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Effect of gamma rays on vegetative growth and biochemical composition of chlorophyll and carotenoid in vM$_1$ generation of chrysanthemum (*Dendranthema grandiflora* Tzvelve)

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Abstract

Gamma irradiation stimulates a broad range of change in physiology and biochemical alterations in plants by modulating specific defensive and metabolic pathways. Pre-planting cutting irradiation is deliberated as an effective method of increasing production, yield and chemical compositions in plants. In the current study, two varieties of *Dendranthema grandiflora* Tzelev (Chrysanthemum), namely Lalpari and DFRC-3, were subjected to gamma irradiation with absorbed doses of 10 Gy, 15 Gy, 20 Gy, 25 Gy, 30 Gy, and 35 Gy, along with a control group. Exposure to these varying doses of gamma radiation resulted in consistent changes in the growth and development of the plants. The current study examines the vegetative growth and biochemical composition of chlorophyll and carotenoids in both irradiated and non-irradiated plants of the vM1 generation. The results of the study indicate that gamma irradiation has a significant effect on vegetative growth, chlorophyll and carotenoid content in the vM$_1$ generation of chrysanthemum. The data reveal that vegetative growth, chlorophyll, and carotenoid content decrease significantly in the first generation after gamma irradiation compared to the control group. The highest plant height, number of leaves, and levels of chlorophyll (chl a, chl b, and total chl) and carotenoids were recorded in non-irradiated plants for both varieties. Among the gamma-irradiated plants, those exposed to 10 Gy exhibited the highest vegetative growth, chlorophyll, and carotenoid content, while those exposed to 30 Gy showed the lowest levels in both varieties. This indicates that vegetative growth, chlorophyll, and carotenoid content decrease with increasing gamma doses. Exposure to the higher dose of 35 Gy of gamma rays resulted in plant mortality, with no plants surviving at this dose in both varieties. The biochemical analysis confirms that photosynthetic components are highly responsive to gamma irradiation and serve as a good indicator of persistence, providing substantial evidence of the effects of gamma irradiation. Therefore, gamma rays show promise as a key tool for enhancing breeding efficiency and increasing the frequency of regeneration.

Keywords: Chrysanthemum, chlorophylls, carotenoid content, genetic variability, gamma irradiation

Introduction

Chrysanthemums (*Dendranthema grandiflora* Tzvelve) are highly significant on a global scale, ranking second only to roses among flower crops. These woody, herbaceous perennials, part of the Asteraceae family, produce a wide variety of blooms. The genus is characterized by a basic chromosome number of n=9, with cultivars exhibiting various levels of polyploidy, including 2n=18, 36, 45, 47, 51 and 75. Chrysanthemums are valued both for their ornamental appeal and their commercial potential. The rich cultural history of chrysanthemums has driven extensive breeding programs, resulting in numerous cultivars that are among the top ten in categories such as cut flowers, potted plants and garden plants globally (Kishi-Kaboshi *et al.*, 2017; Din *et al.*, 2020; Shahrajabian *et al.*, 2019) [21, 12, 33]. In India, chrysanthemums are particularly popular, with widespread cultivation across many states, despite the absence of indigenous varieties (Kazaz *et al.*, 2020) [19]. Enhancing genetic diversity is crucial for developing new cultivars (Patil *et al.*, 2017; Datta, 2020) [29, 11]. The expanding chrysanthemum market drives breeders and researchers to develop new varieties with enhanced aesthetics, improved stress tolerance, and superior quality traits. The primary techniques for generating novel chrysanthemum cultivars are crossbreeding and mutation breeding. Crossbreeding, which includes both sexual and asexual reproduction, can occur naturally but faces challenges due to the self-incompatibility and cross-pollination characteristics of chrysanthemums. These factors complicate parent selection and the identification of superior hybrid progenies (Anderson *et al.*, 2017; Zhang *et al.*, 2018; Kumari *et al.*, 2019; Baghele, 2021) [1, 43, 22, 4].

