

Effects of gamma radiation (^{60}Co) on the *in vitro* mutation of the Son La ancient chrysanthemum variety (*Chrysanthemum* sp. “Cuc Son La”)

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ABSTRACT

The Son La ancient chrysanthemum, a woody-stemmed variety, is prized for pot cultivation and bonsai, reflecting the cultural significance of chrysanthemums as symbols of longevity. This study, conducted from February to November 2024 at Nong Lam University, Ho Chi Minh City, aimed to evaluate the impact of gamma radiation (^{60}Co) on the *in vitro* propagation and mutation induction of Son La ancient chrysanthemum plants, thereby identifying the optimal radiation dose for mutation breeding. The experiment was carried out using a completely randomized design with a two-factor setup: tissue type (shoot clusters, callus) and radiation dose (0, 20, 30 and 40 Gy). Results showed significant impacts on survival, growth, and mutation frequency. The LD_{50} (lethal dose, 50%) values at 50 days after irradiation (DAI) were 36.8 Gy for shoot clusters and 16.0 Gy for callus, indicating higher callus sensitivity. In shoot clusters, 20 Gy significantly stimulated growth (6.1 shoots, 6.2 cm height, 43.4 leaves), while 40 Gy severely inhibited it (2 shoots, 1.1 cm height, 8.9 leaves); callus survival sharply declined at 20 Gy (31.1%) and reached 0% at 30 and 40 Gy. A dose of 20 Gy induced diverse morphological mutations in shoot clusters (leaf color, shape, stem), while 40 Gy caused 100% yellowing and stunting; callus primarily showed chlorophyll loss (6.9%) and shoot regeneration failure (15.6%). Statistical analysis revealed a significant tissue type-dose interaction ($P < 0.01$). A dose of 20 Gy was optimal for mutation induction in shoot clusters, whereas callus being too sensitive. This study provides new LD_{50} data, tissue-specific radiation sensitivity, and mutation potential, recommending 20 Gy for future breeding studies focusing on shoot clusters, and suggesting further investigation into shoot cluster radiation tolerance.

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1. Introduction

In the agricultural industry, alongside staple crops, the flower-growing sector has gained attention for economic potential. Chrysanthemums, particularly the Son La ancient variety, are highly favored, ranking second in the global cut flower industry. The woody-stemmed Son La ancient chrysanthemum is ideal for pot cultivation and bonsai, aligning with current ornamental trends. However, the variety requires new strains with diverse flower colors, blooming periods, and adaptability to different climates to meet consumer demands. Therefore, development of the Son La ancient chrysanthemum should focus on rapid propagation and creating mutant lines with varied flower colors, stem shapes, and leaf characteristics to meet market needs and adapt to ecological conditions, especially in southern regions.

Among the methods of inducing mutations, radiation treatment is an effective approach that allows for changes in the genetic structure and promotes a high mutation frequency through irradiation. Specifically, the use of gamma rays from a ^{60}Co source has proven effective in generating plant lines with new traits (Beyaz et al., 2017). However, factors such as the type of specimen, dosage, and irradiation duration still need further research to optimize the mutation induction process. The combination of tissue culture techniques and gamma radiation mutation treatment offers promising prospects for improving chrysanthemum varieties and targeting new mutation traits.

2. Materials and Methods

2.1. Time and place of the study

The study was conducted from February to November 2024 at the tissue culture laboratory of the Department of Physiology and Biochemistry, Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam.

Gamma ray irradiation with a ^{60}Co source was performed at Ho Chi Minh City Biotechnology Center. The plant materials used in this study included shoot clusters with a diameter of approximately 1 cm and square-shaped callus samples with a side length of 0.5 cm.

2.2. Experimental design

The experiment had 2 two factors: tissue type factor (T): shoot clusters, callus and irradiation dose factor (I): 0, 20, 30 and 40 Gy. The experiment consisted of 8 treatments, with 3 replicates, arranged in a completely randomized design. Each treatment in a replicate had 2 petri dishes. Each petri dish had 10 samples.

2.3. Experimental method

The callus formation medium was MS (Murashige & Skoog, 1962) with 7 g/L agar, 30 g/L sucrose, 1.0 mg/L BA, and 0.1 mg/L NAA. The basic shoot multiplication medium was prepared using the same MS formulation, supplemented with 7 g/L agar, 30 g/L sucrose, and 1.5 mg/L BA, as identified in the previous experiments.

For *in vitro* shoot cluster samples: shoot clusters with a diameter of approximately 1 cm are cultured in petri dishes (10 cm in diameter; 2 cm in height), with each petri dish containing 20 mL of culture medium and 10 shoot clusters per

dish. The irradiation procedure was conducted at the Ho Chi Minh City Biotechnology Center. The samples were irradiated with gamma rays from a ^{60}Co gamma radiation source at a dose rate of 2.5 kGy/h. The non-irradiated treatment serves as the control. After 10 days of irradiation, the shoot clusters were transferred to the shoot multiplication medium.

