



**Full Length Article**

## Evaluation of Flower Diversity of Selected *Phalaenopsis* Orchids Mutant Irradiated by Gamma Ray

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### Abstract

*Phalaenopsis* orchids, widely distributed as potted plants globally, face an escalating consumer demand for the development of new varieties. This demand compels breeders to continuously enhance genetic diversity in response to consumer preferences. One approach to augmenting genetic diversity involves inducing mutations through gamma-ray irradiation. The objective of this research is to analyze the phenotypic diversity of selected mutant *Phalaenopsis* resulting from gamma-ray irradiation. This process conducted at the National Nuclear Energy Agency of Indonesia. The experimental design employs a Randomized Block Design with three doses of gamma-ray irradiation, namely 0, 25 and 35 Gy. The irradiated materials consist of plants that have produced flower stalks, comprising LM 114, GL 021651, GL 354, GL 04616, GL 070179, JN 0017 and GL 151224 genotypes. Each treatment dose is replicated 10 times. The results indicate that mutation induction using gamma-ray irradiation on the flower stalk is capable of enhancing the genetic diversity of *Phalaenopsis* orchids. Six mutants at 35 Gy exhibiting genetic variability were identified, showing variation in sepal and petal tip shape. So that, gamma irradiation doses at 35 Gy can be applied to induce genetic variability in the *Phalaenopsis* orchid. However, there is currently no evidence of an increased genetic diversity in terms of color or pattern of flowers. © 2024 Friends Science Publishers

**Keywords:** Mutations; Phenotypic diversity; Selection

### Introductions

*Phalaenopsis*, commonly known as the moon orchid holds a prominent position in the world of ornamental plants due to several key attributes and characteristics that make it an important ornamental plant (De and Kumar 2014; Magar *et al.* 2020). *Phalaenopsis* orchids come in a wide range of diversity of flower colors and patterns such as white, pink, purple, yellow, and various shades in between (Nascimento *et al.* 2015). They also exhibit diverse patterns, such as spots, stripes, and solid colors, making them versatile for decorative purposes (Wang *et al.* 2017).

Enhancing genetic diversity in ornamental plants variation contributes to the creation of new and unique ornamental plant varieties. A diverse array of ornamental plant varieties allows consumers to choose from a wide range of options, catering to different preferences in terms of color, fragrance, and overall appearance. This diversity supports the horticultural industry and satisfies consumer demand for novelty and uniqueness (Hajizadeh *et al.* 2022).

Gamma ray irradiation has played a important role in revolutionizing the field of plant breeding by offering a potent tool to augment genetic diversity (Habibullah *et al.* 2022). As a form of mutagenesis, gamma ray irradiation involves exposing plants to controlled doses of gamma

radiation, which induces random genetic mutations. This process has been instrumental in broadening the genetic pool of plant species, facilitating the development of new cultivars, and enhancing the adaptability and resilience of plants (Lestari *et al.* 2018). Gamma ray irradiation had a significant in bolstering genetic diversity within the realm of plant breeding, shedding light on its contributions to agriculture, horticulture, and scientific research (Yani *et al.* 2018). Gamma rays, a form of ionizing radiation, can penetrate plant cells and disrupt their DNA structure (Agisimanto *et al.* 2016). When plants are exposed to controlled doses of gamma radiation, it can lead to the random induction of mutations in their genetic material. These mutations can result in a wide range of genetic changes, including alterations in traits like size, shape, color, disease resistance, and other desirable characteristics (Magdalita *et al.* 2022). The findings from the research conducted by Widiarsih and Dwimahyani (2013) showed that gamma-ray irradiation-induced mutations can accelerate the flowering of orchids and lead to a range of leaf color variations, ultimately resulting in variegation.

The study on enhancing genetic diversity in *Phalaenopsis* was previously conducted by Kurniadi *et al.* (2023), utilizing UV<sub>254</sub> light and Ethyl Methane Sulfonate on protocorm-like bodies (PLBs). Additionally, at the

research of Aloysius *et al.* (2017) indicated that gamma ray irradiation at doses ranging from 12 to 18 rads can enhance the diversity of *Spathoglottis plicata* in terms of leaf, root, and shoot characteristics. However, research on the impact of gamma ray irradiation applied to the flower spike of *Phalaenopsis* orchids has not been conducted yet. The objective of this research is to reveal the diversity of flower characteristics in selected *Phalaenopsis* mutants resulting from gamma ray irradiation, with the expectation of enhancing the plant's aesthetic value.

## Materials and Methods

### Plant material

Four-year-old plants of six hybrid *Phalaenopsis* consisted of LM 114, GL 021651, GL 354, GL 04616, GL 070179, JN 0017, GL 151224 genotype, that had flower spikes without buds were exposed to gamma ray irradiation at doses of 25 and 35 Gy.

