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Effect of gamma irradiation on median lethal dose for mutation induction in *Zinnia elegans* and *Cosmos bipinnatus*

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Abstract Zinnias and cosmos are popularly grown as decorative flowers in homes, gardens, and as potted plants. In this study, the appropriated gamma radiation doses for inducing mutations in zinnias and cosmos were investigated. It was found that gamma radiation significantly improved the seed germination parameters of both zinnias and cosmos. Vegetative growth traits showed that both zinnias and cosmos had decreased survival rate, fewer shoots, and reduced plant height as the irradiation dose increased. At a dose of 800 Gy, seeds were able to germinate, but seedlings grew to be stunted and eventually died. The median lethal dose (LD_{50}) of zinnias and cosmos was determined as 459.6 and 345.5 Gy, respectively. Gamma radiation also affected the development of flowers as it delayed the flowering time in zinnias. In addition, gamma irradiation induced morphological changes in both plants, including stunted stems, curled leaves, smaller flowers and light green variegated leaves in zinnias, and the asymmetry and curling petals, in cosmos. Our findings provide crucial information for optimizing the gamma radiation dose to induce mutagenesis in zinnias and cosmos while minimizing other deleterious effects.

Keywords: Ionizing radiation, Induction mutation, Ornamental plants, Seed irradiation

Introduction

Gamma radiation has long been recognized as a potent mutagenic agent, exerting its influence on the genetic makeup of living organisms. Understanding the effects of gamma radiation on the induction of mutations is of paramount importance in the fields of agriculture and genetics. Previous investigations have established gamma radiation as a powerful tool for inducing genetic variations in plants, providing valuable insights into the underlying mechanisms of mutagenesis (Riviello-Flores *et al.,* 2022). Gamma irradiation has been demonstrated to promote plant growth and improve the economic traits and productivity of various plants by altering cytological, biochemical, and genetical

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processes within the plant cells (Sparrow, 1961; Sharma and Rana, 2007; El Sherif *et al*., 2011; Wu *et al*., 2019).

Gamma irradiation-induced mutation is also successfully used in ornamental industry as it provides sources for improving flowers with exotic patterns, including the variations in flower colors and shapes (Yamaguchi, 2018). Despite being an important tool for plant breeding, gamma rays may cause serious damages to plants. Depending on the irradiation level, they can disturb protein synthesis, enzyme activity, and the regulation of plant hormones (Surakshitha *et al*., 2017). Moreover, the impact of gamma radiation on plant physiology varied depending on the specific plant species or varieties and irradiation dosage (Roslim *et al*., 2015). Although extensive research in this area has accumulated over the past years, species-specific responses to gamma radiation and the determination of median lethal doses remain relatively unexplored aspects of mutagenesis.

Thus, the present study delves into the impact of gamma radiation on the median lethal dose for mutation induction in two ornamental plant species, *Zinnia elegans* and *Cosmos bipinnatus*. These two plant species were selected based on their significance in floriculture, primarily for decorative purposes. Zinnias and cosmos are cultivated for their colorful flowers, enhancing the beauty of many gardens, landscapes, and public areas in Thailand. Moreover, these two plant species are easy to manage, disease-resistant, and tolerant of tropical climates in Thailand. Investigating the effects of gamma radiation on these species will not only contribute to our understanding of the basic principles of mutagenesis but also provide practical implications for the cultivation and breeding of ornamental plants.

The determination of the median lethal dose (LD_{50}) for mutation induction is a critical aspect of assessing the mutagenic potential of gamma radiation. The LD_{50} represents the dose at which 50% of the exposed organisms exhibit lethal mutations, offering a quantitative measure of the radiation's impact on the genetic stability of a population. Such information is essential for optimizing radiation-based breeding strategies and minimizing undesirable effects. The study aimed to explore the LD_{50} for mutation induction in *Zinnia elegans* and *Cosmos bipinnatus*.