For in vitro callus samples: leaves from 5-week-old in vitro plants cultured on MS medium are used, specifically the third and fourth leaves. These leaves are cut into square pieces (0.5 cm per side) and cultured in the optimal medium determined in previous experiment to induce callus formation. After 30 days, callus samples that are uniform in color are selected and cut into squares (0.5 cm per side). The samples were treated with irradiation as shoot cluster samples. After 10 days of irradiation, the calluses were transferred to the shoot multiplication medium.

2.4. Parameters

2.4.1. Determination of LD_{50} (lethal dose, 50%) value at 50 DAI (days after irradiation)

The damage rate of callus samples (%) was evaluated based on visual changes in color before and after irradiation. Observations were recorded every 10 days over a 50-day period. The survival rate of shoots and callus (%) was calculated as (number of surviving samples / total initial cultured samples) $\times$ 100, recorded at 10 and 50 DAI.

2.4.2. Growth parameters at 50 DAI

For shoot clusters, the number of shoots (shoot) was counted when they reached 0.5 cm in height. Shoot height (cm) was measured from the medium surface to the highest point of the

cluster, and the total number of leaves per plant (leaf) was calculated as (total number of leaves per shoot/total number of shoots surveyed). For callus tissue samples, the shoot regeneration rate (%) was determined as (number of samples regenerating shoots/total initial samples) $\times$ 100, and the number of shoots per sample (shoot) was calculated as (total number of shoots/the total number of samples with shoots).

2.4.3. Mutation parameters at 50 DAI

For shoot clusters, mutations in leaves and stems were observed, including leaf arrangement, shape, and color, as well as stem morphology and color. For callus tissue samples, mutations such as chlorophyll loss, failure to regenerate shoots, or death were recorded. The mutation frequency for both groups was calculated as (number of mutated individuals/total number of individuals) $\times$ 100.

2.5. Data processing and analyses

Data were processed with Excel 2019 (Microsoft, USA). R (version 4.4.2) was used to perform ANOVA and rank the treatments with Duncan's multiple range test at $\alpha = 0.01$ or $\alpha = 0.05$. The damage rate at 10 and 50 DAI, the survival rates of shoot clusters and calluses at 10 DAI, the shoot regeneration rate, and the number of shoots at 50 DAI were transformed using the formula $\sqrt{x+0.5}$ before statistical analysis. The survival rates of shoot clusters and calluses at 50 DAI were transformed using the formula $\arcsin\sqrt{x}$ before statistical analysis, the value 0 replaced with $1/4n$ and 100 replaced with $100 - 1/4n$ (with $n = 15$ samples) before the formula $\arcsin\sqrt{x}$ was applied (Gomez & Gomez, 1984).

3. Results and Discussion

Gamma rays, a type of high-energy electromagnetic radiation, have unique characteristics that allow them to penetrate plant tissues and induce genetic mutations. The biological effects are influenced by various factors, including the source, type of radiation, dose rate, absorbed dose, and the sensitivity of tissues and organs. Gamma rays have been successfully applied to various plant species, creating superior new mutations for cultivation. Gamma radiation has diverse effects on plants, and its impact depends on factors such as dosage, exposure time, and plant species. Gamma radiation can induce beneficial mutations in plants, leading to changes in growth, development, and physiological characteristics (Bharath et al., 2024).

3.1. Survey of LD₅₀ Value

3.1.1. Effects of ⁶⁰Co gamma radiation on the damage rate of callus samples

The results in Table 1 indicate that the damage rate in the calluses is directly proportional to the radiation dose. The higher the dose, the greater the damage rate, and the damage in the callus samples increased over time.

At 10 DAI, the 40 Gy dose resulted in the highest damage rate of 51.9%, which was statistically significant compared to the control treatment (0%) and the other treatments (Figure 1).

At 20 DAI, the 40 Gy dose still showed the highest damage rate at 58.8%, with no statistically significant difference compared to the 30 Gy dose (40.1%). However, the difference was statistically significant compared to the control treatment (0.5%) and the 20 Gy treatment (34.1%).

At 30 DAI, the 40 Gy radiation dose resulted in the highest damage rate of 83.3%, with no statistically significant difference compared to the 30 Gy radiation dose (68.5%), but a highly significant difference compared to the control (2.3%) and the 20 Gy dose (56.9%).