### Time and location of research

The gamma irradiation was carried out at the National Nuclear Energy Agency's Center for Application of Isotope and Radiation Technology. The study was conducted from March to August 2023. After that, these plants were cultivated in the screen house of the Faculty of Agriculture at Jenderal Soedirman University in Purwokerto, Indonesia.

### Experimental design

The experimental design was a factorial Randomized Block Design with ten times replications. There are two factors tested, namely gamma ray irradiation dose and *Phalaenopsis* genotype. The gamma irradiation doses tested were 0 Gy, 25 Gy and 35 Gy. *Phalaenopsis* genotype consisted of LM 114, GL 021651, GL 354, GL 04616, GL 070179, JN 0017, GL 151224 genotype. Plants with flower stalks were exposed to gamma rays at doses of 0 Gy, 25 Gy, and 35 Gy in the Gamma Chamber.

### Data collection

Data were collected from the flower at 16 weeks after irradiation. Variables observed were qualitative characteristics namely flowering position, flower shape, petal shape, petal arrangement, petal tip shape, sepal tip shape, cross section of petals and sepals; and quantitative characteristics namely inflorescence length, number of flowers, flower length and width, dorsal sepal and the petal, encompass measurements guided by the UPOV (International Union for the Protection of New Varieties of Plants) *Phalaenopsis* Descriptors. The morphological traits were contrasted with those of the *Phalaenopsis* plants that had not been treated.

## Statistical analysis

To assess the performance of the observed traits, the collected data was subjected to variance analysis at an error level of 5% (Table 1). In the significance data, further analysis was carried out using Duncan's Multiple Range Test at a 5% error level.

## Results

### Diversity of flower qualitative traits

Mutations can induce genetic variation within a population. This variation can affect traits such as flower color, size, shape, fragrance, and other characteristics. For example, a mutation might result in a flower with a slightly different hue or a unique pattern. Qualitative traits are those that can be categorized into discrete groups. These traits are usually controlled by one or a few genes, and they often follow Mendelian inheritance patterns.

The diversity observed in *Phalaenopsis* mutants irradiated at 25 and 35 Gy revealed the new traits (Table 2). These selected mutants can subsequently be propagated through vegetative means to perpetuate these desired characteristics in the following generation. In this research, each mutant exhibiting distinct new characteristics was generated from the LM 114, GL 021651, GL 04616, GL 070179 and JN 0017 genotypes.

The tip of the petals underwent a change in shape in LM 114, GL 021651, GL 04616 and JN 0017 mutants (Table 3). In the untreated plants, the petal tip was originally blunt, but after gamma irradiation, it became serrated. However, the GL 070179 mutant displayed a different alteration, with a change in the overall petal shape, which originally slightly rounded to rhombus.

Flower color did not exhibit a significant increase in diversity. The LM 114 mutant displayed a change in labellum color; untreated plants exhibited a red hue, whereas irradiated plants displayed a pale purple tint. The GL 021651 mutant demonstrated an uptick in yellow coloration, indicating heightened expression of carotenoid pigments in both the petals and labellum. For the GL 04616 mutant, there was a slight reduction in purple pigment at the base of the petals, but it was more evenly distributed compared to untreated plants. The GL 070179 mutant, akin to the LM 114 mutant, exhibited a faded labellum color. In untreated plants, the labellum was red, while in irradiated plants, it transitioned to a slightly paler shade of purple. The JN 0017 genotype mutant displayed an increase in anthocyanin expression, evidenced by a multitude of purple spots on the flowers; This aligns with Magdalita *et al.* (2022), which found that gamma ray irradiation led to the emergence of striped purple pigments in *Phalaenopsis* orchids.

### Diversity of flower quantitative traits

Quantitative traits in plants refer to measurable characteristics that exhibit a continuous range of variation

**Table 1:** Recapitulation of analysis of variance on the characteristics of several orchid genotypes at various doses of gamma ray irradiation

Characteristics	Genotype (G)	Irradiation dose (D)	G x D
Length of inflorescence	*	ns	ns
Number of flowers	*	ns	ns
Length of leaf	*	ns	ns
Width of leaf	*	ns	ns
Inflorescence diameter	*	ns	ns
Flower length	*	*	*
Flower width	*	ns	*
Length of dorsal sepal	*	ns	*
Wide of dorsal sepal	*	ns	ns
Length of lateral sepal	*	ns	ns
Wide of lateral sepal	*	ns	ns
Petal length	*	*	*
Petal width	*	*	ns

Note: ns = not significant; \* = significant

**Table 2:** Flower performance of *Phalaenopsis* mutants compared to untreated plants

No.	Genotype	Normal Plant	Mutant
1.	G1 (LM 114)		
2.	G2 (GL 021651)		
3.	G3 (GL 354)		
4.	G4 (GL 04616)		
5.	G5 (GL 070179)		
6.	G6 (JN 0017)		
7.	G7 (GL 151224)		

within a population. These traits are often influenced by the combined effects of multiple genes and environmental factors. Unlike qualitative traits, which are categorical and follow Mendelian inheritance patterns, quantitative traits show a spectrum of phenotypic expression.