Mutation induction is a powerful tool for developing novel plant germplasms, with radiation being a prominent method in mutation breeding. Radiation-induced mutation breeding yields superior cultivars compared to conventional breeding, which is often time-consuming, labor-intensive, and limited in genetic diversification (Beyaz and Yildiz, 2017; Anne and Lim, 2020) [6, 3]. In ornamental horticulture, radiation has been employed to modify various traits such as flower colour, shape, inflorescence type, fertility and leaf colour or variegation (Brunner, 1995) [7]. Many flower cultivars have arisen from both spontaneous and induced mutations through gamma irradiation, ion beam irradiation, X-rays, and chemical mutagenesis. These methods can alter flower colour, shape, and petal and leaf colour, making them beneficial for chrysanthemum mutation breeding (Yamaguchi *et al.*, 2008; Matsumura *et al.*, 2010) [41, 24]. Exposure of chrysanthemum plants to various doses of gamma irradiation can change inflorescence color by altering pigment content, thereby enhancing the decorative value of the cultivars (Datta and Gupta, 1981) [10]. According to the Mutant Variety Database (IAEA, 2022) [17], there are 400 recorded chrysanthemum mutants among vegetatively propagated plants. The majority of these mutants are floricultural plants, with a smaller proportion being fruit trees (Kumari *et al.*, 2019; Melsen *et al.*, 2021) [22, 25].

Chlorophyll is a green photosynthetic pigment primarily found in the green tissues of plants, such as leaves, stems, flowers, and roots (Srichaikul *et al.*, 2011; Hasanuzzaman *et al.*, 2013; Kamble *et al.*, 2015) [36, 15, 18]. This antioxidant compound resides within the chloroplasts. The main pigments in plant photosystems are chlorophyll a and chlorophyll b (Kamble *et al.*, 2015; Richardson *et al.*, 2002) [18, 31]. The concentration of chlorophyll in leaves serves as an indicator of a plant's physiological state, reflecting its chloroplast content, photosynthetic efficiency, and overall metabolic condition (Kamble *et al.*, 2015) [18]. Typically, chlorophyll a is present at concentrations 2-3 times higher than chlorophyll b (Srichaikul *et al.*, 2011) [36]. Carotenoids, a class of pigments, play multiple roles in plants, including photoprotection and light harvesting. Plants are sensitive to radiation, with high levels of irradiation significantly inhibiting growth and potentially proving lethal. Physiological alterations resulting from irradiation show a correlation between chlorophyll and carotenoid content and the effects of gamma irradiation.

Thus, mutations can exhibit valuable and sophisticated efficiencies. Numerous nuclear techniques are utilized in agriculture, with gamma rays employed to develop tolerance to biotic and abiotic stresses and to enhance plant characteristics in vegetatively propagated plants. In this context, varying doses of gamma rays were applied as induced mutagens to study their effects on vegetative growth, chlorophyll a, b and carotenoid content of chrysanthemum (*Dendranthema grandiflora*) leaves.

Materials and Methods

The present investigation titled "Effect of gamma rays on vegetative growth and biochemical composition of chlorophyll and carotenoid in vM_1 generation of chrysanthemum (*Dendranthema grandiflora* T.)" was conducted at the experimental field of the Model Floriculture Centre, Department of Horticulture, G. B. Pant University of Agriculture and Technology (GBPUA&T), Pantnagar, U. S. Nagar, during the period of 2022-2023. Rooted cuttings mutagenesis was carried out at the Department of Physics, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar.

Field trial

The cuttings were collected from the mother block of chrysanthemum at the Model Floriculture Centre, Pantnagar, U.S. Nagar. After one-month, healthy rooted cuttings of two varieties, Lalpari and DFRC-3, were irradiated with gamma-rays at different doses (10, 15, 20, 25, 30, and 35 Gy). Non-irradiated cuttings served as controls, with 25 cuttings taken for each gamma dose. The field experiment was situated at approximately 29° North latitude and 79.3° East longitude, nestled in the Tarai region of the Shivalik range in the Himalayas, at an altitude of 243.14 meters above mean sea level. Irradiated cuttings of each dose were planted in poly bags filled with a uniform mixture of vermicompost, sand and garden soil at a ratio of 1:1:1 per polybag, and placed under shade nets. After one week of hardening, the cuttings in polybags were transplanted into the main experimental field. The experiment was arranged in a randomized block design consisting of 14 treatment combinations with three replications. Rooted cuttings were planted at 30 × 30 cm spacing. The data were recorded on vegetative parameters which includes plant height (cm), number of leaves per plant, leaf colour and in biochemical parameters chlorophyll a, b, total chlorophyll and carotenoid content at the time of bud stage.

