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Agronomic characteristics and radiosensitivity level of the Rubaru shallot irradiated by gamma rays

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ABSTRACT
The Rubaru shallots are resistant to various abiotic and biotic stresses, such as high salinity and Fusarium wilt disease. However, this shallot was unable to flower even after flowering was induced by cold temperatures and increasing day length, as well as polyploidy methods. Therefore, efforts are needed to increase the genetic diversity of the Rubaru variety of shallots through the induction of physical mutations with gamma ray irradiation. This research aims to determine the agronomic characteristics and radiosensitivity level of the Rubaru shallot (Allium cepa L.) resulting from gamma-ray irradiation. The research used a Randomized Complete Block Design (RCBD) with one factor, namely the dose of gamma ray irradiation (G), which consists of 5 levels as follows: G0 (control), G1 (2 Gy), G2 (4 Gy), G3 (6 Gy), G4 (8 Gy). The gamma-ray irradiation treatment had a very significant effect on agronomic characteristics, namely plant length and number of leaves, number of bulbs per cluster, bulb diameter, bulb fresh weight per cluster, bulb dry weight per cluster, and weight of the largest bulb per cluster. Gamma irradiation treatment with a dose of 2 Gy produced the highest number of bulbs and bulb diameter, with the largest weight of < 3g. Meanwhile, a dose of 8 Gy had a real effect on all parameters in inhibiting the growth of the Rubaru shallot. The calculated lethal dose (LD50) value is 6.18 Gy.
Keywords: irradiation, physical mutation, radiosensitivity, shallot

1. Introduction

Rubaru Shallot, known as the Sumenep Shallot, is a variety that is resistant to Fusarium wilt disease and tolerant to stress. It has a more distinctive and sharper aroma than other shallot varieties [1]. Apart from that, the Rubaru variety has wider adaptability, so it is suitable in low and highlands and can be planted in the dry and rainy seasons [2], [3]. Generally, shallots cultivated in Indonesia can flower and produce seeds with varying flowering rates, except for the Rubaru variety [4]. This is in line with the research results of Fairuzia et al. [5], that the rubaru variety could not produce flowers even though it had been given flowering induction, such as low temperature (4-6 °C) and longer photoperiod (14 hours), as well as polyploidization.

Given its powerful traits to mitigate the adverse negative impact of abiotic and biotic stress, the Rubaru shallot variety makes an important contribution to shallot and onion breeding, enhancing tolerance to environmental stress such as salinity and drought deprivation, and increasing resistance to diseases. Eventually, it will increase the shallot and onion production as well as the production loss due to pathogen attack and abiotic stress on shallots and onions. However, due to its inability to produce flowers, an alternative approach should be utilized in conjunction with physical treatments, such as low temperatures and longer photoperiods, which cannot induce flowering in Rubaru, as well as chemical mutations, like polyploidization, which cannot produce a mutated plant. This is caused by the diplontic selection effect that converts the mutated cells into normal cells [5]. Moreover, the Rubaru variety is also considered in the smaller bulb group with around small bulbs (diameter ≤ 1.5 cm or weight < 5 g), which affects the nutrient storage needed in the flowering stage. Khokhar also mentioned that onions with a fresh weight of around 4 – 7 grams can initiate florescence, and bulbs with a weight of less than 4 grams are limited in their capacity to initiate flowers [6]. Therefore, another mutation method should be used to induce the flowering potential and other agronomical traits, and one of the mutations that can be used is Gamma-ray radiation.

Gamma-ray physical radiation has a short wavelength with high energy, which can change the structure of plant DNA, so it can be used to improve the genetic characteristics of the Rubaru variety. It is safer than chemical mutations because it does not cause residue [7]–[9]. Utilization of gamma rays can be used to produce mutant plants that are resistant to biotic stress, tolerant to abiotic stress, and produce characters that increase growth and production characteristics in plants resulting from generative and vegetative propagation [10]–[12]. Studies regarding the effect of gamma-ray irradiation on the Rubaru variety of shallots have yet to be published. Therefore, it is important to study the effect of various doses of gamma irradiation on the diversity of agronomic characteristics of local Rubaru shallots (*Allium cepa* L.), especially to increase bulb diameter.