The analysis of variance (Table 1) showed that there

are interactions between genetic and irradiation dose on flower length and width, dorsal sepal and petal length. In addition, gamma ray irradiation affects petal width. This shows that there is different radiosensitivity in each genotype. Based on Makhziah and Soedjarw (2023), the radio sensitivity of varieties to gamma rays refer to the

**Table 3:** Qualitative characteristics of *Phalaenopsis* mutants compared to untreated plants

No.	Mutant	Characters	Normal Plant	Mutant
1.	G1 (LM 114)	Flower shape	(1) Round	(1) Round
		Petal shape	(8) Slightly rounded	(8) Slightly rounded
		Petal arrangement	(1) Open	(1) Open
		Form the petal tips	(5) Blunt	(10) Serrated
		Form the sepal tips	(5) Blunt	(1) Taper, sharpened to the tip
2.	G2 (GL 021651)	Cross section of petals and sepals	(2) Flat	(2) Flat
		Flower shape	(1) Round	(1) Round
		Petal shape	(8) Slightly rounded	(8) Slightly rounded
		Petal arrangement	(1) Open	(1) Open
		Form the petal tips	(5) Blunt	(10) Serrated
3.	G3 (GL 354)	Form the sepal tips	(5) Blunt	(5) Blunt
		Cross section of petals and sepals	(2) Flat	(2) Flat
		Flower shape	(1) Round	(1) Round
		Petal shape	(8) Slightly rounded	(8) Slightly rounded
		Petal arrangement	(1) Open	(1) Open
4.	G4 (GL 04616)	Form the petal tips	(5) Blunt	(10) Serrated
		Form the sepal tips	(5) Blunt	(5) Blunt
		Cross section of petals and sepals	(2) Flat	(2) Flat
		Flower shape	(1) Round	(1) Round
		Petal shape	(8) Slightly rounded	(8) Slightly rounded
5.	G5 (GL 070179)	Petal arrangement	(1) Open	(1) Open
		Form the petal tips	(5) Blunt	(1) Taper/Sharpens to the tip
		Form the sepal tips	(1) Taper, sharpened to the tip	(1) Taper/Sharpens to the tip
		Cross section of petals and sepals	(2) Flat	(2) Flat
		Flower shape	(1) Round	(1) Round
6.	G6 (JN 0017)	Petal shape	(8) Slightly rounded	(4) Like a rhombus
		Petal arrangement	(1) Open	(1) Open
		Form the petal tips	(5) Blunt	(1) Taper/Sharpens to the tip
		Form the sepal tips	(5) Blunt	(1) Taper/Sharpens to the tip
		Cross section of petals and sepals	(2) Flat	(2) Flat
7.	G7 (GL 151224)	Flower shape	(1) Round	(1) Round
		Petal shape	(4) Like a rhombus	(4) Like a rhombus
		Petal arrangement	(1) Open	(1) Open
		Form the petal tips	(1) Taper/Sharpens to the tip	(1) Taper/Sharpens to the tip
		Form the sepal tips	(1) Taper/Sharpens to the tip	(1) Taper/Sharpens to the tip
		Cross section of petals and sepals	(2) Flat	(2) Flat

**Table 4:** Leaf length and width, length of inflorescence, number of flowers, and diameter of inflorescence (mm) of *Phalaenopsis* mutant irradiated by gamma ray

Factors	Length of leaf (cm)	Width of leaf (cm)	Length of inflorescence (cm)	Number of flowers	Diameter of inflorescence (mm)
Irradiation dose					
0 Gy	22.06	8.40	21.59	14.28	5.93
25 Gy	22.08	8.50	22.66	16.76	5.91
35 Gy	21.40	8.49	23.04	14.66	6.00
Genotype					
LM 114	25.66 a	8.12 cd	18.34	13.11 b	5.43 d
GL 021651	22.57 c	8.78 b	22.83	9.55 b	6.26 bc
GL 354	21.22 d	9.46 a	19.07	7.88 b	6.03 bc
GL 04616	20.68 d	8.58 b	22.91	11.11 b	5.71 cd
GL 070179	24.04 b	7.74 d	20.45	9.66 b	6.37 b
JN 0017	20.62 d	8.16 cd	19.05	8.33 b	7.16 a
GL 151224	18.12 e	8.38 bc	23.24	47.00 a	4.65 e