Table 1. Effects of ⁶⁰Co gamma radiation on the damage rate (%) of callus sample

Irradiation dose (Gy)	Observation time (DAI)				
	10	20	30	40	50
0 (Control)	0 ^c	0.5 ^c	2.3 ^c	2.3 ^c	2.6 ^c
20	15.3 ^c	34.1 ^b	56.9 ^b	73.8 ^b	95.7 ^b
30	34.2 ^b	40.1 ^{ab}	68.5 ^{ab}	90.7 ^{ab}	100 ^a
40	51.9 ^a	58.8 ^a	83.3 ^a	95 ^a	100 ^a
CV (%)	22.4	24.7	12.9	10.4	7.2
F value	40.4 ^{**}	26.0 ^{**}	80.9 ^{**}	119.3 ^{**}	249.3 ^{**}

Within the same group, mean values with the same letter following indicate no significant statistical difference.
**: significant difference with $P \leq 0.01$. DAI: days after irradiation.

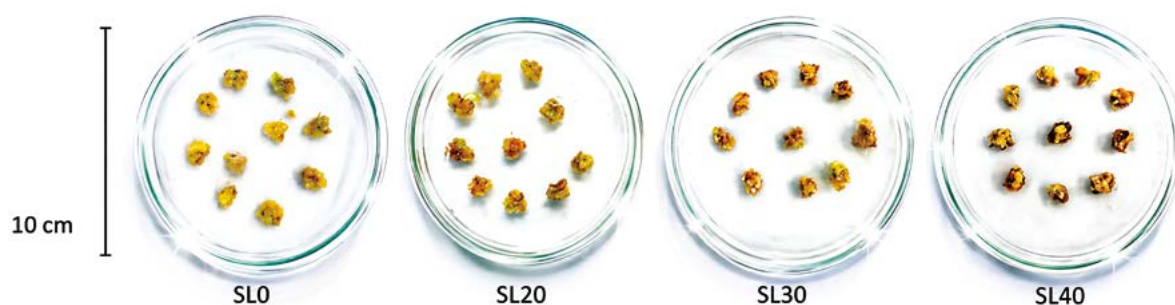


Figure 1. Callus samples at 10 days after irradiation following gamma radiation exposure.

SL: corresponding irradiation doses of 0, 20, 30, or 40 Gy for the callus samples.

At 40 DAI, the 40 Gy radiation dose resulted in the highest damage rate of 95%, with no statistically significant difference compared to the 30 Gy dose (90.7%), but a highly significant difference compared to the control (2.3%) and the 20 Gy dose (73.8%).

At 50 DAI, both the 40 Gy and 30 Gy doses resulted in the highest damage rate of 100%, with

a highly significant difference compared to the control (2.6%) and the 20 Gy dose (95.7%).

As the radiation dose increases, the damage rate of the samples increases. At 50 DAI, the damage rate reached 100% for the 40 and 30 Gy doses, with the samples completely devoid of life.

3.1.2. Effects of ^{60}Co gamma radiation on the survival rate of Son La ancient chrysanthemum variety

Table 2. Effects of ^{60}Co gamma radiation on the survival rate (%) of Son La ancient chrysanthemum variety

Observation time	Tissue type	Irradiation dose (Gy)				Average (T)
		0 (Control)	20	30	40	
10 DAI	Shoot cluster	100 ^a	100 ^a	100 ^a	100 ^a	100 ^A
	Callus	100 ^a	95.6 ^a	86.7 ^b	75.6 ^c	89.5 ^B
	Average (I)	100 ^A	97.8 ^A	93.4 ^{AB}	87.8 ^B	
CV (%) = 3.6%; $F_I = 4.1^*$; $F_T = 15.3^{**}$; $F_{I \times T} = 4.1^*$						
50 DAI	Shoot cluster	100 ^a	100 ^a	57.8 ^b	46.7 ^c	76.1 ^A
	Callus	100 ^a	31.1 ^d	0 ^e	0 ^e	32.8 ^B
	Average (I)	100 ^A	65.6 ^B	28.9 ^C	23.4 ^C	
CV (%) = 4.9%; $F_I = 1048.6^{**}$; $F_T = 1370.4^{**}$; $F_{I \times T} = 159.5^{**}$						

*Within the same group, the mean values with the same letter following indicate no statistically significant difference. *: significant difference at $P \leq 0.05$; **: significant difference at $P \leq 0.01$. DAI: days after irradiation.*

The results in Table 2 at 10 DAI indicate that, considering the tissue type factor, the survival rate of the Son La ancient chrysanthemum variety in shoot cluster samples was higher at 100%, with a statistically significant difference at $P < 0.05$ compared to callus samples (89.5%). Regarding the irradiation dose factor, the survival rate of the non-irradiated samples were the highest at 100%, showing a statistically significant difference at $P < 0.01$ compared to the survival rate at 40 Gy (87.8%). In terms of the interaction between tissue type and irradiation dose, the survival rate of the non-irradiated samples (control) and the shoot cluster samples irradiated at 20, 30, and 40 Gy remained at 100%, whereas the callus samples irradiated at 40 Gy had a significantly lower survival rate of only 75.6%, with a statistically significant difference at $P < 0.05$ (Figure 2).

