors and production factors caused still have different variations with control plants (not irradiated) so they still require selection tests in generation M2 and so on. Soeranto (2003) stated that mutations may not be directly expressed in phenotype, which is if the mutation occurs in a recessive direction and is in the heterozygous genotype structure or also called silent mutation. In addition, mutation treatment sometimes does not appear in generation M1 but only appears in generation M2 and so on (Soedjono, 2003).

Standard Deviation Value

Standard deviation is a statistical measure used to measure the degree to which the values in a dataset spread or vary from the mean. Standard deviation in plant breeding is one of several statistical measures that can be used to describe plant diversity. Standard deviation measures and increases genetic diversity that can increase plant resistance to environmental change or disease. This is in accordance with the opinion

Table 4.5. indicates the standard deviation value in each parameter has a different value. In the plant height parameter, the standard deviation value of the treatment dose of 200 Gy (7.66) was higher compared to the control (6.46). The leaf count parameter of the standard deviation value of 400 Gy (5.05) was higher compared to the control (3.27). While in the parameters of the number of pods and weight of 100 seeds, the treatment dose of 100 Gy has a higher standard deviation value compared to the control, namely 12.96 and 2.

Standard deviation can be used to measure genetic or phenotypic variation within a plant population. A high standard deviation value indicates large variation, while a low value indicates more limited variation. In the observed parameters, edamame plants that were treated with 60Co gamma ray radiation and had the highest standard deviation value showed a large variation in each plant. This was shown by the presence of a marked difference in plant height at the treatment dose of 200 Gy with the control. On the parameter of the number of leaves, the edamame plant treatment dose of 400 Gy showed a marked difference with the control. This is in accordance with the opinion of Trustinah and Iswanto (2013) which states that the higher the value of diversity, the greater the chance of obtaining a better plant genotype through selection.

CONCLUSION

The dose of 60Co gamma ray irradiation that can cause lethal dose 20 (LD20) and lethal dose 50 (LD50) in edamame Local Varieties of Jember is 143.32 and 551.881, respectively, and this irradiation affects the growth and yield of edamame plants of Jember Local Varieties, with notable differences in plant height and leaf count at certain doses

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