Note: Numbers followed by the same letter in the same column and variable are not significantly different based on DMRT analysis with an error rate of 5%

degree to which different plant or crop varieties react or respond to exposure to gamma ray irradiation. Different plant varieties may exhibit varying degrees of radiosensitivity, influencing how they respond to gamma ray treatment. In other hand, other characters are entirely

influenced by genetic factors. A trait is any genetically determined characteristic in plants. In plants, traits are the observable features or properties that result from the expression of specific genes. Each gene carries the instructions for the synthesis of a particular protein or plays

**Table 5:** Flower length and width of *Phalaenopsis* mutant irradiated by gamma ray

Genotype	Flower length (cm)			Flower width (cm)		
	0 Gy	25 Gy	35 Gy	0 Gy	25 Gy	35 Gy
LM 114	8.50 a	7.97 a	8.40 a	7.90 a	8.77 a	7.70 a
GL 021651	7.60 a	7.20 a	6.63 b	8.70 a	7.63 ab	7.36 b
GL 354	10.40 a	8.70 b	8.13 c	8.70 b	10.93 a	9.50 ab
GL 04616	7.15 b	8.06 a	7.00 b	7.65 a	8.43 a	8.60 a
GL 070179	7.60 a	7.23 a	7.06 a	9.00 a	7.60 b	7.20 b
JN 0017	6.65 b	7.20 b	8.10 a	7.30 a	8.40 a	7.50 a
GL 151224	3.50 a	3.33 a	3.76 a	3.90 a	3.67 a	3.76 a

Note: Numbers followed by the same letter in the same row and variable are not significantly different based on DMRT analysis with an error rate of 5%

**Table 6:** Dorsal sepal and petal length of *Phalaenopsis* mutant irradiated by gamma ray

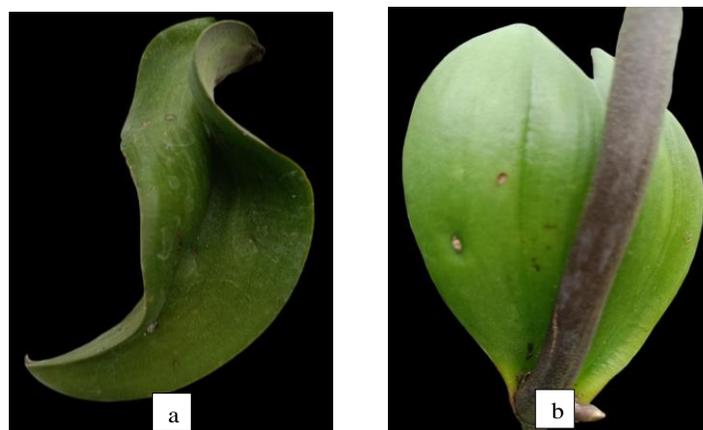
Genotype	Dorsal sepal length (cm)			Petal length (cm)		
	0 Gy	25 Gy	35 Gy	0 Gy	25 Gy	35 Gy
LM 114	4.50 a	4.36 a	4.57 a	4.23 a	4.30 a	4.17 a
GL 021651	4.30 a	4.23 a	4.03 a	4.17 a	3.70 ab	3.50 b
GL 354	5.00 ab	5.10 a	4.67 b	4.90 b	5.40 a	4.43 b
GL 04616	3.90 a	4.20 a	4.00 a	3.70 a	3.97 a	4.10 a
GL 070179	4.90 a	4.03 b	4.10 b	4.30 a	3.80 b	3.56 b
JN 0017	3.70 a	4.00 a	4.10 a	3.67 b	4.20 a	3.60 b
GL 151224	1.20 b	1.93 a	1.90 a	1.80 a	1.77 a	1.87 a

Note: Numbers followed by the same letter in the same row and variable are not significantly different based on DMRT analysis with an error rate of 5%

**Table 7:** Dorsal sepal and petal width (cm), lateral sepal length and width (cm) of *Phalaenopsis* mutant irradiated by gamma ray