At 50 DAI, regarding the tissue type factor, the survival rate of the Son La ancient chrysanthemum variety in shoot cluster samples was higher at 76.1%, showing a statistically significant difference at $P < 0.01$ compared to callus samples (32.8%). In terms of the irradiation dose factor, the non-irradiated samples showed the highest survival rate at 100%, with a statistically significant difference at $P < 0.01$ compared to the survival rate at 40 Gy (23.4%). Regarding the interaction between tissue type and irradiation dose, the survival rate of the non-irradiated samples (control) and the shoot cluster samples irradiated at 20 Gy remained at 100%, whereas the callus samples irradiated at 40 Gy had a significantly lower survival rate of only 23.4%, with a statistically significant difference at $P < 0.01$.

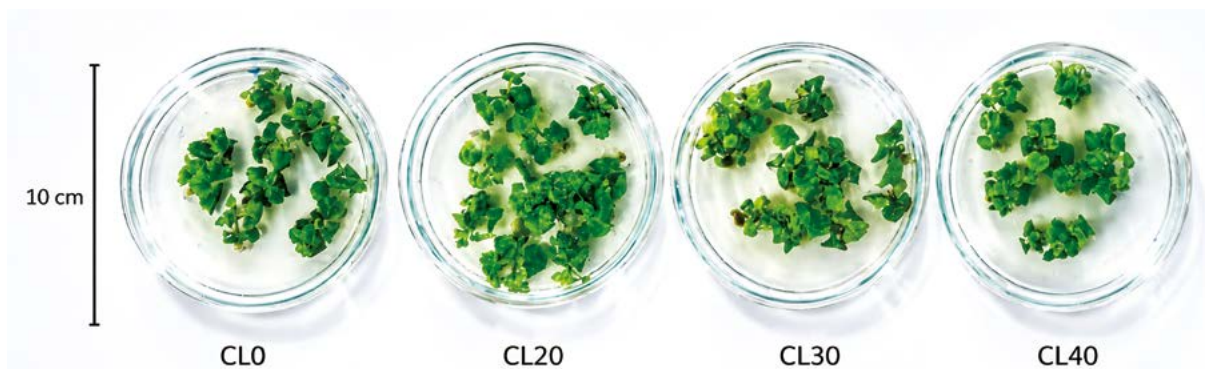


Figure 2. Shoot cluster samples at 10 days after irradiation following gamma radiation exposure.

CL: corresponding irradiation doses of 0, 20, 30, or 40 Gy for shoot clusters samples.

These findings highlight the dose-dependent sensitivity of callus tissue. At 10 DAI, survival gradually decreased with increasing radiation doses, whereas by 50 DAI, callus samples exposed to 30 and 40 Gy were completely nonviable, turning brown-black due to pigmentation loss. This confirms that the lethal irradiation dose for callus tissue is 30 Gy, whereas shoot clusters exhibit greater resistance to radiation stress.

Physiologically, high irradiation doses affect the cell wall and membrane, disrupting the exchange of water, minerals, and growth regulators (Le & Vu, 2021). When gamma irradiation doses increase, the mortality rate in chrysanthemum varieties can rise significantly.

Vucic et al. (2006) indicated that irradiation doses ranging from 20 to 50 Gy can cause high

mortality in plant individuals under *in vitro* conditions. The mortality rate can reach up to 100% at excessively high doses, rendering the plants unable to develop.

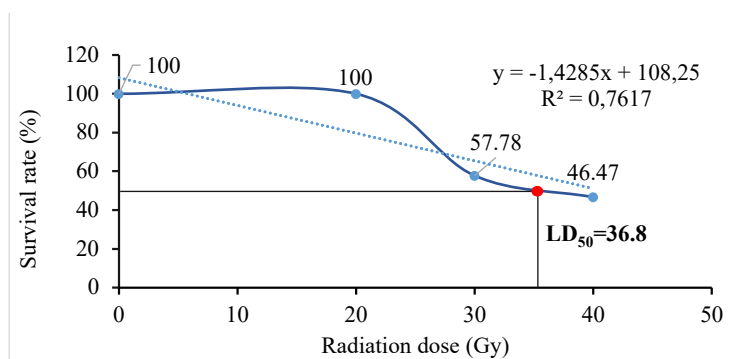


Figure 3. The correlation between survival rate and irradiation dose of shoot cluster samples at 50 days after irradiation.

