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Original Article

Analysis of morphological diversity, phenolic content, and antioxidant activity of mutant MV1 shallots treated with gamma irradiation

Análise de diversidade morfológica, teor de compostos fenólicos e atividade antioxidante de mutantes de chalota MV1 tratados com irradiação gama

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Abstract

The importance of this study lies in identifying functional mutants as potential seed sources for shallot cultivation. This study aimed to evaluate morphological diversity, total phenolic content, and antioxidant activity of MV1 shallot (*Allium cepa* var. *aggregatum*) mutants subjected to gamma-ray irradiation. A completely randomized design was used with six gamma irradiation doses (0, 25, 50, 75, 100, and 125 Gy), each replicated three times. Field trials were conducted at the Center for Integrated Farming, University of Bengkulu, Indonesia. Gamma irradiation significantly affected *A. cepa* growth, yield, and biochemical composition, with the LD_{50} estimated at 125 Gy. Although higher doses reduced most growth parameters, the sprouting percentage declined by only 3%. Gamma doses between 25 and 125 Gy increased bulb number in *A. cepa*, reaching a maximum of 5.7 bulbs per plant at 100 Gy. The 100 Gy treatment also produced the highest total phenolic content (76.99 mg GAE/100 g DW) and antioxidant activity (2.7653 µmol TE/g DW). Overall, gamma irradiation significantly influenced the morphological traits and metabolic profiles of *A. cepa* mutants. Notably, the 100 and 125 Gy treatments formed distinct mutant groupings, indicating pronounced morphological and metabolic alterations.

Keywords: *Allium cepa* var. *aggregatum*, gamma irradiation, DPPH, FRAP, phenolic compounds, shallot mutant.

Resumo

A importância deste estudo reside na identificação de mutantes funcionais com potencial para uso como fontes de sementes no cultivo de chalotas. O objetivo deste trabalho foi avaliar a diversidade morfológica, o teor total de compostos fenólicos e a atividade antioxidante de mutantes de chalota MV1 (*Allium cepa* var. *aggregatum*) submetidos à irradiação gama. Utilizou-se um delineamento inteiramente casualizado com seis doses de irradiação gama (0, 25, 50, 75, 100 e 125 Gy), com três repetições. Os experimentos de campo foram conduzidos no Centro de Agricultura Integrada da Universidade de Bengkulu, Indonésia. A irradiação gama afetou significativamente o crescimento, a produtividade e a composição bioquímica de *A. cepa*, sendo o valor de LD_{50} estimado em 125 Gy. Embora doses mais elevadas tenham reduzido os parâmetros de crescimento, a porcentagem de brotação diminuiu apenas 3%. Doses entre 25 e 125 Gy aumentaram o número de bulbos, atingindo um pico de 5,7 bulbos por planta a 100 Gy. Essa dose também resultou no maior teor de fenólicos totais (76,99 mg GAE/100 g de peso seco) e na maior atividade antioxidante (2,7653 µmol TE/g de peso seco). De modo geral, a irradiação gama influenciou significativamente as características morfológicas e os perfis metabólicos dos mutantes de chalota. Notadamente, os tratamentos com 100 e 125 Gy resultaram em agrupamentos distintos, indicando alterações morfológicas e metabólicas mais pronunciadas.

Palavras-chave: *Allium cepa* var. *aggregatum*, irradiação gama, DPPH, FRAP, compostos fenólicos, mutante de chalota.

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1. Introduction

Genetic diversity is a crucial foundation for plant breeding, allowing the development of cultivars with desirable traits such as higher yield, stress resilience, and improved nutritional quality. Among the various approaches used to enhance genetic variability, gamma-ray irradiation has emerged as an effective and widely accepted technique. With its high energy and deep penetration, gamma irradiation induces mutations at the DNA and chromosomal levels, potentially generating novel genetic combinations that are difficult to achieve through conventional breeding methods (Anpama et al., 2022). This technique is widely applied because of its efficiency and its ability to induce mutations without leaving harmful residues, unlike chemical mutagens (Kurniajati et al., 2020; Sari et al., 2020). In shallot (*Allium cepa* var. *aggregatum*), where conventional breeding is constrained by poor flowering capacity (Marlin et al., 2019), induced mutation represents an important alternative strategy to broaden the genetic base.

