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GAMMA RAY-INDUCED *IN VITRO* MUTAGENESIS FOR ENHANCED DROUGHT TOLERANCE IN SHALLOT (*Allium ascalonicum* L.)

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SUMMARY

Gamma ray irradiation combined with *in vitro* Polyethylene Glycol (PEG 6000) selection sought to develop and select mutants with enhance drought tolerance in shallots (*Allium ascalonicum* L.). The research aimed to identify an effective gamma ray dose in inducing mutation in shallot stem bases of the Bima variety and the ideal PEG concentration to select drought tolerance mutant lines. The seven doses of gamma-ray irradiation (0, 2, 4, 6, 8, 10, and 12 Gy) and four PEG concentrations (0%, 10%, 20%, and 30%) were treatments used. The results showed the LD_{50} of gamma-ray irradiation was 5.92 Gy, while the LD_{50} of PEG was 18.57%. The higher the dose of gamma-ray irradiation (> 8 Gy) and PEG (> 20%), the greater the inhibition in the culture's growth. In selection media with 20% PEG, the lowest relative decrease index (RDI) was visible in surviving shoots derived from 6 Gy, which produced the longest roots. The results suggested the 6 Gy gamma-ray irradiation followed by *in vitro* selection using 20% PEG could be effective for developing drought-tolerant shallots. Out of 30 mutant plants, six promising lines derived from explants irradiated by 6 Gy, i.e., B6G-1, B6G-3, B6G-4, B6G-5, B6G-9, and KI-1, were able to form greater bulbs per clump and weight per bulb than the control, Bima variety.

Keywords: Shallot (*A. ascalonicum* L.), drought tolerance, gamma-ray irradiation, PEG 6000, *in vitro* selection

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Key findings: Although research on drought-tolerant shallots (*A. ascalonicum* L.) remains scarce, this study highlights the application of mutagenesis (gamma irradiation) and *in vitro* selection (PEG 6000) to enhance shallot drought tolerance. Six promising mutant lines were selected for subsequent evaluation.

INTRODUCTION

Shallots (*Allium ascalonicum* L.), a subgroup of *A. cepa* (common onion) now officially named *Allium cepa* var. *aggregatum* Don., is one of the invaluable horticultural crops worldwide. It has a considerable contribution to human diets due to nutritional components and medicinal properties (Sun *et al*., 2019). The shallot crop has become a national strategic commodity and a source of income for the farming community in Indonesia, especially as one of the main ingredients in various local cuisines (Saptana *et al.,* 2021). Therefore, the stability of shallot production is crucially essential, requiring sustainability.

Climate change is evidently the primary threat to agriculture globally due to its considerable negative effects on cropping systems (Alotaibi, 2023). Prolonged dry seasons caused by climate pattern variations became a significant challenge to future global food security (Arifah *et al.,* 2022). Irregular rainfall patterns and rising temperatures contribute to increased frequency and severity of droughts, which, in turn, negatively impact crop yields (Kumar *et al*., 2019). Drought stress negatively alters morphological parameters, such as plant height, the number of leaves, and leaf area, which results in critical yield losses of up to 65% in onions (Ghodke *et al*., 2018).

Shallots with a shallow root system limit the plant's ability to get water from deeper zones, which authenticates shallots as a drought-sensitive crop. Almost 90% of shallot roots gather at a depth of 40 cm, with only 2%–3% of the total roots spread below 60 cm (Fanny *et al*., 2020). This root morphology limits soil water availability to the shallot, especially when grown in coarse-textured (sandy) soils where the irrigation water moves below 60 cm (Rahmawati and Yasvi, 2024). Therefore, drought severity tends to be one of the vital factors that affect shallot production. In onions, due to lack of water, the critical period occurred during vegetative growth and bulb formation, which eventually caused reduced production and even crop failure (Gedam *et al.,* 2021).

Drought stress has been reported to influence the physiological and biochemical processes, such as photosynthesis, nitrogen assimilation, and overall metabolism (Qiao *et al.,* 2024). In onions, including shallots, water deficit conditions led to a significant reduction in bulb weight, bulb size, and overall yield (Susilowati *et al.,* 2023). Therefore, to mitigate the impact of climate change, the development of drought-tolerant shallot cultivars is an effective and inexpensive technology choice. Genetic variation is one of the basic strategies for selecting superior mutants and drought-tolerant genotypes in breeding programs. Hence, the highest genetic diversity is crucial in the selection of desirable crop genotypes through breeding programs. However, shallots have low genetic diversity, as their propagation is mostly vegetative in Indonesia (Herlina *et al.,* 2019).