Factors	Dorsal sepal width (cm)	Petal width (cm)	Lateral sepal length (cm)	Lateral sepal width (cm)
Irradiation dose				
0 Gy	2.76	4.59 a	3.87	2.53
25 Gy	2.79	4.39 a	3.77	2.60
35 Gy	2.66	4.02 b	3.85	2.58
Genotype				
LM 114	3.27 a	4.53 bc	4.18 b	2.98 b
GL 021651	3.20 a	4.33 c	4.33 ab	3.27 a
GL 354	3.21 a	6.10 a	4.61 a	2.91 b
GL 04616	2.78 b	4.86 b	4.11 b	2.54 c
GL 070179	2.72 b	4.50 bc	3.63 c	2.32 d
JN 0017	2.66 b	4.43 bc	4.00 b	2.50 c
GL 151224	1.33 c	1.60 d	1.95 d	1.48 e

Note: Numbers followed by the same letter in the same column and variable are not significantly different based on DMRT analysis with an error rate of 5%



**Fig 1:** Abnormal leaf **a)** Wavy leaf; **b)** Leaves growing on flower stalk

a role in regulating cellular functions, ultimately influencing the plant's traits (Sommer 2020).

The results of the analysis of variance indicate that leaf length and width, the number of flowers, and flower

diameter were primarily influenced by genetic factors (Table 1). In Table 4, the LM 114 genotype exhibited the longest leaf at 25.66 cm, while the GL 354 genotype had the widest leaf at 9.46 cm. The GL 151224 genotype displayed

the highest number of flowers, reaching 47.00, as it belongs to the *Phalaenopsis multiflora* category with mini-sized flowers, contributing to its higher flower count compared to other genotypes classified as grandiflora types. The largest inflorescence diameter was recorded in genotype JN 0017, reaching 7.16 mm.

Various genotypes exhibited distinct responses in terms of flower length and width to different doses of gamma ray irradiation (Table 5). The length and width of flowers in LM 114 remained unchanged following gamma ray irradiation. However, in GL 021651, there was a reduction in both the length and width of flowers at a dose of 35 Gy, decreasing from 7.60 cm to 6.63 cm for length and from 8.70 cm to 7.36 cm for width. In GL 354, gamma irradiation led to a decrease in flower length at 25 Gy and 35 Gy, diminishing from 10.40 cm to 8.70 cm at 25 Gy and 8.13 cm at 35 Gy; however, it stimulated an increase in flower width from 8.70 cm to 10.93 cm at 25 Gy and 9.50 cm at 35 Gy. In GL 04616, there was an increase in flower length at 25 Gy, rising from 7.15 cm to 8.06 cm, but it decreased again to 7.00 cm at 35 Gy; however, the flower width remained constant. In GL 070179, flower length was unaffected by gamma ray irradiation, but the flower width decreased at 25 Gy and 35 Gy, diminishing from 9.00 cm to 7.60 cm at 25 Gy and 7.20 cm at 35 Gy. For JN 0017, there was an increase in flower length due to gamma ray irradiation at 35 Gy, rising from 6.65 cm to 8.10 cm, while there was no change in flower width. GL 151224 did not exhibit changes in flower length and width following gamma ray irradiation.

There were variations in the response of different genotypes to different doses of gamma irradiation concerning dorsal sepal length and petal length (Table 6). In LM 114, there were no changes in the length of dorsal sepals and petals due to gamma ray irradiation. However, in GL 021651, there was a reduction in petal length at 35 Gy of gamma ray irradiation, decreasing from 4.17 cm to 3.50 cm; nevertheless, the length of dorsal sepals remained unchanged. In GL 354 exhibited a decrease in dorsal sepal length from 5.00 cm to 4.67 cm at 35 Gy; however, petal length increased at 25 Gy from 4.90 cm to 5.40 cm. Yet, it decreased to 4.43 cm at 35 Gy, indicating that low doses of irradiation can stimulate growth. In GL 04616 did not undergo changes in the length of dorsal sepals and petals due to gamma ray irradiation. In GL 070179 decreased in the length of dorsal sepals and petals at 25 and 35 Gy; dorsal sepal length decreased from 4.90 cm to 4.03 at 25 Gy, while petal length decreased from 4.30 cm to 3.80 cm at 25 Gy. In JN 0017, there was no change in the size of dorsal sepals, but there was an increase in petal length at 25 Gy, going from 3.67 cm to 4.20 cm, although it decreased again to 3.60 cm at 35 Gy. GL 151224 witnessed an increase in dorsal sepal length at 25 Gy and 35 Gy, from 1.20 cm to 1.93 cm; however, the length of the petals did not undergo significant changes due to gamma ray irradiation.