Beyond morphological changes, gamma irradiation also influences physiological and biochemical processes in plants. Its effects include alterations in photosynthetic activity, disruption of cellular structures, and the generation of reactive oxygen species (Kebeish et al., 2015). These physiological responses may stimulate or suppress the synthesis of secondary metabolites such as phenolics and flavonoids, which are widely recognized for their antioxidant potential and are commonly evaluated using different antioxidant mechanisms, including radical scavenging and reducing power assays (Arif et al., 2023; Mardiyanti et al., 2024; Putri et al., 2023; Carocho and Ferreira, 2013). Several studies have demonstrated that these bioactive compounds contribute significantly to plant defense mechanisms and nutritional quality. In *Allium* species, particularly shallots and onions, organosulfur compounds, allicin, and phenolics are major contributors to antioxidant activity (Dhurhania and Novianto, 2016; Ladeska et al., 2020). These antioxidants are essential not only for plant stress tolerance but also for human health, as they help mitigate oxidative stress and reduce the risk of degenerative diseases (Ahmad et al., 2015).

Recognizing these potentials, recent research on mutation breeding in shallots has increasingly focused not only on morphological traits but also on biochemical responses. Gamma irradiation may act as a double-edged sword; while it can enhance the production of beneficial metabolites, excessive doses may impair growth and disrupt physiological balance (Borzouei et al., 2010). Therefore, identifying optimal irradiation doses that enhance genetic diversity and biochemical traits without causing adverse effects is essential. This study was conducted to evaluate the morphological diversity, total phenolic content, and antioxidant activity of MV1 *A. cepa* mutants generated through gamma-ray irradiation. Understanding these responses is expected to facilitate the identification of mutant lines with superior traits for sustainable shallot cultivation and seed development (Alfariatna et al., 2018).

2. Materials and Methods

2.1. Field experimental procedures

The experiment was conducted using true shallot seeds (TSS) treated with gamma irradiation. Irradiation was performed at the National Nuclear Energy Agency of Indonesia (BATAN) using a Gamma Cell 220 machine with cobalt-60 as the energy source and an initial activity of 10.687 curies. The irradiation dose rate was approximately 5.6 Gy per second. The treatment consisted of six levels of gamma irradiation: 0 Gy (control), 25 Gy, 50 Gy, 75 Gy, 100 Gy, and 125 Gy.

Shallot bulbs from the first generation (M1) of irradiated seeds were planted in polybags filled with a soil, manure, and husk mixture (2:1:1). The experiment followed a completely randomized design (CRD) with three replications. The study was conducted at the Center of Integrated Farming, Faculty of Agriculture, University of Bengkulu (3°45′44″ S, 102°16′45″ E). Prior to planting, the top quarter of each bulb was trimmed and treated with fungicide. One bulb was planted per polybag with a 3 cm planting depth.

Plants were fertilized at planting using urea (200 kg/ha), SP-36 (150 kg/ha), and KCl (110 kg/ha), along with liquid organic fertilizer (10 mL/L) applied every 10 days. Manual weeding was conducted to prevent crop damage. Pest and disease control was managed through weekly application of insecticides and fungicides. Shallots were harvested at 65 days after planting (DAP), or when 20–80% of the leaves had yellowed or wilted, the tops drooped, and bulb tips emerged above the soil surface. Bulbs were cleaned and air-dried on bamboo racks for two weeks.

2.2. Observed variables

The observed parameters included plant height, number of leaves, number of bulbs, bulb diameter, fresh and dry plant weight, harvest time, number of stomata, flowering percentage, days to umbel appearance, umbel stalk length, umbel diameter, and number of umbels.

2.3. Sample preparation and extraction

Ten bulbs per treatment were randomly selected as independent biological replicates, cleaned, cut, dried, and ground into powder. A total of 4 g of sample was extracted with 20 mL ethanol using a Microwave Assisted Extraction (MAE) method at 135 W for 3 minutes. The filtrate was collected and adjusted to a final concentration of 0.2 g/mL for analysis.

2.4. Total Phenolic Content (TPC)

TPC was determined following a modified method from Arista et al. (2022). A 20 µL aliquot of extract was mixed with 120 µL of 10% Folin-Ciocalteu reagent and incubated for 5 minutes in the dark. Then, 80 µL of 10% Na_2CO_3 was added, and the mixture was incubated for 30 minutes. Absorbance was measured at 750 nm using a microplate reader. Gallic acid was used as the standard. Results were expressed as mg gallic acid equivalent per 100 g dry weight.