Mutation breeding is very effective in obtaining genetic diversity and variations to develop new genotypes through physical and chemical mutagen treatments (Nilahayati *et al.,* 2024). Mutagenesis, combined with *in vitro* selection, could enhance the opportunities of obtaining new cultivars adapted to specific environments (Sinuraya *et al.,* 2022; Abdelhameed *et al.,* 2024). Induction of mutagenesis through gamma-ray irradiation with a lower dose (0–50 Gy) has been proven efficient in increasing genetic diversity due to chromosomal rearrangements and deletions (Susila *et al.,* 2019; Sinuraya *et al.,* 2022). Whilst, high-radiation doses could induce radio inhibition by affecting growth promoters and, eventually, destroy the tissues in crop plants (Riviello-Flores *et al.,* 2022). According to Mehmandar *et al*. (2023), *in vitro* selection using polyethylene glycol (PEG) with a

molecular weight of 6000 as a stress trigger could simulate drought-stress conditions in the laboratory. Thus, the said methodology is rapid, accurate, and reliable for evaluating and identifying drought-tolerant genotypes in crop plants.

The shallot, Bima variety, is widely cultivated in Indonesia due to its high productivity (9.9 t/ha), moderate resistance to bulb rot (*Botrytis allii*), medium-to-large-sized bulbs, and wider adaptability to lowland areas (Basuki *et al.,* 2017). However, this variety is highly sensitive to water deficit. Research by Hemon *et al*. (2025) showed that drought stress significantly reduced the number of leaves, tillers, bulbs per clump and bulb weight per clump in the Bima variety. Maintaining the stability of national shallot production in the face of climate change underscore the need for drought-tolerant shallot varieties. The research aimed to enhance the tolerance of the Bima variety shallot to drought stress conditions using gamma irradiation and *in vitro* selection under PEG pressure.

MATERIALS AND METHODS

Breeding material and stem base isolation

The planting material (explants) used was the Bima shallot variety obtained from Brebes, West Java, Indonesia. Thus, it is known as "Bima Brebes". Bulbs were cleaned and washed with soap before being sterilized by soaking the explants in sodium hypochlorite solution for 2 h, followed by 96% ethanol for 10 min and 20% Benomyl for 30 min to kill microorganisms on the bulbs' surface. The rinsing of bulbs used sterile water before drying them with sterile filter paper. The sterilized bulb layers are removed by using a scalpel until the three deepest layers are exposed. Then, plant these exposed layers in the media. The materials used were the Murashige Skoog medium base, benzyl amino (BA) purine, naphthalene acetic acid (NAA), polyethylene glycol (PEG 6000), sucrose, phytagel, ethanol, Clorox disinfecting bleach, Tween 80, sterile distilled water, and fertilizers.

Mutation induction using gamma rays and LD_{50} determination (activity 1)

The completely randomized design (CRD) was this experiment's layout. Explants measuring 0.5–1.0 cm were planted into bottles containing MS medium (Murashige and Skoog, 1962) without plant growth regulators. After three days, the explants were treated with gamma-ray irradiation with seven different doses (0, 2, 4, 6, 8, 10, and 12 Gy) using a 60Co Gamma Chamber 4000A at the Irradiation and Instrumentation Laboratory, National Research and Innovation Agency, Jakarta, Indonesia. Each treatment had 10 repetitions, resulting in 70 experimental units. Each experimental unit consisted of five explants. After gamma-ray irradiation treatment, the explants were transferred into MS media supplemented with 1 mg/L BAP and 0.1 mg/L NAA and incubated in a culture room for 16 h of daily illumination under fluorescent 20 W lamps (30–40 μmol/m^2/s). The observations proceeded on the percentage of stem bases alive, plant height, shoot number, and growth performance. The LD_{50} value calculation used the linear regression analysis performed with Microsoft Excel software. The surviving stem base percentage underwent plotting against the radiation doses to generate the regression equation ($y = ax + b$). The LD_{50} estimation was by substituting $y=50$ into the linear equation to find the corresponding dose (x).

PEG LD_{50} dose determination (activity 2)

The CRD was the layout used in this experiment. The explant used was *in vitro* shoots (non-mutants) of the Bima shallot variety. Explants with a size of 1 cm incurred growth in bottles containing liquid MS media added with the BA, NAA, and PEG-6000 with four different concentrations (0%, 10%, 20%, and 30%). Each treatment reached 10 repeats, resulting in 40 experimental units. Each experimental unit consisted of one explant. Shoot selection in media containing PEG continued for six weeks. The explants in the culture bottle, placed in a culture rack for 16 h, sustained daily illumination under fluorescent

20 W lamps (30-40 µmol/m^2/s). Observations were made on the survival percentage of shoots, shoot height, and the visual condition of explants. The LD_{50} dose of PEG determination depended on the percentage of survival shoots.

***In vitro* selection of shallot mutants using the LD_{50} dose of PEG (activity 3)**

The explants used were mutant shoots derived from activity 1, with non-mutated shoots of 1 cm in size serving as the control. *In vitro* selection for drought-tolerant shallot mutants was performed using 20% PEG (the LD_{50} determined from activity 2) added to the selection medium (MS + 1 mg/L BAP + 0.1 mg/L NAA). A selection medium without PEG served as the control. These media were supplemented with 30 g/L of sucrose, and the pH was adjusted to 5.8. The solidifying agent used was 2.5 g/L Phytagel™. There were 10 explants per treatment. Selection was carried out in media containing 20% PEG for six weeks. The cultures were maintained in a culture room at 22 °C under 16 h photoperiod, provided by 20 W fluorescent lamps delivering 30–40 µmol/m^2/s. The relative decrease index (RDI) in survival shoot was calculated, using the following formula:

$$RDI = \frac{Vc - Vs}{Vc} \; x \; 100\%$$

Where RDI = relative decrease index, Vc = variable value on control media (PEG 0%), and Vs = variable on selection media (PEG 20%).

