Determination of Median Lethal Dose and Effects of Gamma Radiation on Seed Germination in *Viola cornuta* L.

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Abstract

Effects of cobalt-60 gamma irradiation on seed germination and seedling survival of 7 cultivars of *Viola cornuta* L. were investigated. The results revealed that low doses of gamma radiation at 100 Gray (Gy) significantly stimulated germination in 'Deep Blue Blotch' and 'Clear Purple' and slightly enhanced germination in 'Clear Yellow' and 'Victoriana'. In contrast, higher doses (> 400 Gy) significantly decreased germination across all cultivars. No germination was observed at 1,000 Gy in the 'Deep Blue Blotch' cultivar. Analysis of mean germination time (MGT) and coefficient of velocity of germination (CVG) showed delays in germination and decreased germination velocity with increasing irradiation dose. The seedling survival percentage also decreased with higher radiation doses, indicating sublethal effects on seedling establishment. The lethal dose for 50 % mortality (LD_{50}) values ranged from 320 to 495 Gy for the Bel Viso series and from 430 to 640 Gy for the Grandissimo series. Our findings highlight the dose-dependent effects of gamma irradiation on seed germination and seedling survival and provide insights into irradiation tolerance and susceptibility among cultivars. Understanding these responses is crucial for optimizing irradiation-induced mutagenesis strategies and developing plant selection plans for crop improvement.

Keywords: Gamma radiation, Induction mutation, Mutagenesis, Radiation sensitivity, Seedling survival

Introduction

Viola cornuta L. belongs to the Violaceae family and is classified as an annual plant or subshrub. This family encompasses over 500 species [1], distributed across 20 genera [2], vary in size, growth habit and color. It is frequently used for flower bedding in gardening and landscaping. Besides the garden decoration, they represent one of the most popular edible flowers which has a refreshing taste and velvety texture. They can be used in fresh and dried form and usually added in salads, soups, drinks, desserts and more [1]. The consumption of edible flowers has significantly increased in recent years due to their value as functional food ingredients. Beside *Viola*, several species have been used and become increasing popular, for example, chrysanthemum, rose, pansy and calendula [3,4]. They enhance the visual appeal, distinctive aromas and exotic flavors of various food products and dishes, while also offering valuable nutrients and medicinal properties [5]. Edible flowers contain vital nutrients and phytochemical compounds, including high levels of antioxidants,

carbohydrate, proteins, vitamins and minerals [6,7]. For instance, a 100 g dry sample of *Viola×wittrockiana* contains approximately 80.27 g of carbohydrates, 10.14 g of crude protein, 1.67 g of fat and 7.92 g of ash [8]. Furthermore, 100 g fresh weight of *Viola × wittrockiana* with white and yellow flowers contained a higher protein content (more than 2.00 g) while the red species had the highest carbohydrate content (8.00 g); additionally, fatty acids such as linoleic acid (predominant), palmitic acid and linolenic acid were present in all cultivars [9]. Likewise, the *Viola betonicifolia* plant powder contains fats, proteins, carbohydrates, dietary fiber and vitamin C [10,11]. Edible flowers exhibit a stunning range of colors, including yellow, orange, red, white, purple and pink, owing to their natural pigments, which can be classified to 4 major classes: Carotenoids, chlorophylls, flavonoids or anthocyanins and betalains [12,13]. These flower pigmentations are rich in bioactive compounds. The red, dark red and dark purple color of flowers show higher concentrations of ascorbic acid, total carotenoid, total anthocyanins and total polyphenols than white and light-colored flowers [7,14]. Previous studies have highlighted the richness of *Viola* flower in flavonoid compounds, with violanthin identified as the major compound [15]. Due to their rich phytochemistry and nutritional composition, *Viola* can be employed in ethnomedicinal practices for treating noncommunicable diseases (NCDs) such as diabetes, asthma, lung disorders and fatigue. Numerous species within this genus have also undergone scientific validation for their pharmacological properties, including neuroprotective effects, immunomodulation, anticancer potential, antihypertensive activity, antidyslipidemic properties, analgesic effects, antipyretic action, diuretic properties, anti-inflammatory activity, anthelmintic effects and antioxidant benefits [16].