2.5. Total Flavonoid Content (TFC)

TFC was measured following Calvindi et al. (2020). A 10 µL sample was mixed with 120 µL distilled water, 10 µL of 10% $AlCl_3$, 10 µL glacial acetic acid, and 50 µL ethanol. After incubation in the dark for 30 minutes, absorbance was recorded at 415 nm. Quercetin was used as the standard. Results were expressed as mg quercetin equivalent per g dry weight.

2.6. DPPH antioxidant assay

The DPPH method followed Nurcholis et al. (2022). A 100 µL sample was mixed with 100 µL of 125 µM DPPH solution, incubated in the dark for 30 minutes, and absorbance was read at 515 nm. Trolox was used as a standard. Results were expressed as micromoles of Trolox equivalent per gram dry weight.

2.7. FRAP antioxidant assay

The FRAP assay followed Nurcholis et al. (2022). FRAP reagent was prepared by mixing acetate buffer (pH 3.6), TPTZ in HCl, and $FeCl_3$ in a 10:1:1 ratio. In a 96-well microplate, 20 µL of sample was combined with 290 µL of FRAP reagent, incubated in the dark for 30 minutes, and absorbance was read at 593 nm. Trolox was used to generate a standard curve. Results were reported as micromoles of Trolox equivalent per gram dry weight.

2.8. Statistical analysis

All quantitative data obtained from morphological, phenolic, and antioxidant analyses were subjected to statistical analysis using GraphPad Prism software (version 10.6.1, GraphPad Software, San Diego, CA, USA). Data were analyzed using one-way analysis of variance (ANOVA) to evaluate the effects of gamma irradiation doses. Statistical significance was determined at $p < 0.05$. When significant differences were detected, mean comparisons were performed using the least significant difference (LSD) test.

3. Result

3.1. Effect of gamma irradiation on growth and yield of shallot mutants (MV1)

The gamma-ray mutation treatment successfully induced morphological variation in shallot plants. Analysis of variance indicated that gamma irradiation significantly affected most morphological parameters, except for fresh weight, dry weight, and days to umbel formation (Table 1). In addition, irradiation had a significant impact on total phenolic content and antioxidant capacity as measured by both the DPPH and FRAP methods (Table 1).

Gamma irradiation reduced plant height. The tallest plants (44.2 cm) were observed in the control (0 Gy), while the shortest (33.03 cm) were found in the 125 Gy treatment (Table 2).

Irradiation also influenced other morphological parameters. The highest number of leaves (21.87) was recorded at 100 Gy, not significantly different from the 125 Gy treatment (21.07). The number of bulbs per plant increased with irradiation and peaked at 100 Gy with an average of 5.7 bulbs. Bulb diameter, however, tended to decrease at higher doses. The largest diameter (28.08 mm) was observed at 50 Gy, though differences among treatments were not always statistically significant.

Table 1. Recapitulation of ANOVA on morphological characteristics, total phenolic content, and antioxidants of first-generation of shallot mutants (MV_1).

Parameter	F value		Coefficient of diversity (%)
	Treatment	Block	
Plant height	3.81 *	0.44 ns	9.19
Number of leaves	17.90 **	1.24 ns	4.25
Number of bulbs	2.26 *	0.60 ns	24.41
Diameter of bulb	1.82 *	2.32 ns	6.61
Plant fresh weight	0.79 ns	0.25 ns	19.62
Plant dry weight	0.87 ns	0.27 ns	21.82
Days of the umbel's formation	0.61 ns	0.00 ns	28.85
Umbel length	3.31 *	0.07 ns	29.20
Umbel diameter	1.46 *	0.00 ns	27.30
Number of umbels	1.43 *	0.09 ns	21.48
Harvest time	8.42 **	0.31 ns	1.57
Total phenolic content	299.05 **	54.60 **	2.60
DPPH	299.96 **	3.26 ns	0.47
FRAP	39.73 **	0.91 ns	6.40

*Significant effects at α= 5%. **Significant effects at α=1%. nsNon-significant.

Neither fresh nor dry weight showed significant differences among treatments (Table 2).

Regarding floral traits, gamma irradiation had no significant effect on umbel formation or number of umbels, but significantly reduced umbel length. The longest umbels were observed in the control group (58.58 mm), while the shortest were in the 125 Gy treatment (27.67 mm). Umbel diameter and number were highest at 75 Gy and 50 Gy, respectively. The shortest harvest duration (81.2 DAP) occurred under the 125 Gy treatment, significantly different from the control (87 DAP), suggesting that gamma irradiation accelerated maturation (Table 3).