The experiment used the CRD layout. Observations were recorded on shoot height, the number of shoots and leaves, root length, and the visual appearance of the explants. All the recorded data underwent the analysis of variance (ANOVA) at the significance level of 0.05. The significant mean differences received further comparison and separation by using Duncan's multiple range test (DMRT) at the significance level of 0.05.

Acclimatization of plantlet mutants and bulb production (activity 4)

The acclimatization began by removing the plantlets (mutant and non-mutant) from the bottle and then washing the roots. The planting of plantlets commenced in polybags containing a mixture of topsoil, organic fertilizer, and rice husk charcoal at a ratio of 4:1:1 (v/v), after which they were covered with plastic for two weeks. Each polybag contained 2 kg of soil and one seedling. Before planting, the application of cow manure (0.50 kg/polybag) and NPK (16:16:16) 1.0 g/polybag as primary fertilizers preceded. Additional NPK fertilization occurred to each 1.0 g/polybag at 10 and 20 days after planting. Plant maintenance continued until harvest. Observations focused on the number and weight of the bulbs.

RESULTS AND DISCUSSION

The effects of gamma-ray irradiation on shallot plants were influenced by the type of culture and the irradiation dose used (Caplin and Willey, 2018). In this study, gamma-ray irradiation treatment on the stem base indicated the varied growth and development changes based on the amount of prescribed dose. The results showed the higher the dose of gamma-ray irradiation, the greater the inhibition of growth and development of the irradiated explants. A gamma-ray irradiation dose of more than 6 Gy affected the vigor of the irradiated stem base of shallot plants, and they became weaker and pale in color. The shallot stem base irradiated with 12 Gy no longer grew, gradually became white, and died after two weeks. Past studies revealed that the plant organelles found to be sensitive to gamma-ray irradiation were chloroplasts, which exhibited swelling and damage to their thylakoid membranes (Riviello-Flores *et al*., 2022). Other research by Bolsunovsky *et al*. (2022) showed the gamma-ray irradiation at a dose of 13 Gy caused chromosome aberrations in the majority of cells in the anaphase and telophase stages of mitosis in onion.

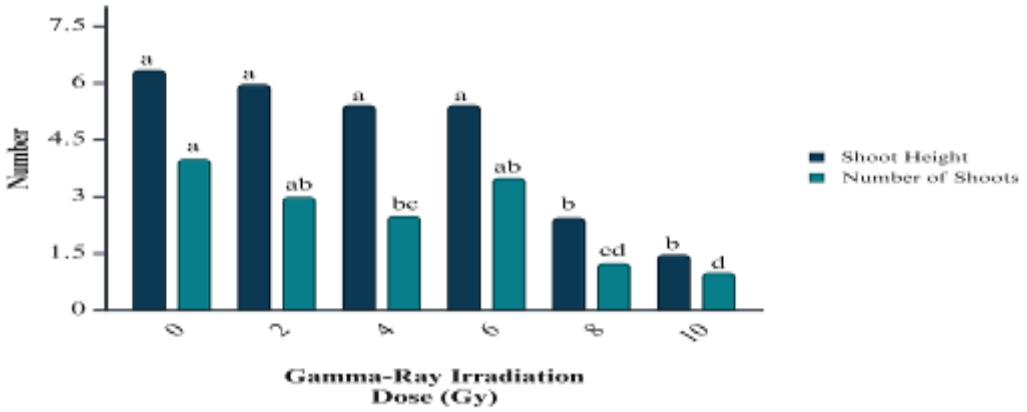

Figure 1. Growth performance of Bima shallot variety after six weeks of gamma-ray irradiation treatment. Note: Values with different letters indicate significant differences between treatments based on the DMRT. The explants irradiated with 12 Gy died at two weeks after treatment.

