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The growth and yield of second-generation of shallot mutants caused by gamma-ray irradiation of *Allium cepa* var. *Aggregatum*

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Abstract Genetic diversity can be enhanced through induced mutation, and gamma-ray irradiation is one effective method for inducing plant mutations. This research aimed to identify functional mutants that can serve as a source of seeds for shallot cultivation. The results indicated that gamma-ray irradiation influenced the growth and yield of second-generation shallot plants. The irradiation treatment led to an increase in the number of leaves, with the 100 Gy dose producing the highest average of 21.9 leaves per plant. The appearance of umbels began gradually following the gamma-ray irradiation, with the time for umbel emergence ranging from 33.4 to 61.5 days. Measurements of the umbel diameter showed an average range of 48 to 56.5 mm, and the average number of umbels per plant varied from 1 to 2.5. The control treatment without gamma-ray irradiation yielded lower results, suggesting that higher irradiation doses resulted in a greater number of umbels. Notably, the irradiation treatment with a dose of 125 Gy led to an increase in the number of stomata, with a maximum of 37.7 stomata observed. In contrast, untreated plants reached a maximum height and umbel stalk length of 44.2 cm and 56.8 cm, respectively. The presence of irradiation treatment is believed to induce changes in the genetic composition of the plants, which may contribute to variations in growth patterns.

Keywords: Cobalt-60 energy, Gamma-ray irradiation, Mutant generation, Mutation breeding

Introduction

The demand for shallots grows every year due to their high economic value as a strategic horticultural commodity. According to the results of the National Socio-Economic Survey (Susenas) in 2021, the average consumption of shallots per person in Indonesia reached 24.91 kg per year. However, the production of shallots in Indonesia has been insufficient to meet the national demand. Meanwhile, shallot production in Indonesia in 2020 was 1,815,445

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metric tons, in 2021 it was 2,004,590 metric tons, and in 2022 it was 1,982,360 metric tons (BPS, 2022), which is unsufficient for national shallot needs.

Efforts to increase shallot productivity can be made by producing new varieties with high productivity. Genetic diversity in shallot plants is important as material for the formation of new cultivars with high productivity, early maturity, and resistance to pests and diseases. The greater the level of diversity in the population, the greater the effectiveness of selection in selecting a character that suits your wishes (Anpama *et al.*, 2022). However, increasing the diversity of shallot genotypes through conventional breeding techniques faces obstacles, as not all shallot plant genotypes are capable of producing flowers (Marlin *et al.,* 2018). Genetic diversity can be achieved through gene recombination, hybridization, genetic engineering, or induced mutations.

The formation of diversity can be achieved through mutations, either through induced mutations or natural mutations (Alfariatna *et al.*, 2018). Mutation is an effective method for increasing the diversity of plants that are generally propagated using bulbs, such as shallots (Anpama *et al.*, 2022). The root cells of germinating seeds show increased inductions of chromosomal aberrations and micronuclei due to gamma-radiation (Bolsunovskya *et al.*, 2019). In the context of crop improvement, mutations can be intentionally used to create genetic variations that can be selected for desired traits, such as disease resistance, increased yield, or adaptation to specific environmental conditions

Physical mutagens can be applied by utilizing physical materials derived from alpha, beta, and gamma rays (Sari *et al.*, 2020). Among the existing physical mutagens, gamma rays are the most widely used due to their higher energy and penetration power. The use of gamma-ray irradiation aims to induce new traits in plants through genetic changes in the parent plant after exposure to gamma radiation at specific doses (Harsanti and Yulidar, 2016). Gamma-ray irradiation has the potential to impact plant physiology and biochemistry, ultimately inhibiting plant growth (Borzouei *et al.*, 2010; Setiawan *et al.*, 2015). Information about plant diversity has implications for determining the development or cultivation program to be used to obtain superior varieties (Mansouri *et al.*, 2015).

This study aimed to analyze the variation in growth and yield of second-generation shallot (MV2) of different doses of gamma radiation.