Extraction of chlorophyll

Chlorophyll and carotenoid content were estimated using the method described by Hiscox and Israelstam (1979) [16]. Fresh young and mature leaf tissues weighing 50 mg each were placed in test tubes, to which 10 ml of dimethyl sulfoxide (DMSO) was added. The test tubes were then incubated at 65°C for three hours in a hot air oven. Following incubation, the absorbance of the DMSO extracts containing chlorophyll and carotenoids was measured at wavelengths of 480 645 and 663 nm using a spectrophotometer with multiple wavelength capabilities. Pure DMSO served as the blank. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content were calculated using the formulas provided by Wellburn (1994) [39].

$$\text{Chl. a (mg g}^{-1}\text{ fresh wt.)} = \frac{(12.47 \times A663 - 3.62 \times A645)V}{1000 \times \text{wt.}}$$

$$\text{Chl. b (mg g}^{-1}\text{ fresh wt.)} = \frac{(25.06 \times A645 - 6.5 \times A663)V}{1000 \times \text{wt.}}$$

$$\text{Total Chl. (mg g}^{-1}\text{ fresh wt.)} = \text{Chl. a (mg g fresh wt.)} + \text{Chl. b (mg g fresh wt.)}$$

$$\text{Carotenoid x + c (mg g}^{-1}\text{ fresh wt.)} = \frac{(1000 \times A480 - 1.29 \times \text{Chl. a} - 53.78 \times \text{Chl. b})/200 \times V}{1000 \times \text{wt.}}$$

Results

Effect of gamma irradiation on vegetative characters

The present study demonstrates that the plant height was significantly decrease when increase the doses of gamma irradiation. The corresponding data are presented in Table 1. In vM_1 generation the highest plant height 38.09 cm was recorded with G_1 (control), followed by 34.36 cm with G_2 (10 Gy) and 30.44 cm with G_3 (15 Gy). Whereas, lowest plant height 16.73 cm was recorded with G_6 (30 Gy). Higher dose of gamma irradiation dose 35 Gy (G_7) no plant survived. The plant height was significantly affected by cultivars. Specifically, in relation to irradiation doses, the highest plant height 27.62 cm was recorded in V_1 (Lalpari). Whereas, lowest plant height 20.95 cm with V_2 (DFRC-3). The interactive effect of gamma irradiation and varieties was statistically significant for plant height. The highest plant height 42.77 cm was recorded in G_1V_1 (control + Lalpari), followed by 39.12 cm in G_2V_1 (10 Gy + Lalpari) and 33.40 cm in G_1V_2 (control + DFRC-3). Whereas, lowest plant height 11.62 cm was recorded with G_6V_2 (30 Gy + DFRC-3), followed by 21.63 cm with G_6V_1 (30 Gy + Lalpari). No plant survived in higher dose (35 Gy) of gamma radiation.