Materials and methods

Plant materials and gamma radiation

Seeds of *Zinnia elegans* ‘Mix’ (Metro Seed Agricultural, Bangkok, Thailand) and *Cosmos bipinnatus* ‘Mix’ (Chia Tai, Bangkok, Thailand) were acutely irradiated with gamma rays generated from Mark I Gamma Irradiator (J. L. Shepherd and Associates, CA, USA) with a Caesium-137 source at the Nuclear Technology Research Center, Kasetsart University, Bangkok, Thailand. The dose rate used in this experiment was 3.74 Gy/min. Thirty seeds for each treatment were irradiated at the doses of 100, 200, 400, 600, and 800 Gy. Non-irradiated seeds (0 Gy) were used as a control. Experiment was conducted using a randomized complete block design (RCBD) with 3 replications. After 14 days, the seedlings were then transplanted into soil bed, amended with lime and soil conditioners at a 1:1 ratio. The seedlings were grown in plots with 20 × 15 cm spacing between plants.

Evaluation of seed germination

The irradiated seeds were sown in a 105-cell seedling tray filled with peat moss, one seed per cell. The effect of gamma radiation dose on seed germination rates was recorded as germination percentage (GP), mean germination time (MGT), and coefficient of velocity of germination (CVG) as shown in the following equations (1-3):

$$GP(\%)=\frac{\text{number of germinated seeds}}{\text{total of seed number}}\times 100 \quad \text{..............(1)}$$

$$MGT\ (days)=\frac{\sum (n_i d_i)}{\text{total of germinated seed}} \quad \text{....................(2)}$$

Where n = number of germinated seed at day i,
d = the day of counting

$$CVG=\frac{\text{total of germinated seed}}{\sum (n_i d_i)}\times 100 \quad \text{.......................(3)}$$

Where n = number of germinated seed at day i,
d = the day of counting

Relative survival percentage and growth parameters

The relative survival percentage from each radiation dose was calculated as relative to survival in control at 30 days and 60 days after transplanting. The median lethal dose (LD_{50}) was calculated using linear regression graph plotted with radiation dose and relative survival percentage. Growth parameters, including plant height, shoot number at 30 and 60 days after transplanting, and days to first flowering were recorded to assess the effect of gamma radiation on plant development. The growth reduction by 50% (GR_{50}) of each plant species was also determined using linear regression graph plotted with radiation dose and relative plant height.

Statistical analysis

A one-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range test (DMRT) at significance levels of $\alpha = 0.05$ was performed using R software to assess the statistical significances among the means within the same plant species.

Results

Effects of gamma ray on seed germination

Irradiated zinnia and cosmos seeds were evaluated for their germination success by measuring the germination percentage (GP), mean germination time (MGT), and coefficient of velocity of germination (CVG). Result indicated that gamma radiation significantly affected the germination percentages only in zinnia (Table 1). Specifically, the lowest germination percentage was observed at an irradiation dose of 100 Gy, (47.78 percent), which was not significantly different from a control group. In contrast, higher irradiation doses of 200, 400, 600 and 800 Gy resulted in significantly higher germination percentages of 73.33, 70.56, 72.22, and 74.44 percent, respectively. For the germination percentages in cosmos, there was no significant differences among radiation doses; however, a trend that GP values tended to decrease as the irradiation dose increased was observed.

In addition to GP, mean germination time and coefficient of velocity of germination were recorded in zinnia and cosmos, both of which showed significant differences among radiation doses. MGT reflects the average time required for seeds to germinate, indicating the day when most seeds were sprouted. CVG measures the speed of germination with a high CVG value enhanced GP and reduced the time needed for seed germination. In this study, MGT values of both plant species increased with higher irradiation doses, where the high doses showed significantly higher MGT values as compared to the control group. Conversely, the CVG values decreased as the irradiation dose increased.