3.2. Effect of gamma irradiation on phenolic content and antioxidant activity

Gamma irradiation significantly altered the total phenolic content in shallot bulbs. The highest total phenolic content (76.99 mg GAE/100 g DW) was recorded at 100 Gy, while the lowest values were observed at 25 and 125 Gy. This variation confirms that radiation dose plays a crucial role in stimulating or suppressing phenolic compound synthesis (Figure 1).

Antioxidant activity measured using the DPPH method showed the highest activity in the control, followed by treatments at 25 and 50 Gy. In contrast, higher doses (100 and 125 Gy) resulted in reduced DPPH antioxidant values, indicating that elevated irradiation may degrade antioxidant compounds or inhibit their biosynthesis (Figure 2A).

Results from the FRAP assay displayed a different trend, with the highest antioxidant capacity observed at 125 Gy, suggesting that this method may reflect the presence of other reducing agents in the extract. Overall, DPPH values were consistently higher than FRAP values, implying that the DPPH assay may be more sensitive for detecting radical scavenging activity, whereas FRAP reflects total reducing power (Figure 2B).

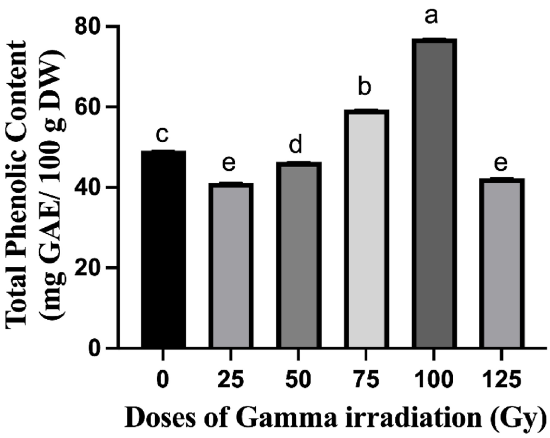

Figure 1. Effect of gamma irradiation doses on total phenolic content of MV1 shallot (*Allium cepa* var. *aggregatum*) mutants. Bars represent mean values. Different letters above bars indicate significant differences among treatments based on the LSD test at α = 5%.

Table 2. The effect of gamma irradiation doses on the growth parameters of first generation shallot mutants.

Gamma- irradiation (Gy)	Plant height (cm)	Number of leaves	Number of bulbs	Diameter of bulb (mm)	Plant fresh weight (g)	Plant dry weight (g)
0	44.20 a	18.67 b	3.47 b	27.96 a	36.98	28.61
25	38.90 abc	18.87 b	3.87 ab	27.16 ab	40.59	33.55
50	41.27 ab	16.63 c	3.80 ab	28.08 a	44.01	37.40
75	35.83 bc	17.73 bc	5.30 ab	24.65 b	34.41	29.18
100	33.03 c	21.87 a	5.70 a	25.49 ab	44.98	37.86
125	37.10 bc	21.07 a	5.33 ab	26.48 ab	41.21	34.04

Numbers in the same column followed by the same letters are not significantly different based on LSD test at α=5%.

Table 3. The effect of gamma irradiation doses on the umbel formation and harvest time of MV_1 shallot mutant.

Gamma- irradiation (Gy)	Umbel formation (dap)	Umbel length (cm)	Umbel diameter	Number of umbels	Harvest time (dap)
0	38.82	58.58 a	48.04 ab	1.00 ab	87.00 a
25	49.44	44.78 ab	53.47 ab	1.22 ab	87.00 a
50	44.64	51.97 ab	48.63 ab	2.14 a	86.77 a
100	33.39	43.28 ab	56.52 a	1.44 ab	85.87 a
125	39.67	27.67 bc	35.19 ab	1.67 ab	81.20 b
150	20.50	13.83 c	18.22 b	0.50 b	86.57 a

Numbers in the same column followed by the same letters are not significantly different based on LSD at α=5%.