Materials and methods

Research procedures

The research was conducted from November 2023 to March 2024 at an experimental site located at an altitude of 10 meters above sea level (3°45′44″ S; 102°16′45″ E). The average monthly rainfall for November 2023, December, January, February, and March 2024 was 17.3, 14, 28.1, 33, and 35.5 mm/month, respectively. The consecutive average air temperatures were 27.2, 27.6, 27.5, 27.7, and 27.5 °C, and the average air humidity was 87.3, 86.9, 85.9, 87, and 85.4%. Additionally, the average daily sunshine duration was 74, 42.1, 68.9, 68.2, 51.7, and 46%. The research uses plant materials obtained from the second generation (MV2) of bulb propagation that have been irradiated with gamma-rays at various doses. Seed irradiation was carried out at BATAN (the National Nuclear Energy Agency of Indonesia). The irradiation process took place in the irradiator room using a Gamma Cell 220 machine. The gamma cell 220 is an irradiator machine that utilizes cobalt-60 as the energy source, with an initial activity of 10.687 curies. The dose rate of the gamma cell 220 irradiator during irradiation is in the range of 5600 gray or 5.6 Gy/second. From this dose rate, it is known that the dose rate per second is 1.55 Gy/second. The prepared shallot seeds are placed inside the chamber of the gamma cell 220 machine, and then exposed to Co-60 gamma rays at predetermined doses.

Experimental design and maintenance

The experiment was performed using Completely Randomized Design (CRD) with 3 replications. The media consisted of a mixture of soil, manure, and husk in a ratio of 2:1:1. The three-component media were thoroughly mixed and placed in polybags measuring 30 × 40 cm. The prepared polybag planting media was arranged according to the predetermined layout. Shallot bulbs were first cut ¼ at the top and then treated with fungicide evenly across the surface of the shallot bulbs. Planting was done by creating planting holes to a depth of ± 3 cm. Planting was carried out by placing the growing point of the shallot bulb on the surface of the soil and then covering it with a thin layer of planting media.

The recommended fertilizers were applied when planting using urea, SP-36, and KCl at 200, 150, and 110 kg/ha, respectively, along with liquid organic fertilizer (10 ml/L). Urea, SP-36, and KCL should be applied together during planting. To apply the fertilizer, create holes around the plants approximately ± 5 cm from the plant, with a depth of ± 2.5 cm, and then sprinkle the fertilizer evenly around the plants. The liquid organic fertilizer was applied every 10 days at a dosage of 10 ml/L.

Weeds are manually removed by pulling them from the polybags, taking care not to disturb or uproot the shallot plants. Insecticides and fungicides are sprayed every week to control pests and diseases. Preventive plant maintenance is necessary to support plant growth and prevent disruptions from harmful organisms affecting shallots.

The harvest process is carried out when the shallot plants are 65 days after planting (DAP) or have met the harvest criteria. The criteria for harvesting shallots are marked by yellowing or wilted leaves on 20% to 80% of the entire plant and most of the bulbs starting to appear on the ground. The top of the plant begins to droop, and the base of the leaves deflates. Harvesting is done by removing the shallot bulbs and cleaning them from the soil. After harvesting, the shallot bulbs are collected based on treatment and placed on bamboo racks to be air-dried for 2 weeks.

Variables and data analysis

The observed variables were plant height, number of leaves, number of bulbs, bulb diameter, plant fresh weight, plant dry weight, harvest time, number of stomata, percentage of flowering, days of the umbels appeared, length of the umbel stalk, umbel diameter, and number of umbels. The data obtained were statistically analyzed using analysis of variance (ANOVA) with a 5% level. If the F test results indicate a significant effect, the LSD test was conducted at the 5% level to identify differences between treatments.

Results

Vegetative growth of plants

During the research, the initial growth of shallots showed normal and favorable conditions. From 1 to 2 WAP, the growth of shallots increased rapidly. When they reached 3 WAP, the shallot plants became infected by fungi. As a result, the plants were sprayed with fungicide every 7 days at a dose of 2 g/L. The vegetative growth of shallots was reflected in the increased size of vegetative organs, such as plant height and number of leaves. There was an increase in plant height and number of leaves from 1 WAP to 8 WAP (Figure 1).

The effect of gamma-ray irradiation on the growth and yield of shallots

The results of the variance analysis examining the effect of gamma ray irradiation doses on shallot growth and yield are presented in Table 1.