Table 1. Seed germination parameters: germination percentage (GP), mean germination time (MGT), and coefficient of velocity of germination (CVG) for zinnia and cosmos seeds treated with different doses (unit: Gy) of gamma radiation

Dose (Gy)	Seed germination parameters					
	Zinnia			Cosmos		
	GP (%)	MGT (days)	CVG	GP (%)	MGT (days)	CVG
0	57.78±2.55^{b}	4.14±0.34^{c}	24.24±2.06^{a}	63.33±4.41	3.13±0.35^{bc}	32.18±3.73^{ab}
100	47.78±6.94^{b}	4.40±0.06^{bc}	22.73±0.33^{ab}	62.78±11.10	2.84±0.11^{c}	35.25±1.41^{a}
200	73.33±4.41^{a}	4.79±0.26^{a}	20.92±1.15^{b}	57.22±6.94	3.10±0.04^{bc}	32.22±0.44^{ab}
400	70.56±0.96^{a}	4.52±0.09^{ab}	22.13±0.43^{b}	57.78±5.85	3.79±0.23^{a}	26.48±1.59^{c}
600	72.22±3.85^{a}	4.56±0.18^{ab}	21.97±0.87^{b}	50.00±16.07	3.49±0.29^{ab}	28.81±2.51^{bc}
800	74.44±11.10^{a}	4.59±0.25^{ab}	21.82±1.14^{b}	53.89±4.19	3.66±0.30^{a}	27.47±2.14^{c}
F-test	**	*	*	ns	**	**
C.V. (%)	17.15	6.06	6.38	15.76	12.02	12.06

[1/]**Significant at p≤0.01, *significant at p≤0.05, ns: not significant
[2/]Mean±SD are shown. Different letters indicate a significant difference by DMRT (p<0.05)

Seedling survival percentage and median lethal dose

The relative survival percentage (RSP) of zinnia and cosmos seedlings was evaluated 30 and 60 days after transplanting by comparing the seedling survival in treatment with the seedling survival in control (0 Gy) (Table 2). The results indicated significant differences in RSP among radiation doses in both species. In zinnia, the highest RSP was recorded at a radiation dose of 200 Gy, which was not significantly different from the 0 Gy and 100 Gy treatments. In contrast, the lowest RSP was observed at an irradiation dose of 800 Gy, which was significantly lower than other treatments. It is also noted that all zinnia plants irradiated at 800 Gy showed abnormal growth and subsequently died (Table 2).

In cosmos, the highest RSP was recorded at a radiation dose of 100 Gy, which was not significantly different from the control group (0 Gy). On the other hand, an irradiation dose of 400, 600, and 800 Gy resulted in significantly lower RSP values compared to the 0, 100, and 200 Gy treatments.

It is noted that the RSP of both plants decreased as the irradiation doses increased. To further establish the appropriate dose of gamma irradiation for mutation induction, the lethal dose for 50% mortality (LD_{50}) was determined using a linear regression graph plotted with radiation dose and RSP, as shown in Figure 1. The regression equations, coefficients of determination (R^2), and LD_{50} of both plants are listed in Table 3. In zinnia, the LD_{50} at 30 and 60 days after transplanting was 467.2 Gy and 459.6 Gy, respectively. In cosmos, the LD_{50} at 30 and 60 days after transplanting was 359.2 Gy and 345.5 Gy, respectively.

Table 2. Relative survival percentage of zinnia and cosmos due to different doses of gamma radiation at 30 and 60 days after transplanting

Dose (Gy)	Survival percentage (% relative to control)			
	Zinnia		Cosmos	
	30 days	60 days	30 days	60 days
0	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[ab]	100.00±0.00[ab]
100	98.85±1.99[a]	98.85±1.99[a]	113.41±27.22[a]	116.53±40.88[a]
200	101.15±1.99[a]	101.15±1.99[a]	78.50±26.55[b]	76.37±25.24[b]
400	79.69±6.30[b]	78.55±8.94[b]	19.91±3.96[c]	21.47±13.73[c]
600	15.67±4.90[c]	14.48±10.12[c]	3.51±3.04[c]	3.60±3.23[c]
800	2.30±3.98[d]	0.00±0.00[d]	1.71±2.96[c]	1.04±1.80[c]
F-test	**	**	**	**
C.V. (%)	64.35	66.53	92.95	95.46