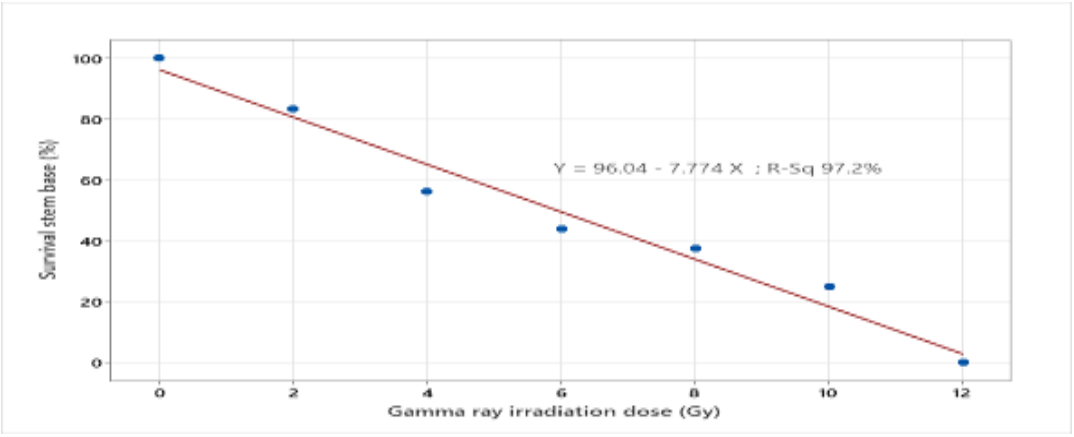

Figure 2. The LD_{50} value of the Bima shallot variety based on the percentage of survival of the stem base after six weeks of mutagenesis treatment.

After gamma-ray irradiation, the surviving shallots showed different growth responses (Figure 1). Generally, shoot height and the number of shoots that grew from the stem base of shallots treated with 2–10 Gy were lower than the control (0 Gy). The reduction in shoot height and the number of shoots was highly significant at the gamma-ray doses of 8 and 10 Gy. According to Bolsunovsky *et al*. (2022), the reduced growth development and morphological variations in onions exposed to high gamma-ray irradiation were caused by DNA damage that interfered with cell division and enlargement.

Measuring sensitivity to radiation relies on parameters such as the lethal dose (LD) and growth reduction (GR). The optimum dose for mutation induction is generally obtained around the LD_{50} or GR_{50}. These values are essential indicators in mutagenesis studies because they help determine the tolerance threshold of explants to the mutagen (Nilahayati *et al.,* 2024). The results indicated that the LD_{50} of gamma-ray irradiation treatment for the shallot Bima variety was 5.92 Gy (Figure 2). However, at a higher irradiation dose (12 Gy), explant mortality reached 100%, revealing that this level was too high for this shallot variety.

Gamma irradiation with increased dose generally inhibited cell division and affected the explants' growth due to damage to the plant meristem cells, which emerged as sensitive to irradiation (Mullins *et al.,* 2021). Total death occurred because the stress induced by gamma irradiation with this dose exceeded the cell's capacity to repair itself, resulting in complete failure in growth and regeneration. However, previous research showed the LD_{50} dose of gamma-ray irradiation using whole shallot bulbs of the Bima variety was identified at the dose of 7.55 Gy (Kurniajati *et al*., 2020). The contrary findings about the LD_{50} values might be due to the different types of explants (full bulb vs. stem base) used and the moisture conditions that influence the sensitivity of plant cells in responding to mutagens. Persada *et al*. (2025) used the Rubaru shallot variety bulbs as explants and found that the lethal dose (LD_{50}) of gamma-ray irradiation was 6.18 Gy.

The *in vitro* selection technique is an *in vitro* culture of plant cells, tissues, and organs on the medium containing selective agents that offers the opportunity to induce and select tolerance for certain targeted traits in crop plants. The technology provides accurate results because the screening points directly to the specific characters being targeted, with no significant influences from environmental factors. The *in vitro* selection for drought tolerance had the general use of polyethylene glycol (PEG) as a selection component. The PEG with a molecular weight of more than 4000 can induce water stress in plants by reducing the water potential in the nutrient solution without causing toxicity. Chaiyapan *et al*. (2023) stated that in *in vitro* culture, the PEG could induce water stress and positively correlate with that in the field or greenhouse test. Therefore, the PEG could simulate drought stress because it retained water, making it unavailable to cells unless the cells have specific mechanisms to attract the moisture (Mehmandar *et al.,* 2023).

The *in vitro* selection in this study showed that increasing PEG concentration could enhance shoot growth inhibition and also decrease the shoots' survival. Using 10% PEG caused 20% death in explants, causing 80% of shoots to survive. Based on the regression equation, the LD_{50} PEG dose for the shallot Bima variety was 18.57% (Figure 3). The results showed the soaking of explants in 18.57% PEG triggered growth inhibition in 50% of the explants used. The osmotic stress produced by PEG disrupted physiological processes, such as water and nutrient absorption. Osmotic stress led to a decrease in the water potential in the medium; hence, the shoots have difficulty maintaining the turgor pressure necessary for growth and development. The shoots grown on the PEG-supplemented medium showed a decrease in growth and a variation in color from green to pale yellowish. Increasing the PEG concentration up to 30% caused the death of