2. Methods

The research was carried out at the Experimental Field of the Faculty of Agriculture, Muria Kudus University, Bae District, Kudus Regency, with an altitude of ± 20 meters above sea level (masl). The research was carried out from January to March 2023. This research design used a one-factor Randomized Complete Block Design (RCBD) with seven replications. The treatment factor is the five levels of gamma-ray irradiation dose (G), namely G0: without gamma-ray irradiation (control), G1: 2 Gy, G2: 4 Gy, G3: 6 Gy, G4: 8 Gy. Each repetition has 12 bulb samples, so there were 420 experimental units. The bulbs were planted in a planting medium containing red soil, husks, and manure in a ratio of 1:1:1.

2.1. Bulb preparation

Rubaru shallot bulbs were obtained from the Mekar Sari Farming Group, Mandala Village, Rubaru District, Sumenep Regency. The selection of bulb seeds is carried out using a selection process, namely that the bulb seeds come from old-harvested bulbs, are bright in color, and have been stored at room temperature for around 3 months with a bulb diameter of ± 1 to 2 cm (medium and uniform size). The irradiation process for shallot bulbs of the Rubaru variety using a 60Co Gamma Chamber 4000A was carried out by the National Nuclear Energy Agency of Indonesia (BATAN) in South Jakarta.

2.2. The field experiment

Characters observed in the mutant generation were plant length, number of leaves per cluster observed at weeks 2-8, number of bulbs per cluster, bulb diameter was counted into two categories, bulb with weight 2-3 g and 3.1-4.2 g, fresh weight of bulbs per cluster, fresh weight The largest bulbs per cluster were carried out at harvest time (63 days after planting), the dry weight of the bulbs per cluster was carried out after the shallot bulbs had been dried for 2 weeks under the sunlight until the weight remained constant.

2.3. Data analysis

Data from observations for each treatment were analyzed using Analysis of Variance (ANOVA), and if there was a real effect, it was continued with the Duncan Multiple Range Test (DMRT) at a real level of 5% using the Statistical Package for the Social Sciences (SPSS) software version 25. Calculation of the level of radiosensitivity uses data on the percentage of live plants at 45 days of age, then the number of live and dead plants is calculated using Curve Expert software to determine the lethal dose 50 (LD50) from the effect of different doses of gamma-ray irradiation.

3. Results and Discussion

3.1. Agronomic characteristics

Based on the results, it shows that gamma-ray irradiation with various doses affects the agronomic character of Rubaru shallots. The various doses of gamma-ray irradiation treatment affect plant length and the number of leaves (Table 1 and Table 2). Gamma-ray irradiation with doses of 6 Gy and 8 Gy produced the lowest plant length and leaf number during the observations, as high doses of irradiation can suppress plant growth. Meanwhile, the plants without Gamma-ray irradiation treatment (0 Gy) have the highest plant length and leaf number because the plant cells were not disrupted by irradiation, and that makes the plant grow normally. Similar results were reported by Batubara [13], who found that the higher the dose of irradiation given to shallots, the more the plant length will be reduced. The decrease in plant length and number of leaves is caused by damage to the plant meristem cells, which function for plant growth and development. The decrease in plant length, number of leaves, and leaf width is due to damage to meristem cells, which function as locations for auxin synthesis [14]–[15]. Meanwhile, the control treatment and gamma irradiation with a dose of 2 Gy were not significantly different due to diplontic selection. Similar results are reported by Ginting [16], stating that the insignificant effect of gamma-ray irradiation treatment is due to the occurrence of diplontic selection towards repairing enzyme systems that are disturbed due to gamma-ray irradiation. Diplontic selection is a competition process between mutated cells and normal cells. If the mutated cells cannot compete within a certain time limit, the plant cells will return to normal growth.