[1/]**significant at p≤0.01

[2/]Mean±SD are shown. The different letter indicates significant difference by DMRT (p<0.05)

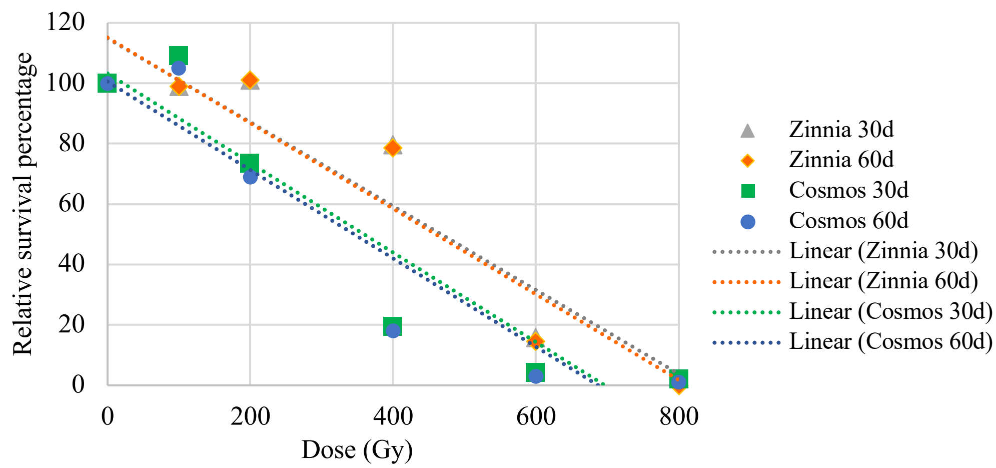

Figure 1. The linear graph shows the relationship between radiation doses and relative survival percentage (RSP) in zinnia and cosmos at 30 and 60 days after transplanting

Table 3. Regression equations, coefficients of determination (R^2), and lethal dose at 50% (LD_{50}) from the linear graph between radiation doses and relative survival percentage (RSP)

Plant	Days after transplanting	Equation	R^2	LD_{50} (Gy)
Zinnia	30	y = -0.139x + 114.89	0.893	467.2
	60	y = -0.142x + 115.13	0.897	459.6
Cosmos	30	y = -0.148x + 103.24	0.878	359.2
	60	y = -0.146x + 100.54	0.883	345.5

The number of shoots

To examine the effect of gamma radiation on plant vegetative growth, the number of shoots per stem was counted at 30 and 60 days after transplanting. The results indicated significant differences in the number of shoots among radiation doses in both plant species only at 30 days after transplanting (Table 4). A trend that higher radiation doses decreased the number of shoots was noted. At radiation doses of 400 and 600 Gy, the number of shoots was significantly lower than the control and lower dose treatments. Specifically, in zinnia, the average number of shoots at 400 and 600 Gy was 4.54 and 1.39, respectively, while in cosmos the average number of shoots at these doses was 5.20 and 3.00, respectively. At 800 Gy, no plants survived for data collection.

Table 4. Average number of shoots of zinnia and cosmos due to different doses of gamma radiation at 30 and 60 days after transplanting

Dose (Gy)	Shoot numbers			
	Zinnia		Cosmos	
	30 days	60 days	30 days	60 days
0	8.32±0.30^{a}	29.64±2.76	9.18±1.15^{a}	34.47±10.57
100	8.45±0.31^{a}	28.84±3.65	7.91±0.93^{ab}	20.54±4.64
200	7.56±1.13^{a}	26.07±1.10	7.20±0.91^{ab}	19.85±8.71
400	4.54±0.51^{b}	26.21±4.26	5.20±1.90^{bc}	18.91±6.29
600	1.39±0.35^{c}	20.47±11.94	3.00±2.12^{c}	16.75±10.96
800	ND	ND	ND	ND
F-test	**	ns	*	ns
C.V. (%)	47.40	23.24	35.69	42.46