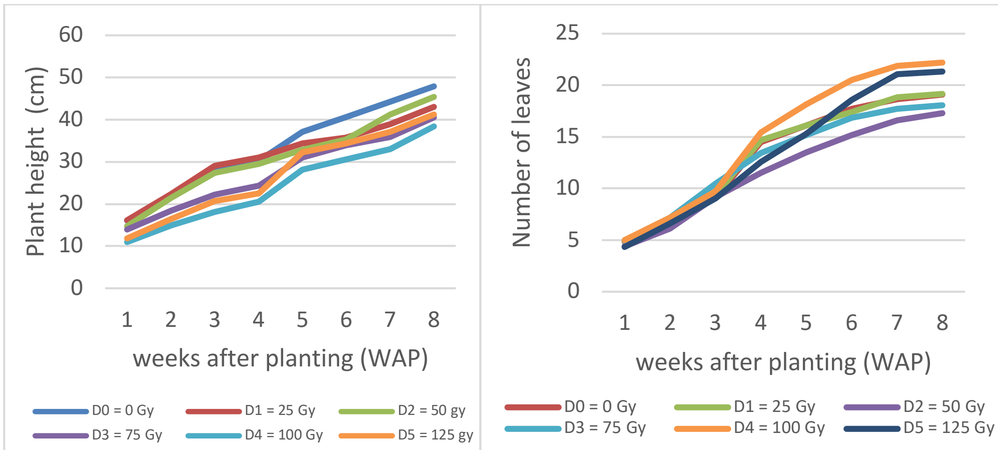

Figure 1. Growth patterns of plant height and number of shallot leaves at several doses of gamma-ray irradiation

Table 1. Analysis of variance at the 5% level of the effect of gamma-ray irradiation dose on shallot growth and yield

Variable	F-value	Coefficient of Variance (%)
Plant height	4.21 *	1.5
Number of leaves	17.22 *	7.7
Number of bulbs	2.42 ^{ns}	23.6
Percentage of flowering	4.28 ^{ns}	9.4
Days of the umbels appeared	0.74 ^{ns}	7.1
Length of the umbel stalk	3.92 *	5.1
Umbel diameter	1.75 ^{ns}	6.0
Number of umbels	1.68 ^{ns}	2.3
Number of stomata	8.56 *	3.3
Harvest time	9.51 ^{ns}	0.2
Bulb diameter	1.49 ^{ns}	1.2
Plant fresh weight	10.70 ^{ns}	3.1
Plant dry weight	0.99 ^{ns}	3.4

Note: *, and ns were significant at α= 5%, and non-significant, respectively.

The analysis of variance showed that the gamma-ray irradiation treatment had a significant effect on the variables of plant height, number of leaves, length of the umbel stalk, and number of stomata. But it has no significant effect on other variables. Further test results to compare the effects between treatments using the LSD test are presented in Table 2.

Table 2. Effect of gamma-ray irradiation dose on plant height (cm) and number of leaves, length of umbel stalk and number of stomata

Gamma-ray irradiation	Plant height (cm)	Number of leaves	Length of the umbel stalk (cm)	Number of stomata
D_0 = 0 Gy	44.2 a ±0.81	18.7 b ±0.69	58.6 a ±1.12	18.4 b ±1.03
D_1 = 25 Gy	38.9 abc ±0.62	18.9 b ±0.47	44.8 ab ±1.64	21.5 b ±1.19
D_2 = 50 Gy	41.2 ab ±0.29	16.7 c ±0.37	52.0 ab ±1.95	18.4 b ±0.90
D_3 = 75 Gy	35.8 bc ±0.31	17.8 ab ±0.58	43.3 ab ±2.56	18.0 b ±0.11
D_4 = 100 Gy	33.0 c ±0.17	21.9 a ±0.44	41.5 bc ±1.50	21.5 b ±0.38
D_5 = 125 Gy	37.1 bc ±2.66	21.0 a ±0.10	41.5 c ±1.83	37.7 a ±0.00

Note : Each value is expressed as the mean plus standard deviation. Numbers in the same column followed by the same letters were nonsignificant difference based on LSD at α=5%

The results of the BNT test at the 5% level showed that the control treatment appeared to have the highest plant height (44.2 cm), although it was not significantly different from treatments D1 and D2 (Table 2). The 100 Gy dose treatment resulted in the shortest plants at 33 cm, and this was not significantly different from treatments D1, D3, and D5. In terms of the number of leaves, the 100 Gy dose treatment had the most leaves at 21.9, although this was not significantly different from treatments D1, D3, and D5. Conversely, the 50 Gy dose treatment had the fewest leaves at 16.7, although it was not significantly different from the other treatments. When it came to umbel stalk length, the control treatment without gamma-ray irradiation had a significantly longer umbel stalk length at 58.6 cm, although it was not significantly different from treatments D1, D2, and D3. On the other hand, the 100 Gy and 125 Gy treatments resulted in the shortest umbel stalk lengths at 41.5 cm, and these were not significantly different from the other treatments.