Gamma radiation serves as an effective method for inducing plant mutation and crop improvement by promoting genetic variation. Gamma radiation, which has the highest energy in the electromagnetic spectrum, is widely used as a mutagenic agent in plant mutation breeding. Previous research has demonstrated that gamma irradiation can successfully improve various plant species with new traits such as changes in flower color and form, leaf color and form, increased stress tolerance and elevated levels of secondary metabolites [17-20]. In crop improvement through radiation-induced mutation, it is crucial to identify the appropriate dose of radiation for a specific crop and cultivar. The radiation dosage should induce the maximum level of variability with the least negative effect. Low doses of radiation typically result in minimal genomic effects, rarely leading to observable phenotypic alterations. Conversely, high doses of radiation tend to induce numerous genomic impacts, consistently resulting in adverse changes [21,22]. Consequently, the primary step of radiation-induced mutation involves determining the optimum dose. Several studies indicated that the highest probability of creating the useful traits for mutation breeding typically occurs at doses where 50 % of the irradiated individuals die, known as the lethal dose 50 % (LD_{50}) [23-25]. Determining LD_{50} is the first step in applying induced mutation. LD_{50} varies depending on the species, varieties and part of plants. For example, exposure to gamma radiation 60 - 70 Gy is identified as the LD_{50} for axillary buds of *Rosa hybrida* 'Apollo' and 'Maroussia' [26], while exposure to gamma radiation 200 Gy is identified as the LD_{50} for cumin seed [27]. The LD_{50} of pea (*Pisum sativum* L.) is 200 Gy [28], for physic nut (*Jatropha crucas* L.) it is 600 Gy [21] and for perennial peanut (*Arachis pintoi* Var. Amarillo) it is 212 Gy [29]. Thus, establishing the LD_{50} for each species, cultivar and genotype is important, as it provides background information for developing plant selection plans and further breeding procedures.

Previous research has shown that when *Viola tricolor* L. flowers were exposed to different doses of cobalt-60 gamma radiation (1.17 and 1.33 MeV), the irradiated samples - especially those treated with 1,000 Gy - displayed the highest phenolic content and antioxidant activity [1]. However, as of now, there have been no reports on inducing mutations using gamma radiation and its effect on seed germination in *Viola* cultivars. The objective of this study is to investigate the effect of cobalt-60 gamma radiation on the germination of *Viola* seeds and to determine the appropriate median lethal dose (LD_{50}) for mutation induction in this species (*Viola cornuta* L.).

Materials and methods

Plant materials

Seven cultivars from 2 series of *Viola*, Bel Viso and Grandissimo, were selected for this study (**Figure 1**) and their natural agronomic traits (**Table 1**). Four cultivars from the Bel Viso series (Deep Blue Blotch, White Jump Up, Victoriana and Yellow) and 3 cultivars from the Grandissimo series (Clear Purple, Clear Yellow and Rose with Blotch) seeds were used. Seeds for all cultivars were purchased from Aga-agro Co. Ltd., Thailand.

| 'Victoriana' | 'Deep Blue Blotch' | 'White Jump Up' | 'Yellow' | 'Clear Yellow' | 'Clear Purple' | 'Rose with Blotch' |

Figure 1 Seven cultivars of *Viola* were used in this study [30].

Table 1 Characteristics of *Viola* plant of the Bel Viso and Grandissimo series.

Characteristics	Bel Viso	Grandissimo
Flower size (cm)	2.5 - 3.0	4.0 - 5.0
Plant height (cm)	10 - 13	15 - 20
Spread (cm)	10 - 15	12 - 15
Days from sowing to transplanting	25 - 30	25 - 30
Days from sowing to full bloom	90 - 100	90 - 95

Experimental design and gamma irradiation

The experiment was designed as a 7×7 factorial experiment in a completely randomized design (CRD) with 3 replicates. The treatment combinations comprised of 7 cultivars of *Viola* and 7 doses of gamma irradiation. The seeds underwent exposure to different acute irradiation doses: 100, 200, 400, 800 and 1,000 Gy. This precise irradiation process employed cobalt-60 gamma radiation within the controlled environment of the Gamma Chamber 5000 (BRIT, India), located at Thailand Institute of Nuclear Technology (TINT) in Ongkharak district of Nakhon Nayok province [31]. The dose rate during the experiment was 1.45 kGy/h. As a reference, non-irradiated seeds (0 Gy dose) served as control. For each dose, 3 replicates were tested, with 20 seeds per replicate.