[1/]**Significant at p≤0.01, *significant at p≤0.05, ND: not detected, ns: not significant
[2/]Mean±SD are shown. Different letters indicate a significant difference by DMRT (p<0.05)

Plant height

Plant height was another growth parameter measured in both species. It showed that plant height at 100 and 200 Gy was not significantly different from the control (Table 5). However, at 400 and 600 Gy, plant height was significantly reduced as compared to the control group, with 600 Gy, resulting in the shortest plants in both zinnia and cosmos. This highlighted the strong negative impact of high radiation doses on plant height. At 800 Gy, no plants survived for data collection.

Table 5. The plant height of zinnia and cosmos due to different doses of gamma radiation at 30 and 60 days after transplanting

Dose (Gy)	Height (cm)			
	Zinnia		Cosmos	
	30 days	60 days	30 days	60 days
0	33.73±0.86[a]	79.09±2.31[a]	34.61±6.35[a]	81.28±11.15[a]
100	32.25±6.18[a]	80.21±4.65[a]	28.09±3.86[ab]	69.76±10.30[ab]
200	30.37±6.45[a]	75.07±3.12[ab]	22.63±0.17[bc]	61.60±6.95[ab]
400	20.19±3.06[b]	64.57±6.74[b]	16.78±3.21[cd]	56.17±11.41[b]
600	7.12±2.24[c]	41.38±10.93[c]	10.35±4.17[d]	31.08±13.12[c]
800	ND	ND	ND	ND
F-test	**	**	**	*
C.V. (%)	44.42	23.33	38.75	29.31

[1/]**Significant at p≤0.01, *significant at p≤0.05, ND: not detected, ns: not significant
[2/]Mean±SD are shown. Different letters indicate a significant difference by DMRT (p<0.05)
[3/]ND: no data observed

Notably, results suggested a clear trend of decreasing plant height with increasing radiation dose in both species. The growth reduction by 50% (GR_{50}) of vegetative growth was determined to establish the adequate irradiation dose, in addition to LD_{50}, using a linear regression graph plotted with radiation dose and relative plant height, as shown in Figure 2. The regression equations, coefficients of determination (R^2), and GR_{50} values of both plants are listed in Table 6. In zinnia, the GR_{50} at 30 and 60 days after transplanting was 442.61Gy and 763.17 Gy, respectively. In cosmos, the GR_{50} at 30 and 60 days after transplanting was 404.82 Gy and 559.39 Gy, respectively.

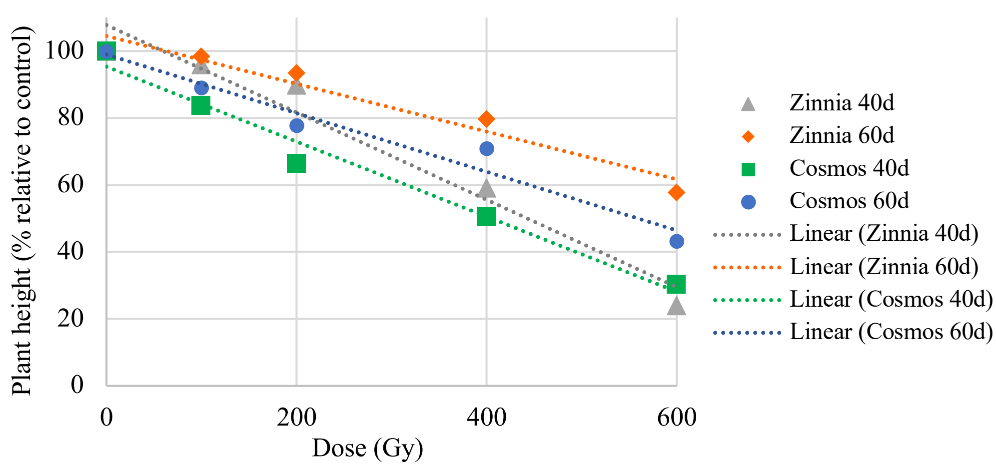

Figure 2. The linear graph shows the relationship between radiation doses and relative plant height in zinnia and cosmos at 30 and 60 days after transplanting