The study demonstrates a significant decreased number of leaves per plant when increase the gamma irradiation doses. Comprehensive data are available in Table 2. In vM_1 generation the maximum number of leaves per plant 126.71was recorded with G_1 (control), followed by 95.96 with G_2 (10 Gy) and 88.13 with G_3 (15 Gy). Whereas, minimum number of leaves per plant 18.10 was recorded with G_6 (30 Gy), followed by 40.40 with G_5 (25 Gy). Higher dose (35 Gy) of gamma irradiation no plants survived in G_7. The number of leaves per plant was significantly affected by cultivars. Specifically, in relation to irradiation doses, maximum number of leaves per plant 62.19 was recorded with V_1 (Lalpari). Whereas, minimum number of leaves per plant 59.02 was recorded with V_2 (DFRC-3). The interactive effects of gamma irradiation and varieties was statistically significant in both the generations for number of leaves per plant. The maximum number of leaves per plant 143.96 was recorded in G_1V_2 (control + DFRC-3), followed by 109.47 with G_1V_1 (control + Lalpari) and 99.03 with G_2V_2 (10 Gy + DFRC-3). Whereas, minimum number of leaves per plant 10.28 was recorded with G_6V_2 (30 Gy + DFRC-3), followed by 25.92 with G_6V_1 (30 Gy + Lalpari) and 28.73 with G_5V_2 (25 Gy + DFRC-3). Interactive effect of G_7V_1 and G7V2 was not found number of leaves per plant because higher dose (35 Gy) of gamma irradiation no plant survived.

Leaf colour variation induced with increased the gamma irradiation dose. The leaf colour (Table 3) dark green were observed in G_1 (Control) and G_2 (10 Gy), green in G_3 (15 Gy) and G_4 (20 Gy) and light green in G5 (25 Gy) and G_6 (30 Gy). No plant survived in G_7 (35 Gy).

Effect of gamma irradiation on biochemical characters

The study illustrates the significant impact of various gamma irradiation doses on chlorophyll a. Detailed data are presented in Table 4. In the vM1 generation, the highest chlorophyll a content of 1.16 mg/g was observed in G_1 (control), followed by 1.13 mg/g in G_2 (10 Gy), and 0.87 mg/g in G_3 (15 Gy). Conversely, the lowest chlorophyll a 0.16 mg/g were recorded in G_6 (30 Gy), followed by 0.80 mg/g in G_5 (25 Gy) and 0.84 mg/g in G_4 (20 Gy). A higher dose of 35 Gy resulted in mortality of all plants. The chlorophyll a content was significantly influenced by cultivars. Specifically, in relation to irradiation doses, maximum chlorophyll a 0.80 mg/g were recorded with V_1 (Lalpari), while minimum chlorophyll a 0.62 mg/g were observed with V_2 (DFRC-3). The interactive effects of gamma irradiation and plant varieties demonstrated statistically significant differences in chlorophyll a. The highest chlorophyll a content 1.29 mg/g was recorded with G_1V_1 (control + Lalpari), followed by 1.24 mg/g with G_2V_1 (10 Gy + Lalpari). Conversely, the lowest chlorophyll a content 0.09 mg/g was recorded with G_6V_2 (30 Gy + DFRC-3). Higher doses of gamma ray radiation (G_7V_1 and G_7V_2) resulted in mortality of all plants.

The study reveals significant effects of varying gamma irradiation doses on chlorophyll b levels. Detailed data are provided in Table 5. In the vM1 generation, the highest chlorophyll b content 0.19 mg/g was observed in G_1 (control), followed by 0.18 mg/g in G_2 (10 Gy) and 0.16 mg/g in G_3 (15 Gy). In contrast, the lowest chlorophyll b content 0.04 mg/g were recorded in G_6 (30 Gy), followed by 0.09 mg/g in G_5 (25 Gy) and 0.14 mg/g in G_4 (20 Gy). Exposure to a higher dose (35 Gy) of gamma ray radiation resulted in the mortality of all plants. The chlorophyll b levels were significantly influenced by cultivars. Specifically, in relation to irradiation doses, the maximum chlorophyll b content 0.14 mg/g was recorded with V_1 (Lalpari), while the minimum chlorophyll b content 0.08 mg/g was observed with V_2 (DFRC-3). The interactive effects of gamma irradiation and plant varieties showed statistically significant differences in chlorophyll b levels. The highest chlorophyll b content 0.24 mg/g was recorded with G_1V_1 (control + Lalpari), followed by 0.22 mg/g with G_2V_1 (10 Gy + Lalpari). Conversely, the lowest chlorophyll b content 0.03 mg/g was recorded with G_6V_2 (30 Gy + DFRC-3). Exposure to higher doses of gamma ray radiation (G_7V_1 and G_7V_2) resulted in mortality of all plants.