Evaluation of seed germination

The irradiated and non-irradiated seeds were sown in 200-cell seedling trays containing moistened commercial substrate (Klasmann Potgrond H, Klasmann-Deilmann, Germany) and placed in greenhouse at Rajamangala University of Technology Tawan-ok, Chanthaburi campus. *Viola* seed germination was then evaluated 20 days after sowing. Seeds were considered germinated when their tiny hypocotyls or cotyledons emerged above the surface of the planting substrate, ensuring both accuracy and reproducibility. Daily counts of germinated seeds were recorded over a 20-day period to assess 3 key parameters: Germination percentage (GP), mean germination time (MGT) and the coefficient of velocity of germination (CVG).

GP was recorded 20 days after sowing. The formula for calculating germination percentage is shown in the Eq. (1) [32]:

$$\text{GP (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \tag{1}$$

MGT and CVG were calculated using Eqs. (2) and (3) [33,34], respectively:

$$MGT \ (days) = \frac{\sum (n_i d_i)}{\text{Total number of germinated seeds}} \qquad (2)$$

$$CVG = \frac{\text{Total number of germinated seeds}}{\sum (n_i d_i)} \times 100 \qquad (3)$$

where n = number of germinated seeds at day i and d = the day of counting.

Determination of relative survival percentage and median lethal dose (LD$_{50}$)

Relative survival is calculated as the ratio between the observed seedling survival proportion in a group of irradiated seeds and the seedling survival proportion in a comparable set of non-irradiated seeds (control group) from the same cultivar and under the same conditions. Seedlings were counted when their first or second leaves emerged above the surface of the planting medium. Relative survival percentage (RSP) was calculated at 30 days after sowing. The formula for calculating RSP is expressed in Eq. (4) [35]:

$$\text{Relative survival (\%)} = \frac{\text{Survival in treatment}}{\text{Survival in control}} \times 100 \qquad (4)$$

The numbers of surviving seedlings were recorded to calculate LD$_{50}$ from the regression graph plotted between radiation dose and relative survival percentage.

Statistical analysis

The data analysis was performed using R software. ANOVA was used to assess group differences and interactions between cultivar type and dose. The multiple comparison of means was performed using the Duncan's New Multiple Range Test (DMRT) at significance levels of $\alpha = 0.05$.

Results and discussion
Seed germination

Seeds from 7 distinct *Viola* cultivars underwent exposure to varying doses of gamma radiation to explore its effects on seed germination as measured by germination percentage (GP), mean germination time (MGT) and coefficient of velocity of germination (CVG). The results (summarized in **Tables 2 - 4** and illustrated in **Figure 2(A)**) indicated that at 20 days after sowing, the low doses enhanced GP in some cultivars, 'Victoriana', 'Deep Blue Blotch', 'Clear Yellow' and 'Clear Purple'. For each cultivar, dose effect was analyzed using ANOVA F-tests to assess the difference among groups. The irradiation with 100 Gy and 200 Gy of gamma radiation significantly enhanced the GP in the 'Deep Blue Blotch' and 'Clear Purple' cultivars, respectively. At 100 Gy, a slight, non-significant increase in GP for 'Victoriana', 'Clear Yellow' and 'Clear Purple' was observed. The exposure to high doses of gamma radiation resulted in a significant decrease in seed germination percentage in all cultivars of *Viola* except 'Victoriana'. At 1,000 Gy, there was no seed germination observed in 'Deep Blue Blotch' (**Table 2**). The stimulation effect on seed germination with the use of low doses of radiation has been reported in various plants such as cumin [27], sunflower [36], cucumber and okra [37]. Gamma irradiation with 100 - 150 Gy increased seed germination percentage in *Lathyrus chrysanthus* [38] and at 150 Gy in tomato seeds [39]. Low-dose irradiation is hypothesized to stimulate growth by altering the hormone signaling or increasing the capacity of cells to neutralize free radicals [40]. The enhanced germination trait observed in plant seeds exposed to low doses of gamma radiation may be attributed to the effects of radiation on gene regulation, hormone stimulation, activation of enzymes involved in the germination process and acceleration of DNA repair and cell division in meristematic tissues [41-43]. The effective radiation doses vary widely depending on the radiation sensitivity of genetic characteristics and seed moisture content [44]. The deleterious effects of high doses of gamma radiation on seed germination and plant growth and development were observed in several previous studies. For instance, in Pigeon pea [45], *Datura innoxia* L. or Pricklyburr [46], Creeping bentgrass [47] and fenugreek [48], high doses of gamma radiation were found to negatively affect seed germination percentage and growth as well as some biochemical constituents.