Table 6. Regression equations, coefficients of determination (R^2), and growth reduction by 50% (GR_{50}) from the linear graph between radiation doses and relative plant height

Plant	Days after transplanting	Equation	R^2	GR_{50} (Gy)
Zinnia	30	y = -0.131x + 107.76	0.958	442.61
	60	y = -0.071x + 104.49	0.949	763.17
Cosmos	30	y = -0.112x + 95.34	0.977	404.82
	60	y = -0.088x + 98.95	0.960	559.39

Floral development

To evaluate the effect of gamma radiation on floral development, the days to first flowering were recorded (Table 7). The data showed that in zinnia, the average time to first flowering at 600 Gy was significantly longer than the control and other radiation doses. This result indicates that higher gamma doses delayed flowering. Although no significant differences were observed in cosmos across the radiation doses, a similar trend was noted. Moreover, no cosmos flowers developed at 600 Gy. At 800 Gy, no plants survived for data collection.

Table 7. Number of days to first flowering of zinnia and cosmos due to different doses of gamma radiation

Dose (Gy)	Days to first flowering	
	Zinnia	Cosmos
0	43.7±0.17[b]	64.8±0.49
100	44.3±1.23[b]	66.3±3.55
200	44.6±2.02[b]	63.9±8.16
400	48.9±2.64[b]	69.6±1.99
600	64.3±5.85[a]	ND
800	ND	ND
F-test	**	ns
C.V. (%)	17.22	6.82

[1/]**Significant at $p \leq 0.01$, ND: not detected, ns: not significant
[2/]Mean±SD are shown. Different letters indicate a significant difference by DMRT ($p<0.05$)
[3/]ND: no data observed

In addition to flowering time, variations in the pattern and traits of zinnia and cosmos flowers were photographed, with representative images shown in Figure 3 and 4, respectively. Since the 'Mix' seeds were used for both species, a variety of flower colors were expected. The untreated flowers developed fully, with symmetrical, well-formed petals that expanded completely. While petal colors varied within the species, they were all vibrant and uniform. In contrast, the treated zinnia flowers showed discoloration and misshapen petals, with lighter color and irregular petal orientation. At higher doses of gamma radiation, some zinnia petals were severely degraded. A similar pattern was also observed in cosmos flowers, where abnormalities such as asymmetrical, narrow, discolored and curled petals were noted. Some cosmos flowers failed to develop at a dose of 200 Gy.

0 Gy 100 Gy 200 Gy 400 Gy

Figure 3. Representative photos of *Zinnia elegans* flowers at different gamma radiation doses

0 Gy 100 Gy 200 Gy 400 Gy

Figure 4. Representative photos of *Cosmos bipinnatus* flowers at different gamma radiation doses

Discussion

Knowing the appropriate radiation dose is crucial for effective mutation induction by gamma rays. Therefore, we tested the physiological effects of different gamma radiation doses on zinnia and cosmos. We found that the gamma radiation has a positive effect on the germination percentage only in zinnia, where 800 Gy resulted in the highest seed germination. The increase of seed germination by gamma radiation was previously reported in several plants such as in wishbone flower (*Torenia fournieri),* arjun tree *(Terminalia arjuna)* and sunflower *(Helianthus annuus* L.*)* (Maherchandani, 1975; Chandrashekar *et al*., 2013; Hussain *et al*., 2017). Gamma rays, which are short-wave photons, generally confer higher energy than light photons, resulting in strong damage to cells on the seed surface, such as the seed coat, and consequently leading to faster germination (Kovács and Keresztes, 2002; Aynehband and Afsharinafar, 2012).