The number of stomata varied with different treatments. The 125 Gy dose treatment resulted in the highest number of stomata, with an average of 37.7 stomata at a magnification of 10x10. However, this was not significantly different from the other treatments. The 75 Gy dose treatment had the lowest number of stomata, averaging 18.0 stomata at 100x magnification, but it also showed no significant difference compared to the D0, D1, D2, and D4 treatments.

The analysis of variance indicated that the treatment with gamma-ray irradiation did not have a significant effect on the number of bulbs, the number of umbels, the days of the umbels appeared, the umbel diameter, and the number of umbels. The average observations for each variable related to umbel formation are presented in Table 3 below.

Table 3. Effect of gamma-ray irradiation treatment on umbel formation

Gamma-ray irradiation	Variable		
	days of the umbels appeared	umbel diameter (mm)	Number of umbels
D_0 = 0 Gy	38.8±1.08	48.0±4.16	1.0±0.00
D_1 = 25 Gy	49.4±1.61	53.5±3.42	1.2±0.11
D_2 = 50 Gy	44.6±2.14	48.6±2.58	2.1±0.07
D_3 = 75 Gy	33.4±0.77	56.5±2.30	1.4±0.29
D_4 = 100 Gy	59.5±1.84	52.8±1.11	2.5±0.88
D_5 = 125 Gy	61.5±2.50	54.7±1.22	1.5±0.50

Note : Each value is expressed as the mean plus standard deviation

In the gamma-ray irradiation dose treatment, the umbel started appearing gradually. It took between 33.4 and 61.5 days for the umbel to appear. The observations of the umbel diameter revealed an average range of 48–56.5 mm, and the average number of umbels ranged from 1–2.5 per plant. The control treatment with gamma-ray irradiation showed low results, indicating that higher doses of irradiation led to a greater number of umbels.

The results of the analysis of variance showed that the gamma-ray irradiation treatment had no significant effect on the variables number of bulbs, harvest time, bulb diameter, plant fresh weight (g), and plant dry weight (g). The average observations for each of these variables are presented in Table 4.

Table 4. Effect of gamma-ray irradiation treatment on the number of bulbs, harvest time, bulb diameter, plant fresh weight, and plant dry weight

Gamma-ray irradiation	Variable				
	Number of bulbs	Harvest time (DAP)	Bulb diameter (mm)	Plant fresh weight (g)	Plant dry weight (g)
D_0 = 0 Gy	3.0±0.43	87.0±0.46	28.0±0.60	37.0±2.80	28.6±2.30
D_1 = 25 Gy	3.9±1.02	87.0±0.71	27.2±1.23	40.6±5.24	33.6±3.49
D_2 = 50 Gy	3.8±0.20	86.8±0.32	28.1±0.84	44.0±5.13	37.4±4.04
D_3 = 75 Gy	5.3±0.87	85.9±1.27	24.6±1.45	34.4±3.39	29.2±2.66
D_4 = 100 Gy	5.7±0.20	81.2±0.00	25.5±0.32	45.0±5.58	37.9±6.73
D_5 = 125 Gy	5.3±0.52	86.6±0.90	26.5±1.67	41.2±2.33	34.0±2.69

Note : Each value is expressed as the mean plus standard deviation

The average number of bulbs ranges from 3.0 to 5.7 bulbs per plant. According to the variety description, the number of bulbs per plant ranges from 2 to 4, indicating the successful growth of shallots during the research. The average harvest time ranges from 81.2 to 87.0 days after planting, and the average bulb diameter ranges from 24.6 to 28.1 mm. Additionally, the average fresh weight of plants ranges from 37.0 to 45.0 g, while the average dry weight ranges from 28.6 to 37.9 g.

Discussion

Growth is a characteristic of a living organism. It is a permanent change that increases the size of the plant. The term 'structural growth' refers to an increase in structural biomass that is irreversible until senescence (Pantin *et al.*, 2012). The plant growth is typically influenced by two factors: internal factors originating from the plant itself (genetics) and external factors arising from the environment. Plant growth is the process of increasing the size or morphological volume of a plant, and this increase is irreversible.