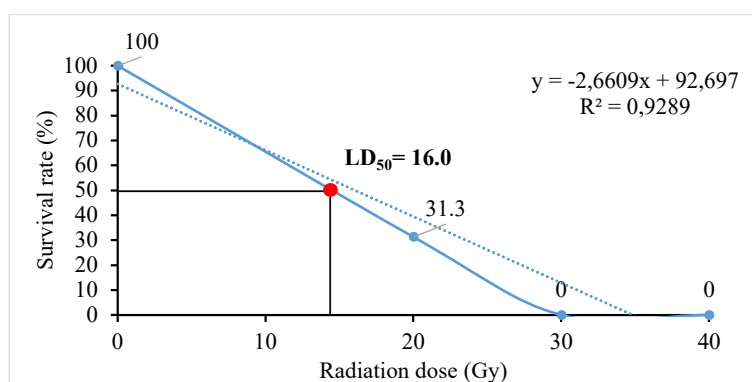


Figure 4. The correlation between survival rate and radiation dose of callus samples at 50 days after irradiation.

The results from Figures 3 and 4 allow for the determination of the LD_{50} values for Son La ancient chrysanthemum samples at 50 DAI. For the shoot cluster sample, the LD_{50} is 36.8 Gy, while for the callus sample, it is 16.0 Gy.

These experimental results confirm that the effects of gamma radiation vary depending on the plant species and the type of tissue. Determining

the LD_{50} value provides a basis for selecting the most effective radiation dose for inducing mutations, achieving a high mutation frequency while ensuring the survival and viability of the mutated plants meets the required standards.

3.2. The effect of gamma radiation from ^{60}Co source on the growth of Son La ancient chrysanthemum *in vitro*

Table 3. The effect of gamma radiation from ^{60}Co source on the growth of Son La chrysanthemum shoot clusters *in vitro* at 50 days after irradiation

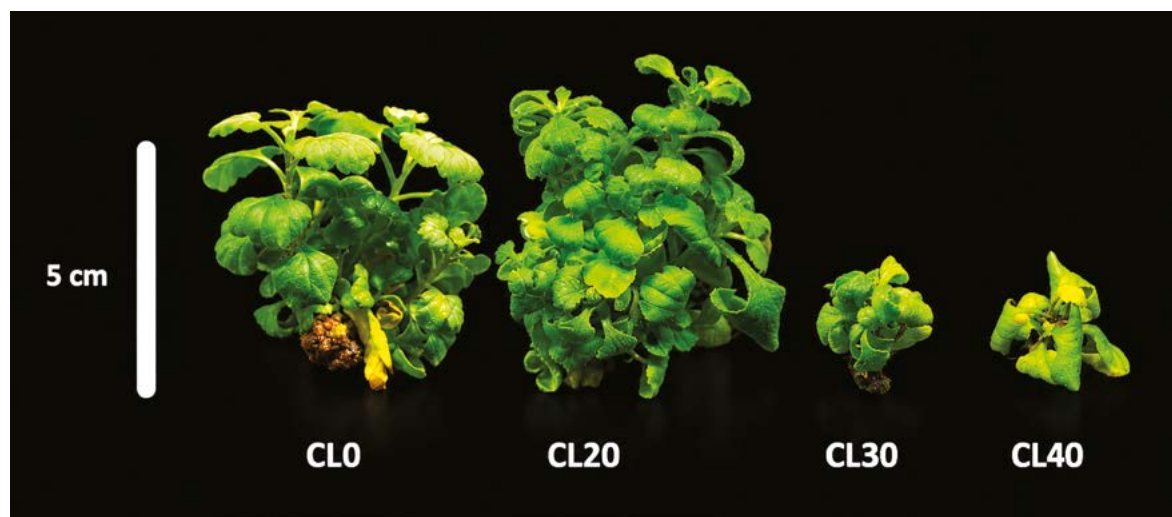
Irradiation dose (Gy)	Observed indicators		
	Number of shoots (shoots)	Shoot height (cm)	Total number of leaves (leaves)
0 (Control)	3.3 ^b	5.7 ^a	21.5 ^b
20	6.1 ^a	6.2 ^a	43.4 ^a
30	3.0 ^{bc}	1.7 ^c	11.7 ^c
40	2.0 ^c	1.1 ^c	8.9 ^c
CV (%)	10.6	17.6	13.7
F value	62.9 ^{**}	50.5 ^{**}	88.0 ^{**}

Within the same group, mean values followed by the same letter indicate no statistically significant difference.

^{**}: significant difference at $P \leq 0.01$.

From the results in Table 3, there was a highly significant difference in the number of shoots, shoot height, and number of leaves among the irradiation doses. The highest number of shoots was observed at the 20 Gy dose, reaching 6.1 shoots, which was statistically significantly different from the control (3.3 shoots) and the other treatments (Figure 5). The formation of new shoots is typically

influenced by growth regulators such as cytokinin and auxin in the culture medium. All treatments were affected by these substances to induce shoot proliferation. However, different irradiation doses led to variations in shoot numbers. In other words, irradiation doses caused damage to some lateral buds, affecting shoot formation (Nguyen et al., 2014).