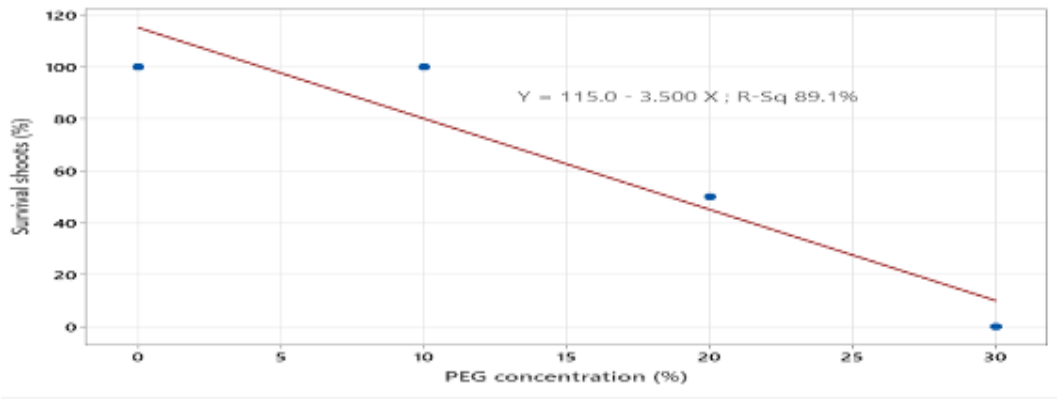

Figure 3. The LD_{50} of PEG based on the percentage of survival shoots after six weeks of PEG treatment.

Table 1. Results of *in vitro* selection of shoot mutants derived from gamma-ray irradiation at two levels of dosage of PEG after six weeks of treatment.

Gamma-ray irradiation dose (Gy)	Survival shoots (%)		Average	Relative decrease index (%)
	0% PEG	20% PEG		
0	100±0	0±0	50.00±0	100.00
2	100±0	40±5	70.00±2.5	60.00
4	85±5	45±5	65.00±2.5	47.06
6	85±5	60±10	72.50±4.3	29.41
8	40±10	20±0	30.00±5	50.00
10	20±10	0±0	10.00±5	100.00
DMRT	R2=10.47	R2=8.75		
	R3=10.97	R3=9.17		
	R4=11.28	R4=9.43		
	R5=11.48	R5=9.61		
	R6=11.63	R6=9.73		

Note: The significant differences between treatments, as determined, used the Duncan multiple range test (DMRT). R = DMRT value of the ranges. Data are given as means ± SD.

all shoots, indicating that the induced osmotic stress by PEG exceeded their survival threshold. Such conditions can lead shoots to severe dehydration, cellular damage, and metabolic failure in crop plants (Yang *et al.,* 2021).

Further study using selection media without (0%) and with 20% PEG showed the decrease in the percentage of survival shoots, along with increasing doses of gamma-ray irradiation. The highest percentage of survival shoots at 0% PEG came from 0 and 2 Gy (100%), followed by 4 and 6 Gy (85%), and with the lowest survival recorded at 10 Gy (Table 1). The results confirmed that the dose of gamma-ray irradiation affected the growth and development of explants after PEG treatment. Selection at 20% PEG resulted in a higher percentage of shoots surviving, ranging from 0% to 60%. The non-mutants (0 Gy) and mutants derived from 10 Gy treatment could not survive and died. The highest percentage of survival shoots of the mutants was evident in the mutants of 6 Gy, which were significantly different from the other mutants, and the lowest percentage of survival shoots appeared from the mutants of 8 Gy. The stress in the 20% PEG selection media was equivalent to -0.71 MPa. PEG causes a decrease in water potential in the media, and the water becomes unavailable for the shoots. Suhesti *et al*. (2019) conducted *in vitro* selection using PEG to mutant callus of sugarcane to obtain callus tolerant to drought, obtaining the LD_{50} at the PEG dose of 10%. These contradictory findings could be due to the different crop plants and types of plant materials used.

On the selection media, the decreasing growth rate of mutant shoots occurred with the relative decreasing index (RDI) value (Table 1). A lower index value revealed a higher level of tolerance to stress in sugarcane crops (Hartati *et al*., 2018). The largest RDI resulted in mutant shoots at 10 Gy and control (0 Gy), while the lowest RDI came from irradiation treatment with a dose of 6 Gy, followed by 4 Gy, 8 Gy, and 2 Gy. The results confirm that shallot mutants derived from gamma-ray irradiation doses less than 10 Gy could survive in 20% PEG selection medium. Thus, plantlets surviving after selection with PEG (20%) were potential for the development of drought-tolerant shallots.

Shallot plants transferred to the medium without PEG appeared to grow well at varying growth rates (Table 2). The shoots, leaves, and roots of mutants derived from 2, 4, and 6 Gy had relatively similar growth and development, while the mutants of 8 Gy had the slowest growth. The mutants of 2, 4, and 6 Gy had shoot height, the number of shoots, and root length significantly different from 8 Gy. Moreover, shoot tolerance to water shortage stress due to PEG can manifest from the morphological traits, viz., root length. Roots are essential for drought

Table 2. The growth and development of mutants after selection using PEG (20%).