## Discussion

Mutation is a process in evolution that can contribute to the diversity of plant species, including flowers characteristics in plant. Mutations are spontaneous changes in the DNA sequence of an organism's genome (Hajizadeh *et al.* 2022). The interaction between gamma ray radiation and genotype (Table 1) shows different effects of gamma rays irradiation in each genotype which then results in different levels of diversity in each genotype. According to Riviello-Flores *et al.* (2022), radiosensitivity in plants refers to the extent to which the plant absorbs the radiation and how likely it is that genetic infections will occur as a result of exposure to ionizing radiation. Radiosensitivity can vary between genotypes. Some plants may be more responsive to gamma radiation, while others may be less responsive. Factors such as the plant's genetic structure, the plant's life cycle and the dose of radiation applied can influence how much radiosensitivity there is.

In this study, gamma irradiation did not cause a significant change in the color of the *Phalaenopsis* flowers (Table 2). This is possible because the unique genetic composition of *Phalaenopsis* may not be highly vulnerable to mutations that alter color induced by gamma irradiation, thus preventing significant changes in color or color patterns.

Gamma irradiation had more of an effect on the shape of the flowers (Table 3). There was an increase in the diversity of flower shapes especially at a dosage of 35 Gy. The dose of 25 Gy exhibited fewer changes compared to the dose of 35 Gy. Increasing the dose of gamma ray irradiation enhances the probability of mutations due to the greater energy and intensity of the radiation. The greater energy delivered with a higher dose increases the chances of inducing mutations by causing more significant disruptions to the DNA structure. These disruptions may include breaks in the DNA strands, cross-linking of DNA molecules, or other alterations that can result in genetic mutations. This is in accordance with the statement Eun and Kim (2022) that as the radiation dose increases, the range of variations gradually expands due to gamma rays have high energy and can penetrate deeply into tissues. However, the optimization of radiation dosage is crucial to balance the induction of mutations with the viability of the organism or cells being irradiated.

The flowers produced by irradiated plants displayed variations in the shape of their petal tips (Table 3). The majority of them exhibit a transformation in the shape of the petal tip, initially blunt, turning serrated, resembling peloric orchids. This result demonstrating that gamma irradiation led to an expanded diversity in this trait. Alterations in flower shape occurred as a result of mutations induced by radiation, particularly within somatic cells. Mutations that occur during the initiation of apical cells produced the stable mutants. Furthermore, the expression of the mutation is influenced by the location of the mutated flower, leading to

noticeable changes in flowers that originate from the tissue with the mutation (Muhallilin *et al.* 2019). Due *et al.* (2019) also added that, when plants are exposed to gamma ray irradiation, the energy can disrupt the DNA structure within the plant cells. The ionizing radiation causes changes in the nucleotide sequence of the DNA, leading to the induction of mutations. These mutations can range from point mutations (changes in a single nucleotide) to larger structural changes in the DNA (Ma *et al.* 2021). Similar transformations occur in *Chrysanthemum* flowers, where gamma irradiation induces alterations in both the form and hue of the flowers (Soliman *et al.* 2014; Wang *et al.* 2020).

This study showed that gamma ray irradiation had no effect on the leaves (Table 4). Leaves exposed to gamma irradiation did not experience significant changes and showed good viability, but there were some leaves that had abnormal growth, such as the appearance of wavy leaves (Fig. 1). The impact of a 35 Gy dose did not impose significant stress on the plants. This is evident in the ability of normally growing plants, which is not lower than the normal plant population. The abnormality in plant growth was because gamma rays belong to the category of electromagnetic wave radiation, and they generate energy by dislodging electrons from target cells. This process can result in direct harm to molecules, as the radiation's energy is absorbed by DNA molecules. Additionally, it can have an indirect effect, where the energy is not absorbed by DNA but instead interacts with other cellular molecules, generating free radicals. These free radicals subsequently induce modifications in DNA molecules, encompassing damage to double strands, loss of nitrogen bases, and chemical alterations in the structure of bases, potentially leading to gene mutations (Li *et al.* 2019). This phenomenon directly impacts living tissues because it can inflict damage or alterations to essential cellular components, subsequently affecting the morphology, anatomy, biochemistry and physiological processes of plants (Choi *et al.* 2015).

Each genotype showed differences in responding to various doses of gamma ray irradiation (Table 5, 6). This is related to the differences in radiosensitivity of each genotype. Certain genotypes might show increased resilience and adaptability, resulting in negligible distinctions at particular doses, such as the LM 114 genotype, display no significant changes in quantitative flower characteristics due to gamma-ray irradiation. But, GL 070179 exhibits a high sensitivity to gamma irradiation, as evidenced by a reduction in flower width, dorsal sepal, and petal length, which decreases upon irradiation starting at a dose of 25 Gy. Nobre *et al.* (2022) said that gamma-ray irradiation can potentially inflict damage on the plasma membrane and cytosolic enzyme systems, resulting in the inhibition of plant growth and development. In GL 354, an irradiation dose of 25 Gy can stimulate the development of flower length, dorsal sepal and petal length, but decreased at 35 Gy dose. According to Kim *et al.* (2021), low doses of gamma irradiation can trigger specific genes responsible for