Table 1. Average length of shallot plants in response to gamma ray irradiation at various ages

Irradiation Doses	Plant length (cm)						
	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP
0 Gy	25.23 a	32.32 a	36.76 a	38.70 a	41.70 a	43.58 a	44.02 a
2 Gy	25.01 a	32.86 a	37.38 a	38.64 a	40.85 ab	42.20 ab	37.90 b
4 Gy	22.68 b	28.67 b	32.18 b	33.85 b	38.43 b	39.63 b	35.23 b
6 Gy	18.08 c	23.50 c	25.86 c	29.26 c	31.94 c	33.21 c	29.86 c
8 Gy	16.15 c	19.14 d	20.57 d	22.04 d	24.65 d	27.08 d	23.40 d

Note: Numbers followed by the same letter in columns indicate that they are not significantly different at the 5% level of the DMRT test.WAP = weeks after planting

Table 2. Average number of shallot leaves in response to gamma ray irradiation at various

Irradiation Doses	Plant length (cm)						
	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP
0 Gy	18.20 ab	23.54 a	26.51 ab	28.93 ab	30.11 a	31.26 a	28.47 a
2 Gy	18.68 a	23.24 ab	26.80 a	30.35 a	28.71 a	27.06 b	21.79 b
4 Gy	17.49 b	21.87 b	25.35 b	27.59 b	22.84 b	21.93 c	18.00 c
6 Gy	15.38 c	19.22 c	22.69 c	24.38 c	20.40 c	21.37 c	18.90 c
8 Gy	14.87 c	17.39 d	21.21 d	23.00 c	18.59 c	16.37 d	12.94 d

Note: Numbers followed by the same letter in column indicate that they are not significantly different at the 5% level of the DMRT test.WAP = weeks after planting

The production parameter of the number of bulbs per cluster, the gamma irradiation treatment with doses of 2 Gy, 6 Gy, and 8 Gy, was significantly different from the control treatment (Table 3).

Table 3. Average of shallot production parameters response to gamma ray irradiation

Irradiation Doses	Number of bulbs	Bulb diameter (mm)		Bulb fresh weight per cluster (g)	Bulb dry weight per cluster (g)	Largest Bulbs Fresh Weight per cluster (g)
		2- 3 g	3.1-4.2 g			
0 Gy	8.97 b	13.24 b	14.06 ab	14.89 a	12.25 a	4.21 a
2 Gy	10.10 a	15.03 a	15.74 a	15.91 a	12.44 a	4.23 a
4 Gy	8.74 b	13.40 b	13.57 b	15.52 a	11.93 a	4.09 a
6 Gy	7.93 c	11.23 c	11.96 c	12.75 b	9.14 b	3.62 b
8 Gy	5.88 c	7.69 d	8.89 d	9.70 c	6.23 c	3.07 c

Note: Numbers followed by the same letter in columns indicate that they are not significantly different at the 5% level of the DMRT test

The decrease in the number of bulbs produced due to high doses of gamma-ray irradiation can disrupt plant development in the bulb formation process. Similar results were reported by Asza et al. [9], that the high doses of gamma-ray irradiation have negative effects in the form of physiological disorders of shallot, thereby reducing the number of bulbs produced. The diameter parameters of shallot bulbs are divided into two groups, namely weighing 2-3 g and 3.1-4.2 g. The diameter of bulbs weighing 2-3 g shows that the gamma irradiation treatment with a dose of 2 Gy produced the largest diameter, namely 15.03 mm, because low doses of gamma-ray irradiation can stimulate cell division and plant growth [8], [16]. Meanwhile, the diameter at 3.1-4.2 g weights for the 2 Gy dose irradiation treatment, namely 15.74 mm, was not significantly different from the control treatment. Gamma-ray irradiation treatment with increasing doses reduced the values of the parameters of fresh weight of bulbs per cluster, dry weight of bulbs per cluster, and fresh weight of the largest bulbs per cluster. Irradiation higher than 4 Gy will decrease the fresh weight of the shallot bulb, and it will decrease the flowering and yield potential. A larger bulb has higher food preservation as a source of nutrients for the flowering development and seed production, and quality of onion [17].