Gamma-ray irradiation dose (Gy)	Shoot height (cm)	Number of shoots	Number of leaves	Root length (cm)	Shoot performance
0	0.00±0.00	0.00 ±0.00	0.00±0.00	0.00±0.00	-
2	6.50±0.58	4.75±0.96	5.50±1.29	2.00±0.16	Green
4	5.98±1.33	2.50±0.58	5.75±1.50	3.00±0.29	Green
6	5.93±0.83	3.50±1.73	7.75±2.63	4.00±0.82	Green
8	2.00±0.41	1.00±0.00	1.75±0.50	0.00±0.00	Greenish yellow
10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
DMRT	R2=4.30	R2=2.85	R2=4.91	R2=2.48	
	R3=4.51	R3=2.99	R3=5,16	R3=2.60	
	R4=4.65	R4=3.08	R4=5.32	R4=2.68	
	R5=4.75	R5=3.14	R5=5.43	R5=2.74	
	R6=4.82	R6=3.19	R6=5.51	R6=2.78	

Note: The significant differences between treatments, as determined, used the Duncan multiple range test (DMRT). R = DMRT value of the ranges. Data are given as means ± SD.

resistance because plants detect water sources under diverse environmental conditions. Therefore, shoots with longer roots were an early indicator that the plant had the potential to be more tolerant to drought conditions because these roots could reach available water in deeper soil layers, during drought stress (Tatar *et al.,* 2020).

However, further evaluation under field conditions is still necessary to ensure the shallot's tolerance to drought stress in more complex natural environments. In this study, shallot subjected to gamma-ray irradiation at 6 Gy produced shoots with the longest roots after selection with PEG (20%); therefore, this mutant shows great potential for high drought tolerance compared to the other. The ability of plants to survive under drought stress conditions seemed to be caused by genetic variations developed by the gamma-ray irradiation treatment. Mutations with the higher gamma-ray doses were mostly destructive; however, at the right dose, the mutants can give rise to new characters that are different from the original ones, resulting in tolerant genotypes to diverse environmental conditions (Seleiman *et al.,* 2021).

The acclimatization success of mutant plantlets under greenhouse conditions reached 90% (data not presented), resulting in 30 mutant plants that grew well in the greenhouse (Figure 4). Acclimatization is considerably the critical stage in plant propagation, where plantlets previously grown in tissue culture media must adapt to the more complex environment in the greenhouse (Wang *et al*., 2021). The acclimatization success reflected mutant plantlets that had the highest survival rate following their transition to an open environment. The mutants derived from different gamma-ray irradiation with 2, 4, and 6 Gy could grow sufficiently, while the mutant derived from the 8 Gy gamma-ray irradiation had a slower growth. According to Gonzalez *et al*. (2020), the higher doses of irradiation had the potential to damage the plant cells and, eventually, caused seedling death.

Out of those 30 shallot mutant lines, six lines (B6G-1, B6G-3, B6G-4, B6G-5, B6G-9, and KI-1) were able to form 4–10 bulbs per clump, while the control (Bima variety) only produced two bulbs. The weight/bulb of those shallot mutant lines ranged from 1.66 to 5.00 g, while the control (Bima variety) was 1.64 g (Figure 4, Table 3). These results outperformed those reported by Persada *et al*. (2025), who found that the Rubaru shallot mutant derived from 2 Gy gamma irradiation produced the most bulbs (10 bulbs per clump), with a maximum weight less than 3 g per clump. Similarly, a higher dose of 8 Gy inhibited the growth of the Rubaru shallot variety.

Further study in subsequent generations of these six lines is necessary to determine whether the observed increase in

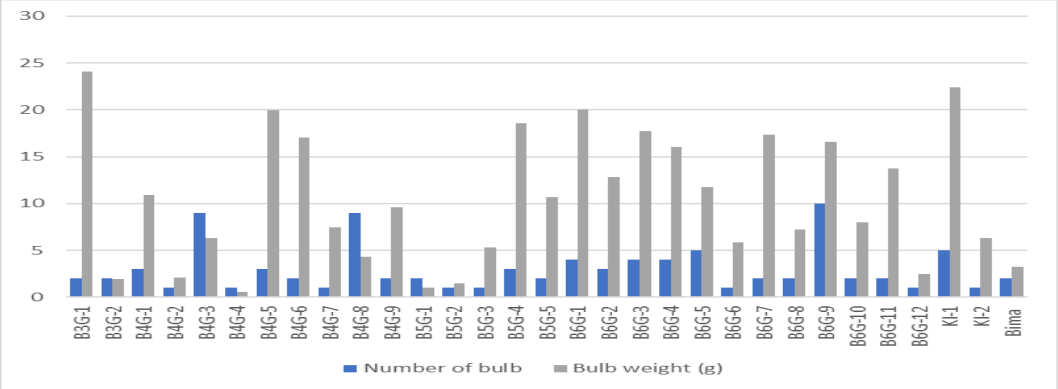

Figure 4. Bulb production of 30 shallot mutant lines under greenhouse conditions.

Table 3. Selected mutants derived from irradiated shallots with 6 Gy.

Mutant lines	Number of bulbs per clump	Bulb weight per clump (g)
B6G-1	4	20.00
B6G-3	4	17.74
B6G-4	4	16.02
B6G-5	5	11.74
KI-1	5	22.39
B6G-9	10	16.56
Cv. Bima (control)	2	3.27

bulb weight is genetically stable or is only a temporary result of physiological radio stimulation. Continuous vegetative propagation is essential to eliminate chimeras and confirm the inheritance of these improved traits.