Mean germination time (MGT) is a measure of the average time taken for seeds to germinate, indicating the day when most seeds have sprouted. In other words, lower MGT values signify faster seed germination (**Table 3** and **Figure 2(B)**). Coefficient of velocity of germination (CVG) is an indicator of the rapidity of germination (**Table 4** and **Figure 2(C)**). A high value of CVG indicates that treatment has increased the germination percentage and reduced the time required to germinate. In this study, MGT increased significantly

for all samples under gamma irradiation except for 'Victoriana', where gamma irradiation had no significant effect on MGT. Meanwhile, CVG showed a significant decrease with increasing radiation dose in all cultivars. However, 'Victoriana' in the Bel Viso series did not exhibit a statistically significant difference from the control. These germination-related indicators suggested that the increase in radiation dose caused delayed seed germination in some cultivars of *Viola cornuta* L. Our results align with the previous observations in soybean and wheat [49-50]. Hong *et al.* [50] also reported a correlation between gamma radiation dose, exposure time, and free radical contents. They found that the level of free radicals in irradiated seeds increased linearly with higher radiation doses. Free radicals are reactive compounds that can damage cell components, including DNA, proteins and lipids [51], potentially causing physiological changes, including disrupting germination speed and reducing plant growth and development. The induction of free radical formation following high gamma radiation dose, leading to severe plant damage, has been previously reported [52].

Table 2 Germination percentage (GP) due to different doses (Unit: Gy) of gamma irradiation.

Dose	Bel Viso Series				Grandissimo Series		
	DB[1/]	VR[1/]	WJ[1/]	Y[1/]	CP[1/]	CY[1/]	RB[1/]
0	80.00 ± 0.00^b	55.00 ± 21.79	53.33 ± 5.77^a	90.00 ± 5.00^a	51.67 ± 7.64^{bc}	48.33 ± 7.64^a	91.67 ± 2.89^a
100	93.33 ± 5.77^a	68.33 ± 15.28	50.00 ± 21.79^{ab}	85.00 ± 5.00^{ab}	63.33 ± 16.07^{ab}	65.00 ± 5.00^a	66.67 ± 15.28^{abc}
200	86.67 ± 12.58^{ab}	50.00 ± 17.32	26.67 ± 12.58^{bcd}	76.67 ± 5.77^{ab}	76.67 ± 10.41^a	55.00 ± 15.00^a	71.67 ± 10.41^{ab}
400	65.00 ± 8.66^c	56.67 ± 10.41	31.67 ± 15.28^{abcd}	70.00 ± 8.66^b	70.00 ± 10.00^{ab}	58.33 ± 17.56^b	50.00 ± 0.00^{bcd}
600	5.00 ± 5.00^d	41.67 ± 10.41	38.33 ± 10.41^{abc}	45.00 ± 8.66^c	38.33 ± 10.41^{cd}	23.33 ± 12.58^b	46.67 ± 23.63^{bcd}
800	3.33 ± 2.89^d	30.00 ± 8.66	21.67 ± 7.64^{cd}	38.33 ± 16.07^c	21.67 ± 7.64^d	18.33 ± 15.28^b	41.67 ± 20.82^{cd}
1,000	0.00 ± 0.00^d	40.00 ± 8.66	13.33 ± 7.64^d	43.33 ± 10.41^c	23.33 ± 10.41^d	18.33 ± 7.64^b	30.00 ± 15.00^d
F-test	**	Ns	**	**	**	**	**

[1/]**Significant at $p \leq 0.01$, ns: Not significant, Mean ± SD are shown. In each column, different letters indicate a significant difference by DMRT ($p < 0.05$). Cultivar abbreviations: CP = Clear Purple, CY = Clear Yellow, DB = Deep Blue Blotch, RB = Rose with Blotch, VR = Victoriana, WJ = White Jump Up and Y = Yellow.

Table 3 Mean germination time (MGT) (Unit: Day) due to different doses (Unit: Gy) of gamma irradiation.