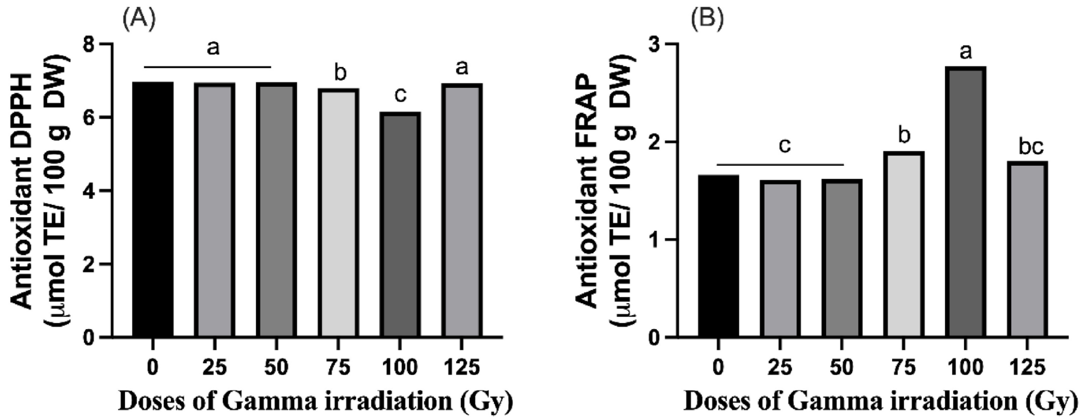

Figure 2. Antioxidant activity of MV1 shallot (*Allium cepa* var. *aggregatum*) mutants subjected to gamma irradiation as measured by (A) DPPH radical scavenging activity and (B) FRAP reducing power. Bars represent mean values. Different letters above bars indicate significant differences among treatments based on the LSD test at α = 5%.

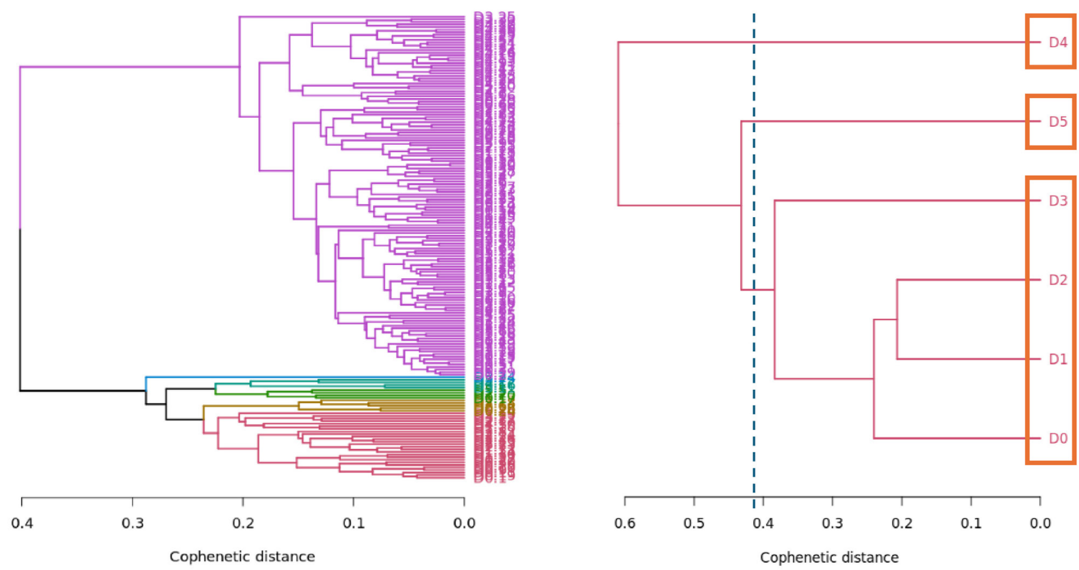

Figure 3. Dendogram of the effect of gamma irradiation on the morphological characters and metabolite content of MV1 shallot mutants. D0, D1, D2, D3, D4, and D5 indicate gamma irradiation doses of 0, 25, 50, 75, 100, and 125 Gy, respectively.

Cluster analysis revealed that shallot mutants formed three distinct groups based on irradiation dose and their morphological and biochemical profiles. The control to 75 Gy treatments clustered together, while 100 and 125 Gy formed separate groups, indicating that higher radiation doses induced more substantial physiological and biochemical differentiation (Figure 3).

4. Discussion

Gamma irradiation effectively induced variations in both morphological and biochemical characteristics of MV1 shallot mutants. The reduction in plant height, particularly at 125 Gy, aligns with previous studies in green bean mutants that reported growth inhibition with increasing doses of gamma rays (Tah, 2006; Singh et al., 2001). High radiation levels are known to suppress cell elongation and division through ionization, excitation, and dissociation processes in plant tissues, potentially damaging meristematic regions (Kovacs and Keresztes, 2002).