CONCLUSIONS

Mutagenesis using 6-Gy gamma-ray irradiation, followed by *in vitro* selection using PEG6000 (20%) can be effective in developing shallots with putative tolerance to drought stress conditions. Six promising shallot mutant lines derived from explants irradiated with 6 Gy were able to form more bulbs per clump and yielded a higher weight per bulb than the control (Bima variety).

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REFERENCES

Abdelhameed AA, Ali M, Darwish DBE, Al Shaqhaa MA, Selim DAFH, Nagah N, Zayed M (2024). Induced genetic diversity through mutagenesis in wheat gene pool and significant use of SCoT markers to underpin key agronomic traits. *BMC Plant Biol*. 24: 673. https://doi.org/10.1186/s12870-024-05345-5.

Alotaibi M (2023). Climate change, its impact on crop production, challenges, and possible solutions. *Not. Bot. Horti. Agrobot. Cluj-Napoca* 51(1): 13020. https://doi.org/10.15833/nbha51113020.

Arifah SD, Yassi A, Bahsar-Demmallino E (2022). Climate change impacts and the rice farmers' responses at irrigated upstream and downstream in Indonesia. *Heliyon 8*: e11923. https://doi.org/10.1016/j.heliyon.2022.e11923.

Basuki RS, Kharіriyatun N, Sembiring A, Arsanti IW (2017). Adoption study of Bima Brebes Shallot from IVEGRI in Brebes District. *J. Hortik***.** 27(2):261–268. https://doi.org/10.21082/jhort.v27n2.2017.p261-268.

Bolsunovsky AY, Trofimova EA, Zueva AV, Dementyev DV, Petrichenkov MV (2022). Effect of gamma radiation on cytogenetic and growth endpoints of *Allium cepa* seedlings in long-term experiments. *Dokl. Biochem. Biophys.* 503: 85–89.

Chaiyapan C, Khairum A, Chueakhunthod W, Pookhamsak P, Siwapithakpong K, Tantasawa PA (2023). In vitro selection of mungbean genotypes for drought tolerance by Polyethylene glycol-induced water deficit. *Chiang Mai J. Sci.* 50(3): 1–11. https://doi.org/10.12982/CMJS.2023.035.

Caplin N, Willey N (2018). Ionizing radiation, higher plants, and radioprotection: From acute high doses to chronic low doses. *Front. Plant Sci.* 9: 847. doi: 10.3389/fpls.2018.00847.

Fanny T, Eliyani, Kurniadinata OF (2020). Can we grow shallot (*Allium ascalonicum* L.) root in hydroponic system with simple growing media? *J. Trop. Hortic*. 3(2): 54–59. http://dx.doi.org/10.33089/jthort.v3i2.50.

Gedam PA, Thangasamy A, Shirsat DV, Ghosh S, Bhagat KP, Sogam OA, Gupta AJ, Mahajan V, Soumia PS, Salunkhe VN, Khade YP, Gawande SJ, Hanjagi PS, Ramakrishnan RS, Singh M (2021). Screening of onion (*Allium cepa* L.) genotypes for drought tolerance using physiological and yield-based indices through multivariate analysis. *Front. Plant Sci***.** 12: 600371. https://doi.org/10.3389/fpls.2021.600371.

Ghodke PH, Andhale PS, Gijare UM, Thangasamy A, Khade YP, Mahajan V, Singh M (2018). Physiological and biochemical responses in onion crop to drought stress. *Int. J. Curr. Microbiol. App. Sci.* 7(1): 2054–2062.

Gonzalez M, Alvarado P, Pérez D (2020). Effects of gamma radiation on seed germination and seedling growth in *Allium cepa* L. *Radiat. Phys. Chem.* 170: 108710.

Hartati RrS, Suhesti S, Yunita R, Syafaruddin (2018). Mutation induction with colchicine and selection of drought tolerant sugarcane using Polyethylene glycol. *J. Penelit. Tanaman Ind*. 24(2): 93–104. https://doi.org/10.21082/littri.v24n2.2018. 93-104.

Hemon AF, Kisman UL, Martia DS, Tufaila H (2025). Performance of shallot local landraces planted in several tuber sizes under drought stress conditions: a study in the Indonesia. *Russ. J. Agric. Socio-Econ. Sci.* 157: 106-116.

Herlina L, Reflinur, Sobir, Maharijaya A, Wiyono S (2019). The genetic diversity and population structure of shallots (*Allium cepa* var. *aggregatum*) in Indonesia based on R gene-derived markers. *Biodiversitas* 20(3): 696–703. https://doi.org/10.13057/biodiv/d200312.

Kumar KM, Sridhara CJ, Hanumanthappa M, Marimuthu S (2019). A review of impacts and mitigation strategies of climate change on dryland agriculture. *Curr. J. Appl. Sci. Technol*. 33(4): 1–12. https://doi.org/10.9734/cjast/2019/v33i430085.