Dose	Bel Viso Series				Grandissimo Series		
	DB[1/]	VR[1/]	WJ[1/]	Y[1/]	CP[1/]	CY[1/]	RB[1/]
0	7.71 ± 0.04^c	7.41 ± 0.65	7.34 ± 0.47^c	8.00 ± 0.92^c	7.63 ± 0.26^c	8.11 ± 0.57^c	6.84 ± 0.01^d
100	7.69 ± 0.34^c	7.20 ± 0.73	7.72 ± 1.04^c	9.07 ± 0.88^c	7.07 ± 0.24^c	8.12 ± 0.77^c	7.35 ± 0.51^{cd}
200	8.30 ± 0.35^c	7.97 ± 1.76	8.35 ± 1.15^{bc}	8.76 ± 0.58^c	7.82 ± 0.58^{bc}	8.89 ± 0.45^{bc}	7.95 ± 0.04^{bc}
400	10.10 ± 0.59^{bc}	8.67 ± 0.96	8.69 ± 0.65^{abc}	11.04 ± 1.13^b	8.83 ± 0.72^b	9.93 ± 0.22^b	8.57 ± 0.59^b
600	13.00 ± 2.83^a	8.66 ± 0.48	10.77 ± 1.20^a	11.47 ± 1.60^{ab}	10.57 ± 0.75^a	12.38 ± 1.12^a	10.43 ± 0.86^a
800	11.00 ± 2.83^{ab}	8.16 ± 1.57	9.92 ± 1.84^{ab}	12.68 ± 0.25^{ab}	11.17 ± 1.04^a	10.30 ± 1.29^b	10.34 ± 0.66^a
1,000	ND	8.89 ± 0.10	9.19 ± 0.88^{abc}	12.92 ± 0.69^a	11.65 ± 0.28^a	12.85 ± 1.26^a	10.81 ± 0.79^a
F-test	**	Ns	**	**	**	**	**

[1/]**Significant at $p \leq 0.01$, ns: Not significant, Mean ± SD are shown. In each column, different letters indicate a significant difference by DMRT ($p < 0.05$), ND: No data observe. Cultivar abbreviations: CP = Clear Purple, CY = Clear Yellow, DB = Deep Blue Blotch, RB = Rose with Blotch, VR = Victoriana, WJ = White Jump Up and Y = Yellow.

Table 4 Coefficient of velocity of germination (CVG) due to different doses (Unit: Gy) of gamma irradiation.

Dose	Bel Viso Series				Grandissimo Series		
	DB[1/]	VR[1/]	WJ[1/]	Y[1/]	CP[1/]	CY[1/]	RB[1/]
0	12.97 ± 0.06^a	13.55 ± 1.14	13.65 ± 0.86^a	12.61 ± 1.36^a	13.12 ± 0.45^a	12.37 ± 0.91^a	14.63 ± 0.01^a
100	13.01 ± 0.58^a	13.99 ± 1.50	13.12 ± 1.87^{ab}	11.09 ± 1.04^a	14.16 ± 0.48^a	12.38 ± 1.11^a	13.66 ± 0.97^{ab}
200	12.07 ± 0.50^a	12.91 ± 2.53	12.12 ± 1.62^{abc}	11.44 ± 0.76^a	12.84 ± 0.99^a	11.26 ± 0.59^{ab}	12.58 ± 0.07^{bc}
400	9.92 ± 0.60^b	11.62 ± 1.21	11.55 ± 0.88^{abcd}	9.12 ± 0.98^b	11.38 ± 0.97^b	10.08 ± 0.22^b	11.71 ± 0.83^c
600	7.88 ± 1.71^b	11.57 ± 0.63	9.36 ± 1.04^d	8.83 ± 1.16^b	9.50 ± 0.70^c	8.13 ± 0.75^c	9.64 ± 0.83^d
800	9.40 ± 2.42^b	12.55 ± 2.30	10.30 ± 1.77^{cd}	7.89 ± 0.16^b	9.01 ± 0.88^c	9.81 ± 1.24^b	9.70 ± 0.60^d
1,000	ND	11.26 ± 0.13	10.95 ± 1.08^{bcd}	7.75 ± 0.40^b	8.59 ± 0.21^c	7.83 ± 0.74^c	9.28 ± 0.66^d
F-test	**	ns	**	**	**	**	**