The study highlights the significant impact of varying gamma irradiation doses on total chlorophyll levels. Detailed data are presented in Table 6. In the vM1 generation, the highest total chlorophyll content 1.36 mg/g was recorded in G_1 (control), followed by 1.30 mg/g in G_2 (10 Gy) and 1.03 mg/g in G_3 (15 Gy). Conversely, the lowest total chlorophyll 0.20 mg/g were observed in G_6 (30 Gy), followed by 0.90 mg/g in G_5 (25 Gy), and 0.98 mg/g in G_5 (25 Gy). Exposure to a higher dose (35 Gy) of gamma ray radiation resulted in mortality of all plants Total chlorophyll content was significantly influenced by cultivars. Specifically, the maximum total chlorophyll 0.94 mg/g was recorded with V_1 (Lalpari), while the minimum of 0.71 mg/g was observed with V_2 (DFRC-3). The interaction between gamma irradiation and plant varieties produced statistically significant differences in total chlorophyll levels. The highest total chlorophyll content 1.53 mg/g was observed with G_1V_1 (control + Lalpari), followed by 1.46 mg/g in G_2V_1 (10 Gy + Lalpari). In contrast, the lowest total chlorophyll content 0.12 mg/g was recorded with G_6V_2 (30 Gy + DFRC-3). Exposure to higher gamma ray doses (G_7V_1 and G_7V_2) led to the death of all plants.

The study reveals a significant effect of gamma irradiation doses on carotenoid content. Detailed data are provided in Table 7. In the vM1 generation, the highest carotenoid content 0.24 mg/g was observed in G_1 (control), followed by 0.19 mg/g in G_2 (10 Gy) and 0.18 mg/g in G_3 (15 Gy). Conversely, the lowest carotenoid content 0.03 mg/g was recorded in G_6 (30 Gy), followed by 0.15 mg/g in G_5 (25 Gy) and 0.16 mg/g in G_4 (20 Gy). Exposure to a higher dose (35 Gy) of gamma radiation resulted in the mortality of all plants.

Carotenoid content was significantly influenced by both cultivar and gamma irradiation doses. Specifically, the highest carotenoid content 0.15 mg/g was recorded with V_1 (Lalpari), while the lowest content of 0.12 mg/g was observed with V_2 (DFRC-3). The interaction between gamma irradiation and plant varieties produced statistically significant differences in carotenoid levels. The highest carotenoid content 0.26 mg/g was noted with G_1V_1 (control + Lalpari), followed by 0.22 mg/g in G_1V_2 (control + DFRC-3). Conversely, the lowest carotenoid content 0.03 mg/g was recorded with G_6V_2 (30 Gy + DFRC-3). Exposure to higher doses of gamma ray radiation (G_7V_1 and G_7V_2) resulted in the death of all plants.

Discussion

Increased gamma irradiation doses significantly reduce plant height number of leaves by inactivating auxin, leading to shorter plants (Banerji and Datta, 1992) [5]. Higher doses of gamma ray irradiation reduced the height of chrysanthemum plants, likely due to damage to the top shoot meristem cells. This is consistent with Srivastava and Kumar (2011) [37], who found that safflower plants exposed to higher gamma irradiation doses were shorter than control plants, attributing this to interference with DNA synthesis. Similar findings were reported by Patil *et al.* (2019) [28], Din *et al.* (2020) [12] and Anne and Lim (2021) [2]. Leaf colour variation may be due to adverse effect of radiation on chlorophyll synthesis process or might be due to reduced levels of substrates affecting biochemical pathways (Singh and Bala 2019) [35].

The significant decrease in chlorophyll content observed with increasing gamma ray exposure likely stems from direct and indirect alterations in biosynthetic pathways associated with flowering physiology (Mahure *et al.*, 2010) [23]. These effects can lead to variations in pigment content, directly influenced by radiation's impact on pigments or indirectly through modifications in biosynthetic pathways (Dohino and Hayashi, 1995) [13]. Similar findings have been reported in studies on other plant species, such as Terminalia arjuna by Chandrashekar *et al.* (2013) [8] and Dianthus caryophyllus by Khatab and Hegazi (2015) [20].