Despite the improvement on seed germination, our results showed the opposite trend for survival rate, number of shoots, and plant height in both zinnia and cosmos. We found that the dose of 400 Gy negatively affected the survival rate of zinnia, while cosmos exhibited greater sensitivity to gamma radiation with its survival rate significantly dropping at a dose of 200 Gy. These findings align with previous studies on cosmos, indicating that ionizing radiation produces various effects on these plants, and cosmos are highly susceptible to gamma rays (Gupta and Samata, 1967; Sinurat *et al*., 2020). The effect of gamma rays on *Zinnia elegans* var. Dreamland was previously reported by Pallavi and collaborators, which they also found a significant decrease in survival percentage and plant height when compared to the control group (Pallavi *et al*., 2017). In the present study, we observed that the survival rate of both plants decreased as the radiation doses increased. Higher levels of radiation can interrupt cell division and elongation process as it can inactivate or reduce auxin accumulation, leading to poor plant establishment and survival (Mahure *et al*., 2010; Surakshitha *et al*., 2017). Thus, it is important to determine the LD_{50} threshold at which the greatest amount of mutation is induced in irradiated plants while minimizing the damage and losses. Since the LD_{50} and GR_{50} for mutation induction in *Zinnia elegans* and *Cosmos bipinnatus* has never been previously reported, we disclosed these parameters of both plants at 30 and 60 days after transplanting in this study.

Some vegetative growth traits such as number of shoots and plant height were significantly decreased with increasing radiation doses. This trend is in line with previous observations of gamma radiation on other ornamental plants such as lilium (Hajizadeh *et al*., 2022), rose (Kahrizi *et al*., 2011), chrysanthemum (Wu *et al*., 2019), jasmine (Ghosh, 2018) and roselle (El Sherif *et al*., 2011). Previous studies have focused on the effects of ionizing radiation on the physiological aspects of plants. Higher doses of radiation induce free radicals that can be harmful to morphology, physiology, and DNA structure of plant cells (Hajizadeh *et al*., 2022). Particularly, auxin biosynthesis is highly sensitive to radiation, which consequently has a major impact on mitotic division of meristematic tissues and directly affects plant height and other traits of vegetative growth (Gordon, 1957; Gupta and Samata, 1967). In addition, ionizing radiation induces cytological changes, including degeneration of nuclei, chromosomal changes, inhibition of cell division, and cell enlargement (Sparrow, 1961; Ghosh, 2018). A recent study on the effects of gamma rays on chrysanthemum reported that gamma rays induce chromosomal aberrations. Specifically, chromosome stickiness was observed in irradiated cells during metaphase I, and the percentage of these abnormal cells positively correlates with the radiation dose (Wu *et al*., 2019). It is also possible that plasma membrane and cytochrome enzymes are damaged by the process, leading to a decline of plant growth and development under intense radiation dose (Anandhi *et al*., 2013).

The floral development was assessed based on the observation of days to first flowering. As the radiation dose increased, the number of days to first flowering tended to increase, indicating a delay in flowering time. A previous study on *Cosmos caudatus* also reported the similar results, especially noting the absence of flowers in plants irradiated with high radiation doses (Sinurat *et al*., 2020). The effect of radiation on extending flowering period has been previously reported in several ornamental plants, including chrysanthemum (Bosila *et al.*, 2019; Wu *et al*., 2019), gladiolus (Patil and Dhaduk, 2009) and tulip (Li *et al*., 2022). Ionizing radiation might cause the alteration of many biosynthetic pathways associated with the flowering process and floral differentiation (Bosila *et al.*, 2019; Kovacs and Keresztes, 2022).

In this study, the LD_{50} of zinnia and cosmos was identified, and the effect of gamma radiation on their physiological aspects were assessed. These results are valuable resources for designing an effective breeding procedure. Since the ionizing radiation technique relies on random occurrences, it is important to select the optimum dosage to obtain the desired traits while minimizing other negative physiological effects on plants.

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