[1/]**Significant at $p \leq 0.01$, ns: Not significant, Mean $\pm$ SD are shown. In each column, different letters indicate a significant difference by DMRT ($p < 0.05$), ND: No data observe. Cultivar abbreviations: CP = Clear Purple, CY = Clear Yellow, DB = Deep Blue Blotch, RB = Rose with Blotch, VR = Victoriana, WJ = White Jump Up and Y = Yellow.
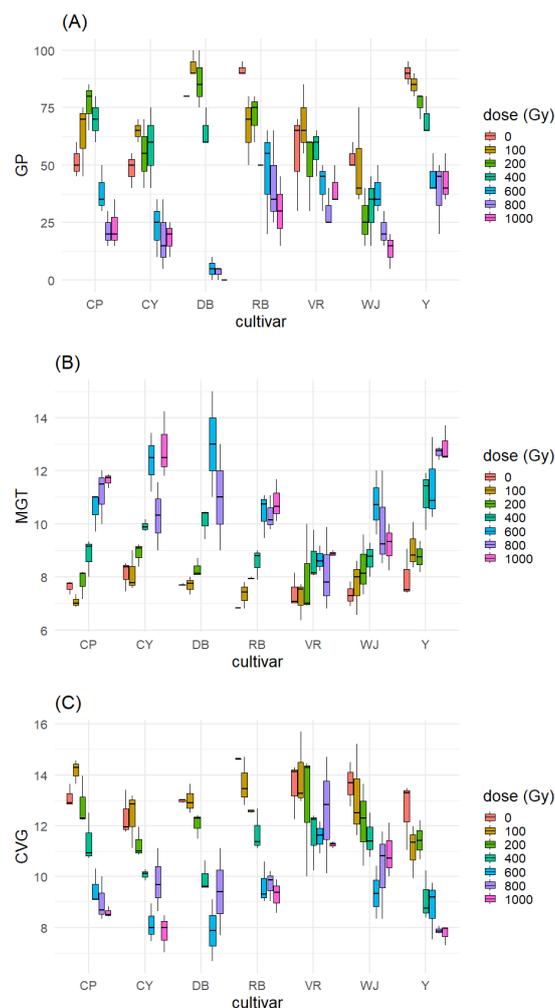

Figure 2 Box distribution plots of germination parameters (A) GP, (B) MGT and (C) CVG, of *Viola* plants. Cultivar abbreviations are arranged alphabetically: CP = Clear Purple, CY = Clear Yellow, DB = Deep Blue Blotch, RB = Rose with Blotch, VR = Victoriana, WJ = White Jump Up and Y = Yellow.

Seedling survival percentage

The relative survival percentage (RSP) of *Viola* seedlings was observed 30 days after sowing, as presented in **Table 4**. For each cultivar, the differences among dose groups were analyzed using ANOVA F-tests. The findings showed that low doses of gamma radiation could slightly enhance RSP in some cultivars, such as 'Victoriana', 'Deep Blue Blotch', 'Clear Yellow' and 'Clear Purple,' but there was no significant difference compared to non-irradiation treatment. The survival percentage decreased with increasing gamma radiation dose. Comparing the results between seed germination percentage and seedling survival percentage, it was observed that some seeds were able to germinate but failed to develop to seedlings. RSP at higher doses of gamma radiation decreased or dropped to 0 in some cultivars, such as 'Victoriana', 'Deep Blue Blotch', 'White Jump Up', 'Yellow' and 'Rose with Blotch'. **Figure 3** shows the representative pictures of *Viola* seedlings germinated from irradiated seeds at varying doses (**Figure 3(A)**). It is noted that a few irradiated plants at high doses (e.g. 400 Gy) could only develop to the cotyledon stage (**Figure 3(B)**) and subsequently died. At high doses of gamma radiation, particularly at 600 Gy and above, RSP significantly

decreased, reaching zero at 1,000 Gy in 5 cultivars (e.g., 'Victoriana', 'Deep Blue Blotch', White Jump Up', 'Yellow' and 'Rose with Blotch). In contrast, 'Clear Yellow' and 'Clear Purple' exhibited RSP values of 7.50 ± 6.61 and 3.33 ± 5.77, respectively. The lower survival rates at higher radiation doses are consistent with previous reports in several ornamental plants, including rose [53], pigeon pea [54], jasmine [55], chrysanthemum [56] and wheat [57]. The reduction in RSP likely results from the cellular damage caused by high radiation doses [58]. Several studies suggest that ionizing radiation induces cytological changes, such as nucleus degeneration, chromosomal alterations, inhibition of cell division and cell enlargement, resulting in poor plant establishment and survival [55,59]. For instance, gamma rays have been shown to induce chromosomal aberrations in chrysanthemums, particularly observed as chromosome stickiness during metaphase I, with the percentage of abnormal cells positively correlating with radiation doses [56]. Furthermore, high radiation doses can damage plasma membranes and cytochrome enzymes. High radiation doses may also increase DNA damage, free radical content and lipid peroxidation, inhibiting the plant growth and development [52,60].

Table 4 Relative survival percentage of 7 *Viola* cultivars due to different doses of gamma irradiation at 30 days after sowing.