This differential impact suggests that gamma irradiation may selectively impair the biosynthesis or degrade the precursors of chlorophyll b. Similar trends have been documented across different plant species, as observed by Fukuzawa *et al.* (1998) [14], Mishra *et al.* (2007) [26] and Neelam *et al.* (2014) [27]. Chanu *et al.* (2020) [9] also reported comparable results in tree tomato (*Solanum betaceum* Cav.), emphasizing the consistent influence of gamma radiation on chlorophyll dynamics in plants. Studied in tree tomato (*Solanum betaceum* Cav.) and *Pisum sativum* show decreased chlorophyll levels with higher radiation doses, linked to cellular damage and impaired chlorophyll biosynthesis (Chanu *et al.*, 2020; Strid *et al.*, 1990) [9, 38]. Gamma rays primarily affect chloroplasts, causing structural damage and reduced chlorophyll through processes such as dephytolization (Wi, 2005; Saha *et al.*, 2010) [40, 32]. Similar effects have been observed in crops like *Medicago truncatula* and *Vigna mungo* (Rejili *et al.*, 2008; Yasim and Arulbalachandran, 2022) [30, 42]. Gamma irradiation affects carotenoids by inducing oxidative changes. Low doses (up to 2 kGy) can enhance carotenoid extractability, but higher doses (above 4 kGy) lead to significant degradation and loss of antioxidant properties due to accelerated oxidative reactions (Siddiq *et al.*, 2013) [34].

Table 1: Effect of gamma irradiation on plant height of chrysanthemum varieties in vM_1 generation

Plant height (cm)								
Gamma ray doses / Varieties	**Control (G_1)**	**10 Gy (G_2)**	**15 Gy (G_3)**	**20 Gy (G_4)**	**25 Gy (G_5)**	**30 Gy (G_6)**	**35 Gy (G_7)**	**Mean**
Lalpari (V_1)	42.77	39.12	33.15	30.32	26.17	21.84	0.00	27.62
DFRC-3 (V_2)	33.40	29.60	27.73	22.68	21.63	11.62	0.00	20.95
Mean	38.09	34.36	30.44	26.50	23.90	16.73	0.00	
Factors			C.D. (5%)			SE(m)±		
Varieties (V)			1.096			0.375		
Gamma ray doses (G)			2.051			0.702		
Interaction (V X G)			2.901			0.992		

Table 2: Effect of gamma irradiation on number of leaves per plant of chrysanthemum varieties in vM_1 generation

Number of leaves per plant								
Gamma ray doses / Varieties	**Control (G_1)**	**10 Gy (G_2)**	**15 Gy (G_3)**	**20 Gy (G_4)**	**25 Gy (G_5)**	**30 Gy (G_6)**	**35 Gy (G_7)**	**Mean**
Lalpari (V_1)	109.47	92.88	80.28	74.69	52.07	25.92	0.00	62.19
DFRC-3 (V_2)	143.96	99.03	95.97	35.16	28.73	10.28	0.00	59.02
Mean	126.71	95.96	88.13	54.93	40.40	18.10	0.00	
Factors			C.D. (5%)			SE(m)±		
Varieties (V)			2.744			0.939		
Gamma ray doses (G)			5.134			1.756		
Interaction (V X G)			7.261			2.484		

Table 3: Effect of gamma irradiation on leaf colour of chrysanthemum varieties in vM_1 generation

Leaf colour							
Gamma ray doses / Varieties	**Control (G_1)**	**10 Gy (G_2)**	**15 Gy (G_3)**	**20 Gy (G_4)**	**25 Gy (G_5)**	**30 Gy (G_6)**	**35 Gy (G_7)**
Lalpari (V_1)	Dark green	Dark green	Green	Green	Ligh green	Light green	-
DFRC-3 (V_2)	Dark green	Dark green	Green	Green	Light green	Light green	-

Table 4: Effect of gamma irradiation on chlorophyll a of chrysanthemum varieties in vM_1 generation