Exposure to gamma-ray irradiation in plants can cause physiological disorders or chromosomal damage caused by mutagens. Gamma rays are ionizing radiation and interact with atoms or molecules to produce free radicals [18]. Free radicals in plant cells can damage cell components, including proteins, lipids, and DNA, through oxidation reactions. Free radicals formed as a result of the administration of gamma rays can disrupt plant cell metabolism because they react with lipids and nucleic acids [19], [20].

Gamma-ray irradiation causes the bonds in the DNA chain to break, which occurs in the double strand of DNA, resulting in incomplete DNA fragments. Apart from that, there are changes in the bases in the DNA chain, such as missing one base, and the sequence of bases being swapped, which causes changes to the nucleotides, which can inhibit the transcription and replication functions of DNA [21]. Devy and Sastra [22] and Hartati et al. [20] added that radioactive rays, when they hit plant tissue, will cause ionization of air molecules, and then they will oxidize the sugars in DNA so that the nucleotide chain will break. Besides, shallot plants irradiated with gamma rays show an inversion phenomenon. Inversion is a change in the location of genes because, during mitosis, the chromosomes are twisted, which can disrupt plant growth and reduce plant vitality [23].

3.2. Radio sensitivity level

Linear regression is shown in Figure 1. which produces a value for this equation Y = 8,464x + (-1,065). The calculation of the lethal dose value is shown in Figure. 2. Calculating the LD50 value shows a value of 6.18 Gy; therefore, it can be rounded to 6 Gy in this study. It means that Rubaru shallots have a higher radiosensitivity level than the Bima Brebes variety, with a value of 7.55 Gy, and Nainggolan has 12 Gy [8]. Meanwhile, the correlation coefficient value shows a value of r = 0.93, where the value is close to 1, which means that increasing the dose of gamma-ray irradiation causes a higher percentage of plant death.

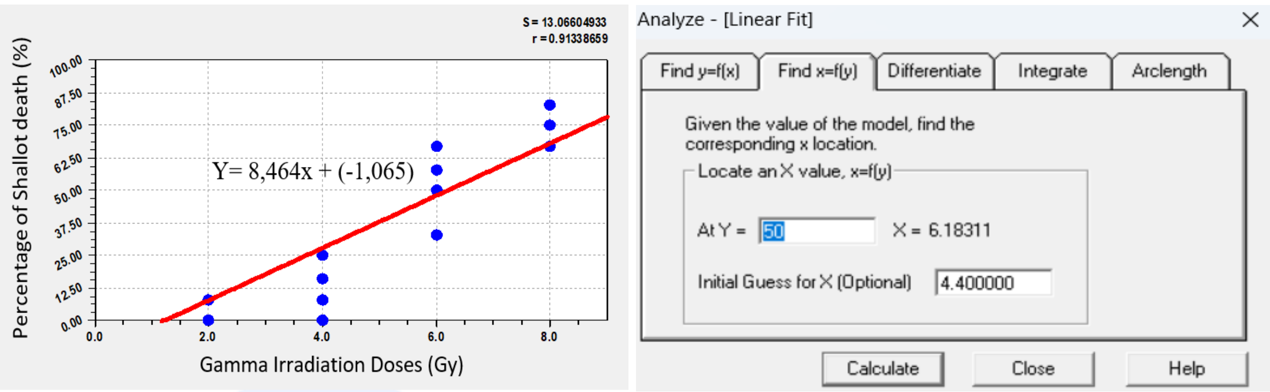

Figure 1. Linear regression of shallot response to gamma ray irradiation; left: correlation coefficient value; right: gamma-ray lethal dosage (LD) value of Rubaru shallot

4. Conclusion

Based on the results and discussion, gamma ray irradiation temporarily gave significantly different results on the agronomic character parameters of Rubaru shallots. The high dose of gamma-ray, namely 8 Gy, significantly inhibited the growth of the Rubaru variety of shallot plants. The lethal dose (LD50) value is 6.18 Gy, Although it does not has any effects to the plant heigth and leaf number. Further research is needed to determine the agronomic characteristics and production of the next generation because this research is still on the first generation.

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