Dose	Bel Viso Series				Grandissimo Series		
	DB[1/]	VR[1/]	WJ[1/]	Y[1/]	CP[1/]	CY[1/]	RB[1/]
0	100.00 ± 0.00^{ab}	100.00 ± 0.00^{ab}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
100	104.64 ± 10.32^{a}	129.70 ± 62.60^{a}	90.91 ± 31.49^{a}	60.05 ± 29.26^{b}	123.89 ± 46.14^{ab}	133.03 ± 21.33^{a}	71.30 ± 14.25^{bc}
200	87.15 ± 1.30^{b}	91.82 ± 53.27^{ab}	33.33 ± 22.88^{b}	89.91 ± 3.05^{a}	145.37 ± 34.95^{a}	116.36 ± 20.97^{a}	83.80 ± 26.58^{ab}
400	45.03 ± 17.47^{c}	59.55 ± 17.86^{bc}	33.33 ± 13.89^{b}	39.03 ± 5.28^{c}	121.48 ± 11.18^{ab}	105.76 ± 19.29^{a}	53.94 ± 14.03^{c}
600	4.04 ± 3.51^{d}	12.45 ± 5.76^{c}	9.09 ± 9.09^{bc}	0.00 ± 0.00^{d}	20.56 ± 12.51^{c}	30.98 ± 18.60^{b}	20.14 ± 12.56^{d}
800	2.38 ± 4.12^{d}	4.17 ± 7.22^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	15.37 ± 8.36^{c}	27.20 ± 30.58^{b}	15.28 ± 2.41^{d}
1,000	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	3.33 ± 5.77^{c}	7.50 ± 6.61^{b}	0.00 ± 0.00^{d}
F-test	**	**	**	**	**	**	**

[1/]**Significant at $p \leq 0.01$, Mean $\pm$ SD are shown. In each column, different letters indicate a significant difference by DMRT ($p < 0.05$). Cultivar abbreviations: CP = Clear Purple, CY = Clear Yellow, DB = Deep Blue Blotch, RB = Rose with Blotch, VR = Victoriana, WJ = White Jump Up and Y = Yellow.

Figure 3 *Viola* plants in a seedling tray at 30 days after sowing (A) and some plants with only cotyledons (no leaves) (B).

To investigate the relationship between cultivar and dose, a 2-way ANOVA analysis was conducted, with the results shown in **Table 3**. The results indicated that there were significant interactions between cultivar and dose factors for GP and MGT, with *p*-values less than 0.01 and 0.05, respectively. However, there was no interaction for CVG, with *p*-value greater than 0.05 (**Table 5**).

Table 5 Two-way analysis of variance (ANOVA) table of 2 factors (cultivar and dose) and interaction (cultivar×dose) for germination parameters and relative survival percentage. (Abbreviations: df = degree of freedom and MS = mean sum of squares).

Source	df	MS	*F-ratio*	*p*-value[1]
Germination percentage (GP)				
Cultivar	6	2076.5	14.8898	< 0.0001**
Dose	6	8671.7	62.1825	< 0.0001**
Interaction	36	559.3	4.0109	< 0.0001**
Error	98	139.5		
Mean germination time (MGT)				
Cultivar	6	13.725	15.3584	< 0.0001**
Dose	6	45.940	51.4086	< 0.0001**
Interaction	35	1.629	1.8235	0.01172*
Error	94	0.894		
Coefficient of velocity of germination (CVG)				
Cultivar	6	16.601	14.630	< 0.0001**
Dose	6	60.658	53.456	< 0.0001**
Interaction	35	1.426	1.257	0.1922ns
Error	94	1.135		
Relative survival percentage (RSP)				
Cultivar	6	4732	12.9653	< 0.0001**
Dose	6	43364	118.8182	< 0.0001**
Interaction	36	918	2.5151	0.0002**
Error	98	365		

[1]**Significant at $p \leq 0.01$, *significant at $p \leq 0.05$, ns: Not significant.