The results showed that gamma-ray irradiation reduced plant height compared to plants without irradiation treatment. However, irradiation treatment increased the number of leaves. The presence of irradiation treatment is thought to cause changes in the genetic composition of plants, so it can play a role in reducing or increasing plant growth. Ionizing radiation is commonly used in plant breeding programs to induce beneficial mutations for crop improvement, including higher yields, semi-dwarf growth, earlier maturity, and increased resistance to biotic and abiotic stress (Li *et al.*, 2007; Zhou *et al.*, 2006). Changes in the genetic composition resulting from gamma-ray irradiation treatment play a role in determining the diversity of appearance, especially in plant height and number of leaves. Differences in average yield of plant height and number of leaves are caused by different genetic characteristics of shallot plants, so they have a direct effect on the morphological appearance of the plants. This is in line with the findings of Bolsunovsky *et al.* (2019), which indicated an increase in the frequency of chromosomal aberrations in germinating seed root cells was first found under exposure to low doses of gamma-ray irradiation (0.05 and 0.1 Gy). The total chromosomal aberrations increased from 1 to 2% in the control to 4–7% in the treatment of electromagnetic fields (EMFs) on the cytogenetic and growth parameters of germinating onion (Tkalec *et al.,* 2009).

Widyaningtyas *et al.* (2024) observed that the number of leaves of fifth-generation shallot plants increased every week at the control doses of 3 Gy and

4 Gy due to gamma-ray irradiation. The increasing doses of gamma-ray irradiation were found to have a direct impact on the increase in the number of leaves. Kurniajati *et al*. (2020) also stated that higher doses of gamma-ray irradiation result in an increase in the average number of leaves.

Alfariatna *et al.* (2018) reported that irradiation treatment does not affect stomata size (both length and width); however, it does affect the number of stomata. The number of stomata can be influenced by external factors, such as light intensity. A lower light intensity leads to a reduction in the number of stomata on a plant.

The gamma-ray irradiation treatment did not increase the number of second-generation shallot bulbs. This contrasts with Kurniajati *et al*. (2020) statement that gamma-ray irradiation can increase the average production of tubers per plant. Sumarni *et al.* (2012) stated that the number of shallot bulbs is more influenced by plant genetic factors than environmental factors and fertilization.

Rizqiani *et al.* (2018) found that gamma-ray irradiation does not significantly affect the time of flower appearance on aster plants. The sunflower seeds treated with 100 and 200 gamma doses resulted in taller plants compared to the control group, while seeds treated with 400 and 500 gamma doses did not survive or produce any seeds for evaluation in further generations (Habib *et al.,* 2022). The gamma-ray irradiation treatment had a significant effect on the length of the umbel stalks of shallots. The fresh weight increase and surviving fraction of in vitro leaf explants of *Saintpaulia ionahta* decreased dramatically with increasing different linear energy transfer (LET) at the same doses (Zhou *et al.,* 2006). However, this differs from the research of Rizqiani *et al.* (2018), which stated that the irradiation dose given had no significant effect on the length of the flower stalks on chrysanthemum plants.

It has been observed that providing gamma-ray irradiation treatment does not impact the harvest time of shallots. Putrasamedja (2010) mentioned that the maturity of the shallot harvest is influenced by environmental factors, such as rainfall and length of exposure. According to Asza *et al.* (2022), increasing the dose of gamma-ray irradiation on shallot bulbs leads to a reduction in fresh bulb weight, dry bulb weight, and bulb diameter.

According to the findings of this study, it can be inferred that second-generation gamma-ray irradiation (MV2) impacts the growth and productivity of shallot plants. The highest plant height recorded without gamma-ray irradiation is 44.2 cm. The treatment with a 100 Gy dose resulted in an increased number of leaves, specifically 21.9 pieces, while the treatment with a 125 Gy dose led to an increase in the number of stomata, with an average of 37.7. Additionally, the parameter of umbel stalk length achieved the best results without gamma-ray irradiation, measuring 56.8 cm. The microbulbs that were irradiated with 8 and 10 Krad showed a higher mean weight and diameter. This indicates that applying gamma irradiation of 8 and 10 Krad can be beneficial in producing mutants with desirable agronomic traits in the Boconó clone (Roldán, 2015).

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