**Figure 5.** Shoot cluster samples at 50 days after irradiation.

CL: corresponding irradiation doses of 0, 20, 30, or 40 Gy for shoot cluster samples.

Lal et al. (2024) indicated that low-dose irradiation (approximately 10 to 20 Gy) can significantly stimulate the growth of chrysanthemum varieties. Chrysanthemum plants irradiated at low doses generally exhibited better growth compared to the non-irradiated control group.

Regarding shoot height, an increase in irradiation dose resulted in a decrease in shoot height. The lowest shoot height was recorded at 40 Gy (1.1 cm) compared to the control (5.7 cm) and other treatments. The highest shoot height was 6.2 cm at the 20 Gy dose, which was not significantly different from the control (5.7 cm) but was highly significantly different from the 30 (1.7 cm) and 40 Gy (1.1 cm) doses. The variations in shoot height at different irradiation doses after 50 days indicate that irradiation caused cellular damage, affecting cell division and expansion, leading to reduced shoot height at higher irradiation doses.

The increase in the number of leaves per shoot also varied among the treatments. However, strong responses to high-dose irradiation resulted in morphological variations such as

smaller leaves and shorter internodes. The highest number of leaves was observed in the control (0 Gy), reaching 43.4 leaves at the 20 Gy dose, which was significantly different from the control (21.5 leaves) and other irradiation doses. Research by Wang et al. (2020) showed that as the irradiation dose increased (above 30 Gy), negative effects started to appear. Irradiation doses higher than 35 Gy significantly reduced shoot height and leaf number.

During *in vitro* culture after irradiation, shoot cluster samples exposed to 30 and 40 Gy doses suffered severe damage to the apical meristem due to radiation effects, preventing further development. At the 40 Gy dose, samples ceased growing and gradually died over time, showing almost no growth or new leaf formation. This confirms that irradiation at 30 and 40 Gy negatively affected the growth and development of Son La ancient chrysanthemum shoot clusters. However, the highest shoot growth was observed at the 20 Gy dose. Gamma irradiation at 20 Gy was found to be the optimal condition for promoting the development of Son La ancient chrysanthemum shoots, leading to stronger growth compared to higher irradiation doses.

Table 4. The effect of gamma radiation from ^{60}Co source on the shoot regeneration rate and number of shoots of callus samples at 50 days after irradiation

Irradiation dose (Gy)	Observed indicators	
	Shoot regeneration rate (%)	Number of shoots (shoots/sample)
0 (Control)	100 ^a	5.5 ^a
20	46.7 ^b	3.12 ^b
30	0 ^c	0 ^c
40	0 ^c	0 ^c
CV (%)	13.7	11.2
F value	163.5**	87.7**

Within the same group, mean values followed by the same letter indicate no statistically significant difference.

** : Significant difference at $P \leq 0.01$.



Figure 6. Callus samples at 50 days after irradiation.

SL: the corresponding radiation doses of 0, 20, 30, or 40 Gy for callus samples.

In plant cell culture, especially in callus culture, mutations often occur at a certain frequency. However, the mutation frequency in somatic cells during plant cell culture is typically very low (Le et al., 2012). Therefore, some scientists have used artificial mutation induction through gamma rays on callus material to increase the mutation frequency, yielding some promising results. This experiment also used callus as the starting material.

The results in Table 4 indicate that at 50 DAI, the highest shoot regeneration rate and number of shoots were observed in the control group, with statistically significant differences compared to the treatments with mutation induction. The 20 Gy irradiation dose resulted in a shoot regeneration rate of 46.7% and an average of 3.12 shoots, showing a highly significant difference compared to the 30 Gy and 40 Gy doses. Notably, no shoot regeneration was observed at 30 Gy and 40 Gy (Figure 6).

3.3. Effect of gamma radiation on the *in vitro* mutation potential of Son La ancient chrysanthemum *in vitro* at 50 DAI

Gamma radiation can cause significant changes in morphological characteristics such

as plant height, leaf area, and leaf number. These changes not only slow down plant growth but can also lead to defects in the structure of leaves and stems, affecting photosynthetic capacity and overall survival (Riviello-Flores et al., 2022).

During the observation, Table 5 indicated that at the 20 Gy irradiation dose, many morphological mutations were clearly expressed through color variations (dark green, light green), with less leaf lobing and tight internodes.