The increase in leaf number and bulb production at 25–125 Gy suggests a positive hormetic effect of moderate irradiation levels. Gamma rays at sub-lethal doses can activate growth-related pathways or enhance physiological responses that promote productivity (Hanafiah et al., 2010). However, the reduction in bulb diameter at higher doses indicates that although organogenesis may be stimulated, overall biomass accumulation may be constrained by cumulative cellular damage.

Floral responses showed that umbel number and diameter were not significantly affected by gamma irradiation, whereas umbel length decreased at higher doses. A reduction in umbel size accompanied by a shortened harvest period, particularly at 125 Gy, may reflect a stress-induced shift in developmental timing. Accelerated maturation under irradiation stress may be advantageous or disadvantageous depending on specific agronomic objectives.

In terms of phytochemical response, the highest total phenolic content was observed at 100 Gy, confirming that gamma irradiation may enhance secondary metabolite production under moderate oxidative stress conditions (Zheng et al., 2020). Phenolic compounds are intrinsic constituents of *Allium* species and have been histochemically identified in *A. cepa* and *A. ascalonicum*, where they contribute to antioxidant defense and quality-related attributes (Syahruni et al., 2024). In contrast, the marked decline in phenolic content at 125 Gy indicates that excessive radiation disrupts biosynthetic pathways or promotes the degradation of phenolic compounds. High irradiation levels intensify oxidative stress through the overproduction of reactive oxygen species (ROS), which can overwhelm the plant's antioxidant defense system and impair the balance between phenolic synthesis and degradation (Pelcaru et al., 2021). Under such conditions, cellular damage may exceed the plant's compensatory metabolic capacity, resulting in reduced phenolic accumulation, a response consistent with reports of decreased phenolic levels under severe stress or high irradiation exposure (Tripathi et al., 2021; Wang et al., 2022). Comparatively, higher phenolic concentrations have been reported in non-irradiated onion extracts under optimized physicochemical conditions, suggesting that irradiation-induced stress modulates, rather than maximizes, phenolic accumulation (Ladeska et al., 2020).

Antioxidant assays revealed a clear divergence between DPPH and FRAP responses. While DPPH activity was highest in the control treatment, FRAP activity peaked at 125 Gy. This divergence reflects differences in antioxidant mechanisms, as DPPH primarily detects radical scavenging capacity, whereas FRAP measures overall reducing power (Miguel-Chavez, 2017). The reduced DPPH response at higher irradiation doses may indicate degradation or reduced synthesis of radical scavengers, while the enhanced FRAP values suggest the accumulation of other redox-active compounds. Similar response patterns have been reported in *Eleutherine palmifolia*, where FRAP was more sensitive than DPPH under stress conditions (Marlin et al., 2022).

Studies on other *Allium* species confirm the antioxidant roles of phenolics, flavonoids, and sulfur-containing compounds in countering oxidative stress (Gawad et al., 2014). However, the phenolic content observed in this study (41–76 mg GAE/100 g DW) remains relatively modest compared with values reported for garlic and onion varieties (Hikmah and Anggarani, 2021; Amalia and Anggarani, 2022), likely reflecting genotypic differences and irradiation sensitivity.

Cluster analysis provided a holistic view of radiation-induced variation among MV1 mutants. Treatments from 0 to 75 Gy clustered together, whereas 100 and 125 Gy formed distinct groups, indicating that higher irradiation doses induce more pronounced morphological and biochemical differentiation. These distinct profiles highlight the potential of high-dose irradiation as a selection strategy in mutation breeding programs, particularly for developing genotypes with enhanced antioxidant-related traits (Marlin et al., 2019; Bolsunovsky et al., 2019).

5. Conclusion

Gamma irradiation proved to be an effective approach for inducing morphological and biochemical variation in MV1 shallot mutants. Moderate irradiation doses, particularly 100 Gy, enhanced key growth traits such as leaf number and bulb formation and resulted in the highest total phenolic content. In contrast, higher doses (125 Gy) tended to suppress growth and reduce DPPH-measured antioxidant activity, although FRAP reducing power remained elevated.

Overall, gamma irradiation influenced both primary and secondary metabolic processes in a dose-dependent manner. Based on the combined morphological and biochemical responses, irradiation doses up to 100 Gy may be considered optimal for generating beneficial traits without substantially compromising plant performance. These findings provide a useful basis for mutation breeding strategies aimed at improving shallot productivity and antioxidant-related traits.

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Data Availability Statement

Not applicable.

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