growth and development. These genes may be involved in processes such as cell division, elongation, and differentiation, leading to enhanced plant growth. Low doses of gamma rays also can stimulate various metabolic processes within plant cells. This includes an increase in enzyme activity and the production of certain growth-promoting substances (Katiyar *et al.* 2022). Hormesis is often associated with stress responses. Low levels of stress can induce plants to adapt and become more resilient. In the case of gamma radiation, a low dose may act as a mild stressor, prompting the plant to activate adaptive responses that benefit growth (Agathokleous *et al.* 2019). In GL 021651 gamma-ray irradiation at 35 Gy reduced the development of flower length and width, and petal length. The findings of this study align with those of Susila *et al.* (2019), who observed that safflower plants exposed to higher doses of gamma irradiation exhibited shorter growth compared to control plants. This effect was attributed to gamma irradiation interfering with DNA synthesis. Similarly, Hong *et al.* (2022) reported that gamma ray irradiation on mulberry plants led to the inhibition of auxin synthesis and cell division.

Gamma ray irradiation caused a decrease in petal width at a dose of 35 Gy (Table 7), but had no effect on dorsal sepal width, lateral sepal length and width. High dose of gamma-ray irradiation can suppress cell division in plants. This inhibition of mitosis and cell proliferation hampers the ability of the plant to generate new tissues. According to Xie *et al.* (2019), high doses of radiation induce physiological stress in plants. This stress response can trigger the production of reactive oxygen species (ROS), leading to oxidative damage to cellular components. Oxidative stress contributes to growth inhibition and can impair the overall health of the plant.

## Conclusion

The presence of variations in radiosensitivity among each *Phalaenopsis* genotype irradiated with gamma-rays results in a variability of quantitative characteristics of flowers. However, overall, gamma irradiation has the potential to enhance the diversity of petal shapes in *Phalaenopsis*.

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## Author Contributions

Each author actively contributed to the development, discussion, and composition of this paper and collectively bears responsibility for its content.

## Conflict of Interest

The authors collectively state that they have no conflicts of interest.

## Data Availability

The data from this study will be accessible upon a reasonable request to the corresponding author.

## Ethics Approval

Not applicable to this paper.