At 50 DAI, Son La chrysanthemum shoot clusters exhibited leaf and stem mutations at doses ranging from 20 to 40 Gy. Observations revealed differences in shape between the irradiated plants and the control plants, such as darker leaves, yellowing leaves, fewer lobes on the leaf edges, tight internodes, and lack of height growth. The frequency of leaf morphological mutations due to *in vitro* irradiation was relatively high, with leaf color being particularly sensitive to gamma rays, leading to various chlorophyll mutations within the 20 - 40 Gy dose range.

At the 20 Gy dose, several mutations were observed, particularly small leaves and tight internodes, with high frequencies (35.4 and 24.5%), along with other forms such as darker

leaves, lighter leaves, and stems with grooves at lower frequencies. The 30 Gy dose resulted in dark leaves and tight internodes with high frequencies (27.8 and 34.2%), but caused slower growth in terms of height, shoot number, and leaf number. The 40 Gy dose had a negative impact on growth potential, damaging the apical meristem and preventing the development of stems, leaves, and new shoots, while leaves turned yellow and no height growth occurred at 50 DAI.

Table 5. *In vitro* mutation patterns of shoot cluster samples at 50 days after irradiation

Irradiation dose (Gy)	Total number of shoots monitored	Mutation type	Number of mutated plants	Mutation frequency (%)
20	274	Dark green leaves	12	4.4
	274	Light green leaves	5	1.8
	274	Slightly lobed leaves	4	1.5
	274	Small leaves	97	35.4
	274	Compact internodes	67	24.5
	274	Grooved stem	2	0.7
30	79	Dark green leaves	3	3.8
	79	Light green leaves	0	0
	79	Slightly lobed leaves	2	2.5
	79	Small leaves	22	27.8
	79	Compact internodes	27	34.2
	79	Grooved stem	0	0
40	60	Yellow leaves	60	100
	60	Stunted height	60	100

For shoot cluster samples, gamma radiation with a ⁶⁰Co source at doses of 20, 30, and 40 Gy was suitable for creating a wide and diverse spectrum of mutations, with significant effects on the growth characteristics, morphological mutations, and color changes in various plant parts such as stems and leaves. However, at the 30 and 40 Gy doses, there was a relatively high mortality rate and a high rate of damage to the samples (Figures 7 and 8).

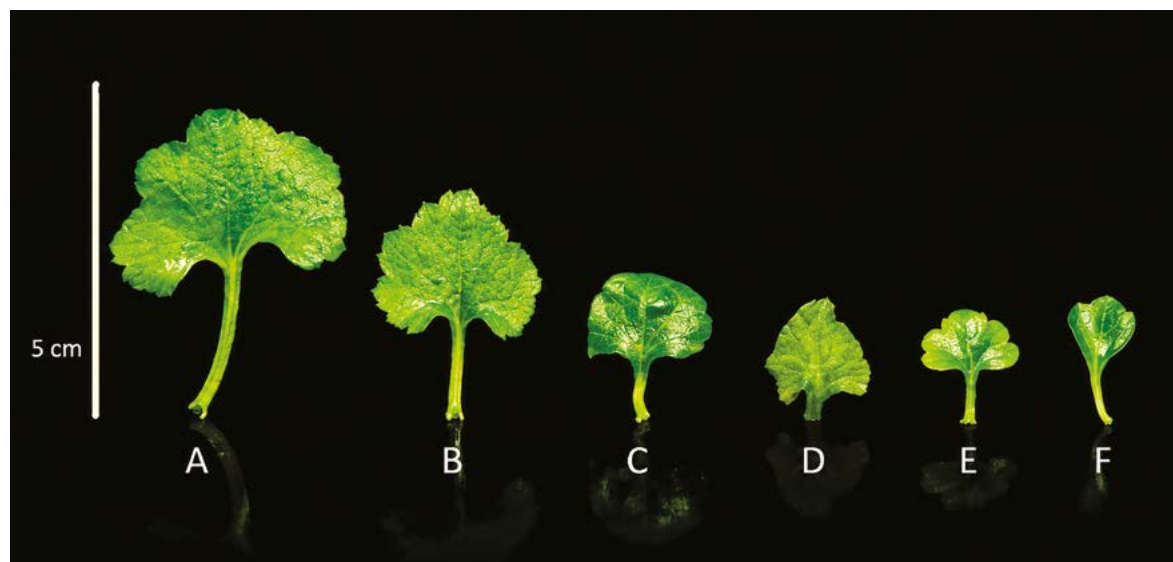


Figure 7. Some morphological leaf mutations observed in shoot cluster samples.

A) Control sample, B) Light-colored leaves, C) Yellow leaves, D) Dark-colored leaves, E) Small leaves, F) Leaves with few lobes.