Lethal dose for 50 % mortality (LD$_{50}$) determination

To determine the appropriate dose of gamma irradiation, the lethal dose for 50 % mortality (LD$_{50}$) was calculated using the linear regression graph plotted between irradiation dose and relative survival percentage, as shown in **Figure 4**. The median lethal dose is a parameter used for establishing the appropriate radiation dose to induce mutations and allow the selection for plants with new traits that are not found in nature [24]. Due to the effect of irradiation on plant viability varies, depending on the type and dose of radiation, species and part of plant.
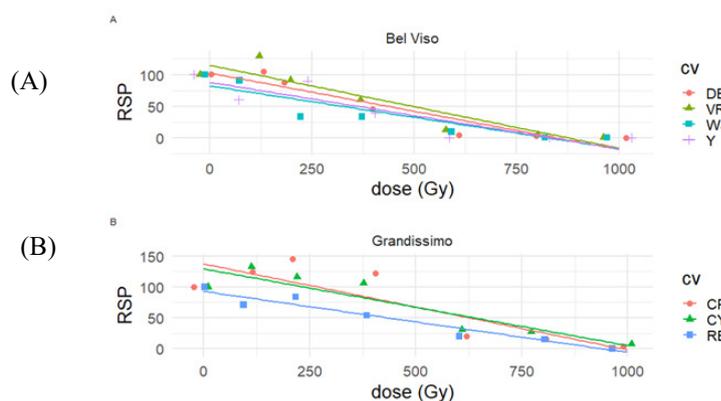
Figure 4 Relative survival percentages (RSP) in *Viola* cultivars, (A) Bel Viso series and (B) Grandissimo series. Cultivar abbreviations: CP = Clear Purple, CY = Clear Yellow, DB = Deep Blue Blotch, RB = Rose with Blotch, VR = Victoriana, WJ = White Jump Up and Y = Yellow. (The data points have a small number of horizontal offsets applied, to avoid overlaps.)

The linear regression equations, coefficients of determination (R^2), and median lethal dose (LD$_{50}$) are presented in **Table 6**. For the Bel Viso series, the LD$_{50}$ ranged from 320 to 495 Gy, whereas for the Grandissimo series, the LD$_{50}$ ranged from 430 to 640 Gy. The results indicate that LD$_{50}$ of *Viola* depends on the cultivar, as different cultivars exhibit different levels of sensitivity to gamma radiation. The Bel Viso series tended to be more radiosensitive than the Grandissimo series. This observation aligns with previous studies on other plants, such as watermelon [61], artichoke [62] and mung bean [63]. The variation in radiosensitivity among different cultivars may result from differences in seed composition [63]. Seeds with higher water content tend to be more sensitive to ionizing radiation due to the increased likelihood of gamma rays interacting with water molecules, leading to the formation of free radicals [64]. Representative pictures of *Viola* plants in **Figure 5** show that irradiated plants have a stunted appearance, asymmetric and misshapen leaves, further supporting the claim that different cultivars respond differently to gamma radiation.

Table 6 Linear regression equations, coefficients of determination (R^2) and lethal dose at 50% (LD$_{50}$).

Series	Cultivar	Equation	R^2	LD$_{50}$ (Gy)
Bel Viso	Victoriana	y = −0.132x + 115.086	0.885	494.62
	Deep Blue Blotch	y = −0.122x + 102.872	0.899	434.92
	White Jump Up	y = −0.100x + 82.294	0.801	323.58
	Yellow	y = −0.106x + 88.037	0.827	360.29
Grandissimo	Clear Yellow	y = −0.124x + 129.383	0.836	639.44
	Clear Purple	y = −0.139x + 137.448	0.747	627.32
	Rose with Blotch	y = −0.099x + 92.993	0.940	434.83

Figure 5 Representative images of *Viola* plants at 68 days after transplanting to pots. (A) 'Yellow' plant grown from non-irradiated seeds, (B) 'Victoriana' plant grown from seeds irradiated at 400 Gy and (C) 'Yellow' plant grown from seeds irradiated at 400 Gy.

Conclusions

In this study, the effect of varying gamma radiation doses on germination and survival of *Viola cornuta* was investigated. Low radiation doses slightly induced seed germination in some cultivars of *Viola* ('Deep Blue Blotch', 'Victoriana', 'Clear Purple' and 'Clear Yellow'), while higher doses reduced germination percentages. The mean germination time (MGT) increased and the coefficient of velocity of germination (CVG) decreased for all 7 cultivars, indicating delayed seed germination in a dose-dependent manner. Seedling survival percentages declined with higher radiation doses, suggesting negative physiological changes caused by the gamma rays. The LD_{50} of 7 cultivars of *Viola cornuta* L. were calculated using relative survival percentage, revealing the variation in radio-sensitivity among different cultivars of *Viola cornuta* L. These findings enhance our understanding of radiation sensitivity in different *Viola* cultivars, which could be valuable for future mutation breeding programs and open up exciting avenues for further research into underlying mechanisms, exploring radiation tolerance, studying genetic variation and conducting field experiments that may yield practical insights.

Acknowledgements

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