## References

- Agathokleous E, M Kitao, EJ Calabrese (2019). Hormesis: A compelling platform for sophisticated plant science. *Trends Plant Sci* 24:318–327
- Agisimanto D, NM Noor, R Ibrahim, A Mohamad (2016). Gamma irradiation effect on embryogenic callus growth of *Citrus reticulata* cv. Limau madu. *Sains Malays* 45:329–337
- Aloysius S, A Purwantoro, K Dewi, E Semiarti (2017). Improvement of genetic variability in seedlings of *Spathoglottis plicata* orchids through X-ray irradiation. *Biodiversitas* 18:20–27
- Choi JI, M Yoon, S Lim, GH Kim, H Park (2015). Effect of gamma irradiation on physiological and proteomic changes of Arctic *Zygnema* sp. (Chlorophyta, Zygnematales). *Phycologia* 54:333–341
- De LC, R Kumar (2014). Tropical and Subtropical Orchids. *Intl J Sci Appl Res* 1:1–9
- Due MS, A Susilowati, A Yunus (2019). The effect of gamma ray irradiation on diversity of *Musa paradisiaca* var. sapientum as revealed by ISSR molecular marker. *Biodiversitas* 20:1416–1422
- Eun CH, IJ Kim (2022). The citrus mutant Jedae-unshiu induced by gamma irradiation exhibits a unique fruit shape and increased flavonoid content. *Plants* 11:1–8
- Habibullah M, TS Wahyudiningsih, G Haryono, M Rahmiyah, N Farid, (2022). The appearance of mutations from three varieties of longan used gamma rays. *IOP Conf Ser: Earth Environ Sci* 1018:012034
- Hajizadeh HS, SN Mortazavi, F Tohidi, H Yildiz, M Helvacı, T Alas, V Okatan (2022). Effect of mutation induced by gamma-irradiation in ornamental plant liliun (*Lilium longiflorum* cv. Tresor). *Pak J Bot* 54:223–230
- Hong MJ, DY Kim, YD Jo, H Choi, JW Ahn, SJ Kwon, SH Kim, YW Seo, JB Kim (2022). Biological effect of gamma rays according to exposure time on germination and plant growth in wheat. *Appl Sci* 12:3208
- Katiyar P, N Pandey, S Keshavkant (2022). Gamma radiation: A potential tool for abiotic stress mitigation and management of agroecosystem. *Plant Stress* 5:100089
- Kim SM, YD Jo, JI Chun, JB Kim, JH Kang (2021). Chronic gamma irradiation changes phenotype and gene expression partially transmitted to next-generation tomato seedlings. *Agronomy* 11:1638
- Kumiadi AS, F Irawati, SED Putra, PH Hardjo (2023). Induction of protocorm-like bodies (plbs) *Phalaenopsis* spp. hybrids mutation through Ultraviolet Irradiation (UV254) and Ethyl Methane Sulfonate (EMS). *Agripr J Appl Agric Sci* 7:1–15
- Lestari EP, A Yunus, S Sugiyarto (2018). Diversity induction of *Dendrobium sylvanum* orchid through in vitro irradiation of gamma ray. *Biosaint J Biol Biol Educ* 10:691–697
- Li F, A Shimizu, T Nishio, N Tsutsumi, H Kato (2019). Comparison and characterization of mutations induced by gamma-ray and carbon-ion irradiation in rice (*Oryza sativa* L.) using whole-genome resequencing. *G3 Genes Genomics Genet* 9:3743–3751
- Ma L, F Kong, K Sun, T Wang, T Guo (2021). From classical radiation to modern radiation: Past, present, and future of radiation mutation breeding. *Front Publ Health* 9:768071
- Magar YG, S Koshioka, A Noguchi, W Amaki (2020). Effects of light quality during cultivation on the flowering and floret arrangement in *Phalaenopsis amabilis*. *Acta Hort* 127:135–140
- Magdalita PM, AO San-Pascual, RL Villareal (2022). Evaluation of plant and flower characteristics of selected 15-gy irradiated *Phalaenopsis aphrodite*. *Mind J Sci Technol* 20:236–249
- Makhziah M, DP Soedjarwo (2023). Radiosensitivity of two local chili varieties to gamma rays. *J Tek Pert Lamp J Agric Eng* 12:423–430
- Muhallilin I, SI Aisyah, D Sukma (2019). The diversity of morphological characteristics and chemical content of *Celosia cristata* plantlets due to gamma ray irradiation. *Biodiversitas* 20:862–866
- Nascimento MF, NFF Nascimento, ER Rêgo, CH Bruckner, FL Finger, MM Rêgo (2015). Genetic diversity in a structured family of six generations of ornamental chili peppers (*Capsicum annum*). *Acta Hort* 1087:395–401
- Nobre DAC, CS Bonfá, AFD Silva, V Arthur, CS Sediyaama (2022). Soybean generations under gamma rays and effects on seed quality. *Chil J Agric Anim Sci* 38:287–296
- Riviello-Flores ML, J Cadena-Iñiguez, LDM Ruiz-Posadas, ML Arévalo-Galarza, I Castillo-Juárez, MS Hernández, CR Castillo-Martínez (2022). Use of gamma radiation for the genetic improvement of underutilized plant varieties. *Plants* 11:1161
- Soliman TMA, S Lv, H Yang, B Hong, N Ma, L Zhao (2014). Isolation of flower color and shape mutations by gamma radiation of *Chrysanthemum morifolium* Ramat cv. Youka. *Euphytica* 199:317–324
- Sommer R (2020). Phenotypic plasticity: From theory and genetics to current and future challenges. *Genetics* 215:1–13
- Susila E, A Susilowati, A Yunus (2019). The morphological diversity of *Chrysanthemum* resulted from gamma ray irradiation. *Biodiversitas* 20:463–467
- Wang HM, CG Tong, S Jang (2017). Current progress in orchid flowering/flower development research. *Plant Signal Behav* 12:e1322245
- Wang L, J Wu, F Lan, P Gao (2020). Morphological, cytological and molecular variations induced by gamma rays in *Chrysanthemum morifolium* “Donglinruixue”. *Fol Hort* 32:87–96
- Widiarsih S, I Dwimahyani (2013). Gamma irradiation application for mutation breeding in early flowering moth orchid (*Phalaenopsis amabilis* Bl.). *J Ilm Aplik Isot Dan Rad* 9:59–66
- Xie L, KA Solhaug, Y Song, DA Brede, OC Lind, B Salbu, KE Tollefsen (2019). Modes of action and adverse effects of gamma radiation in an aquatic macrophyte *Lemma minor*. *Sci Total Environ* 680:23–24
- Yani RH, N Khumaida, SW Ardie, M Syukur (2018). Analysis of variance, heritability, correlation and selection character of M1V3 generation cassava (*Manihot esculenta* Crantz) mutants. *Agrivita* 40:74–79