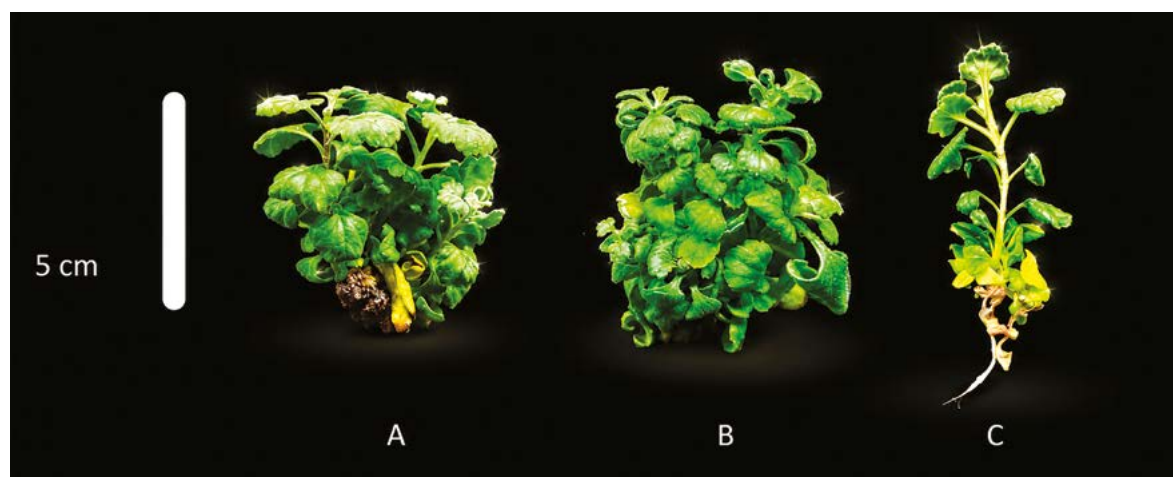


Figure 8. Some morphological stem mutations observed in shoot cluster samples.

A) Control sample, B) Tight internodes, C) Stem with grooves.

3.3.2. Effect of gamma radiation on the *in vitro* mutation potential of callus samples at 50 DAI

In plant cell culture, especially in callus culture, mutations often occur at a certain frequency. However, the mutation frequency in

somatic cells during plant cell culture is generally very low. Therefore, some scientists have used artificial mutation induction through gamma rays on callus material to increase the mutation frequency, yielding some promising results (Le & Vu, 2021).

Table 6. *In vitro* mutation potential of callus samples at 50 days after irradiation by a dose of 20 Gy

Total number of samples monitored	Mutation type	Number of mutated samples	Mutation frequency (%)
45	Callus regenerating shoots without chlorophyll	2	6.9
45	Callus failing to regenerate shoots	7	15.6
45	Callus regenerating normal shoots	5	11.1
45	Dead callus	31	68.9

In this experiment, as the irradiation dose increased from 20 to 40 Gy, the survival rate of the callus decreased over time. After 50 days of irradiation treatment, at doses ranging from 20 to 40 Gy, the callus lost its pigmentation, turning brown-black, and the survival rate of the callus was lower than that of the shoot samples at the same irradiation dose and cultivation time. This indicates that callus is more sensitive to irradiation intensity than shoot material. At the 20 Gy dose, color mutations in the shoots (loss of chlorophyll) appeared with a low frequency (6.9%). As shown in Table 6, the mortality rate of the callus was 68.9% of the total samples, with a higher percentage of non-regenerating callus (15.6%) compared to the normal shoot regenerating callus (11.1%), and the survival rate of the samples was low (31.1%). At the 30 and 40 Gy doses, the calluses died completely.

Many previous studies have also demonstrated the effects of gamma radiation from a ^{60}Co source on survival and growth in other plant species. According to Le et al. (2016), when high gamma doses were used, the survival rate of the callus decreased significantly. The negative effects of radiation can cause cellular damage and inhibit

cell division, leading to reduced survival ability of the tissue.

Therefore, it can be seen that for Son La ancient chrysanthemum, irradiation doses ranging from 20 to 40 Gy caused significant damage to the cells. While the 20 Gy dose induced chlorophyll mutations, the survival rate of the samples decreased significantly.

4. Conclusions

Gamma radiation is a valuable tool for inducing mutations in Son La ancient chrysanthemum, but its effectiveness is highly dependent on both the irradiation dose and the tissue type. The 20 Gy dose proved optimal for inducing a wide spectrum of mutations in shoot clusters while maintaining viable growth. Callus tissue exhibited extreme sensitivity to radiation, limiting its utility for mutation breeding in this variety. The significant interaction between tissue type and irradiation dose highlights the importance of considering these factors in future studies. The newly identified LD_{50} values, the documentation of specific mutation types, and the clear differences in radiation tolerance between

tissue types, provide essential information for developing new chrysanthemum varieties with enhanced ornamental characteristics and adaptability.

Conflict of interest

We certify that this is our own research work. The data and results presented in the study are genuine and have never been published in any other journal.

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