Kurniajati WS, Sobir, Aisyah SI (2020). Penentuan dosis iradiasi sinar gamma dalam meningkatkan keragaman untuk perbaikan karakter kuantitatif bawang merah (*Allium cepa* var. *aggregatum*). *J. Ilm. Appl. Isotop Radiasi*. 16(2): 83–89.

Mehmandar MN, Rasouli F, Giglou MT, Zahedi SM, Hassanpouraghdam MB, Aazami MA, Tajaragh RP, Ryant P, Mlcek J (2023). Polyethylene glycol and sorbitol-mediated in vitro screening for drought stress as an efficient and rapid tool to reach the tolerant *Cucumis melo* L. genotypes. *Plants* 12: 870. https://doi.org/10.3390/plants12040870.

Mullins E, Bresson JL, Dalmay T, Dewhurt IC, Epstein ME, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Naegeli H, Nogue F, Serrano JS, Savoini G, Veromann E, Veronesi F, Casacuberta J, Lenzi P, Guajardo IM, Raffaello T, Rostok N (2021). In vivo and in vitro random mutagenesis techniques in plants. *EFSA J***.** 19(11): 6611. https://doi.org/10.2903/j.efsa.2021.6611.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15(3): 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Nilahayati, Handayani RS, Harahap MSA, Irawan G, Rosmaina (2024). Determination of lethal dose 50 for induced mutagenesis in soybean [*Glycine max* (L.) Merril] cv. Gepak Kuning through ethyl methane sulfonate mutagen. *Agric. Sci. Dig.* 44(5): 892–897. https://doi.org/10.18805/ag.DF-544.

Persada O, Fairuzia F, Yuliani F, Arini N (2025). Agronomic characteristics and radiosensitivity level of the Rubaru shallot irradiated by gamma rays. *Indonesian J. Agric. Res*. 8 (3): 129–133.

Qiao M, Hong C, Jiao Y, Hou S, Gao H (2024). Impacts of drought on photosynthesis in major food crops and the related mechanisms of plant responses to drought.

Plants 13: 1808. https://doi.org/10.3390/plants13131808.

Rahmawati N, Yasvi AP (2024). Improvement in physio-biochemical characteristics of shallot plants with nano silica at several levels of drought stress. *IOP Conf. Ser. Earth Environ. Sci.* 1302: 012032. https://doi.org/10.1088/1755-1315/1302/1/012032.

Riviello-Flores MdL, Cadena-Iñiguez J, Ruiz-Posadas LdM, Arévalo-Galarza MdL, Castillo-Juárez I, Hernández MS, Castillo-Martínez CR (2022). Use of gamma radiation for the genetic improvement of underutilized plant varieties. *Plants* 11: 1161. https://doi.org/10.3390/plants11091161.

Saptana, Gunawan E, Perwita AD, Sukmaya SG, Darwis V, Ariningsih E, Ashari (2021). The competitiveness analysis of shallot in Indonesia: A policy analysis matrix. *PLoS ONE* 16(9): e0256832. https://doi.org/10.1371/journal.pone.0256832.

Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Dindaroglu T, Abdul-Wajid HH, Battaglia ML (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* 10(2): 259. https://doi.org/10.3390/plants10020259.

Sinuraya M, Rosmayati, Hasanuddi, Hanafiah DS (2022). Gamma irradiation induced high yield mutant genotype Samosir local shallot in the third generation. *Trends Sci***.** 19(9): 3681. https://doi.org/10.48048/tis.2022.3681.

Suhesti S, Syafaruddin, Ardana IK, Hadipoentyanti E, Hartati RrS (2019). Increasing drought tolerance of sugarcane through gamma ray irradiation and in vitro selection. *AIP Conf. Proc.* 2462: 050007. https://doi.org/10.1063/5.007615.

Sun W, Shahrajabian MH, Cheng Q (2019). The insight and survey on medicinal properties and nutritive components of Shallot. *J. Med. Plants Res.* 13(18): 452–457. https://doi.org/10.5897/JMPR2019.6836.

Susila E, Susilowati A, Yunus A (2019). The morphological diversity of *Chrysanthemum* resulted from gamma ray irradiation. *Biodiversitas* 20(2): 463–467. https://doi.org/10.13057/biodiv/.

Susilowati RD, Sulistyaningsih E, Murti RH (2023). Increasing the growth and yield of shallot (*Allium cepa* L. *Aggregatum* group) by using methyl jasmonic acid (MeJA) concentrations under drought condition. *Agric. Sci.* 8(1): 55–68. https://doi.org/10.22146/ipas.71747.

Tatar I, Shamsiah A, Rahim HA (2020). In vitro screening of gamma irradiated rice variety MR263 for drought tolerance using Polyethylene glycol. *Food Res*. 4(5): 11–20. http://www.myfoodresearch.com.

Wang L, Chen C, Zhou Y (2021). Plant tissue culture and its applications in plant propagation. *Plant Biotechnol. Rep.* 15(3): 135–150.

Yang X, Lu M, Wang Y, Wang Y, Liu Z, Chen S (2021). Response mechanism of plants to drought stress. *Horticulturae* 7: 50. https://doi.org/10.3390/horticulturae7030050.