Chlorophyll a (mg/g)								
Gamma ray doses / Varieties	**Control (G_1)**	**10 Gy (G_2)**	**15 Gy (G_3)**	**20 Gy (G_4)**	**25 Gy (G_5)**	**30 Gy (G_6)**	**35 Gy (G_7)**	**Mean**
Lalpari (V_1)	1.29	1.24	0.99	0.95	0.87	0.24	0.00	0.80
DFRC-3 (V_2)	1.03	1.01	0.75	0.73	0.73	0.09	0.00	0.62
Mean	1.16	1.13	0.87	0.84	0.80	0.16	0.00	
Factors			C.D. (5%)			SE(m)±		
Varieties (V)			0.003			0.001		
Gamma ray doses (G)			0.005			0.002		
Interaction (V X G)			0.007			0.002		

Table 5: Effect of gamma irradiation on chlorophyll b of chrysanthemum varieties in vM_1 generation

Chlorophyll b (mg/g)								
Gamma ray doses / Varieties	**Control (G_1)**	**10 Gy (G_2)**	**15 Gy (G_3)**	**20 Gy (G_4)**	**25 Gy (G_5)**	**30 Gy (G_6)**	**35 Gy oG_7)**	**Mean**
Lalpari (V_1)	0.24	0.22	0.19	0.15	0.13	0.06	0.00	0.14
DFRC-3 (V_2)	0.15	0.14	0.12	0.12	0.06	0.03	0.00	0.08
Mean	0.19	0.18	0.16	0.14	0.09	0.04	0.00	
Factors			C.D. (5%)			SE(m)±		
Varieties (V)			0.003			0.001		
Gamma ray doses (G)			0.006			0.002		
Interaction (V X G)			0.009			0.003		

Table 6: Effect of gamma irradiation on total chlorophyll of chrysanthemum varieties in vM_1 generation

Total chlorophyll (mg/g)								
Gamma ray doses / Varieties	**Control (G_1)**	**10 Gy (G_2)**	**15 Gy (G_3)**	**20 Gy (G_4)**	**25 Gy (G_5)**	**30 Gy (G_6)**	**35 Gy (G_7)**	**Mean V**
Lalpari (V_1)	1.53	1.46	1.18	1.10	1.00	0.29	0.00	0.94
DFRC-3 (V_2)	1.18	1.15	0.87	0.85	0.80	0.12	0.00	0.71
Mean G	1.36	1.30	1.03	0.98	0.90	0.20	0.00	
Factors			**C.D. (5%)**			**SE(m)±**		
Varieties (V)			0.006			0.002		
Gamma ray doses (G)			0.011			0.004		
Interaction (V X G)			0.015			0.005		

Table 7: Effect of gamma irradiation on carotenoid content of chrysanthemum varieties in vM_1 generation

Carotenoid content (mg/g)								
Gamma ray doses / Varieties	**Control (G_1)**	**10 Gy (G_2)**	**15 Gy (G_3)**	**20 Gy (G_4)**	**25 Gy (G_5)**	**30 Gy (G_6)**	**35 Gy (G_7)**	**Mean**
Lalpari (V_1)	0.26	0.19	0.21	0.20	0.17	0.04	0.00	0.15
DFRC-3 (V_2)	0.22	0.20	0.15	0.13	0.13	0.03	0.00	0.12
Mean	0.24	0.19	0.18	0.16	0.15	0.03	0.00	
Factors			C.D. (5%)			SE(m)±		
Varieties (V)			0.004			0.002		
Gamma ray doses (G)			0.008			0.003		
Interaction (V X G)			0.012			0.004		

Conclusion

It can be concluding increasing gamma ray doses reduction in plant height, number of leaves chlorophyll a, b, total, carotenoid content and induced leaf colour variations in chrysanthemums. The study shows that gamma irradiation can create genetic variability, useful for improving plant traits. Therefore, applying physical mutagenic treatments at lower frequencies can be a viable method for inducing superior genotypes with notable alterations in plant growth and metabolism. Though, further investigations are needed to verify these results in subsequent generations of the chrysanthemums studied.

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