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Induced mutations in chrysanthemum through gamma rays

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ABSTRACT

The research was conducted to induce variations in the chrysanthemum variety 'Mother Teresa' in 2020-21. The purpose was to generate and detect the desirable variants concerning altered flower colour, form and flower yield from the treated population. The terminal rooted cuttings were irradiated with different γ-ray doses (0, 5, 10, 15, and 20 Gy) using Cobalt 60 (^{60}Co) as the source and evaluated further along with the control population, i.e. without irradiations. The observations on various morphological and floral parameters were recorded and compared with the control population (non-irradiated). Marked floral changes about altered flower colour, shape, form and changes in leaf shape, colour and sizes were observed with higher doses of gamma irradiations. Percent flower colour variations were recorded as 4.10% at 10 Gy, 1.56% at 15 Gy and 4.25% at 20 Gy dose. The present study, 11 variants were detected with altered flower colour and form with 10 to 20 Gy-ray doses. The study developed an efficient protocol for the induction of mutations using γ-rays in chrysanthemum.

Key words: *Chrysanthemum morifolium* Ramat., Cv. Mother Teresa, γ-irradiation, LD_{50}, Vegetative and floral Characters.

INTRODUCTION

Chrysanthemum is derived from two Greek words, 'C*hrysos', which* means 'gold' and *'Anthos'* (flower) and is completely known as 'Golden flower'. It is an herbaceous perennial plant belonging to the 'Asteraceae' family, also known as 'Queen of East', 'Glory of East', or 'Autumn Queen. The major chrysanthemum growing states are Maharashtra, Tamil Nadu and Karnataka. It has been reported that about 24.05 thousand hectares in India are under commercial cultivation, with 456.11 thousand metric tons of loose flower and 15.74 thousand metric tons of cut flower production (Anonymous, 2). Chrysanthemum has wide diversity in flower colour, shape, form, growth habit, maturity group and commercial uses. Flowers are used for loose flowers for garland making, worship, cut flowers for flower arrangements and bouquet making, pot culture, and bedding/ garden decoration.

In the national and international flower markets, the quests for novelty always exist and are generally preferred by consumers. Although many varieties are available in the market, the uniqueness of commercial characteristics like prolonged duration of flowering, response/maturity group, wide adaptability, changes in flower colour, shape, size and post-harvest keeping quality are always valued. The conventional methods in chrysanthemums to create variability are hybridization and open-pollinated seedling selection. Most of the chrysanthemum cultivars yield low seed settings, which might be due to longer outer ray florets, which prevents pollination, and another reason might be protandry or sporophytic type of self-incompatibility (Kher, 10).

Mutation breeding, an established technique, has drawn considerable attention in chrysanthemums as it can be easily detected in the vegetatively propagated crops in the M_1 generation. Thus, the selection of desirable improved traits is straightforward. Chrysanthemum is a cross-pollinated crop. Therefore, only seedling selection developed through natural or cross-pollination is generally used to create variability (Datta, 6). Induced mutagenesis has special significance in creating variability within a shorter time, especially in vegetatively propagated crops with a highly complicated and heterozygous nature. An optimum level of gamma irradiation also helps improve flower shape, colour and size, earliness, delay in flowering and alteration of plant stature (Broertjes, 5). In modern-day chrysanthemum cultivars, mutation breeding is the only complementary and helpful tool to improve existing cultivars within a shorter period. The study was planned and conducted to induce variability through gamma irradiations in the chrysanthemum variety 'Mother Teresa'.

MATERIALS AND METHODS

The research was conducted at the Research Farm, Department of Floriculture & Landscaping, PAU, Ludhiana (Punjab), from June 2020 to January 2021. The climate is semi-arid and sub-tropical, with an average rainfall of 609 mm in 2020. The average highest and lowest temperatures were recorded as 31.05° C and 19.67° C, respectively, and agro-meteorological data were recorded from June 2020

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to January 2021 (Fig. 1). The selected variety was 'Mother Teresa', which is no pinch or stake variety. Flowers are creamish in colour, anemone type and used for pot plantation and decoration.

The terminal cuttings of uniform size (5-7 cm) of selected variety were prepared during 1st week of July and treated with IBA @400 ppm solution to induce rooting. The cuttings were planted in plug trays having BRH (burnt rice husk) as rooting medium and kept under a shade net (50%). New roots were formed 15-20 days after planting, and rooted cuttings were exposed to different levels (0, 5, 10, 15 and 20 Gy) of γ-rays (CO^{60} source) at the Department of Fruit Science, PAU, Ludhiana (irradiator reading 2000 ANSI-N 433.1). Critical cultural practices recommended by PAU, Ludhiana, were followed to grow healthy plants. Irradiated cuttings were transplanted in plastic pots (8-inch size) in the first week of August 2020, along with non-irradiated (control) plants immediately after treatment. The potting media used was 2:1:1 (garden soil: sand: leaf mould). The observations on various growth and flowering parameters were recorded. Data were analyzed statistically following Analysis of variance (ANOVA) with SPSS-21 software (Statistical Product and Service Solution, USA). Their means were calculated based on 'Tukey HSD at a probability level of 5%.

RESULTS AND DISCUSSION

The results related to plant height, spread, survival percentage, lethal dose, branches per plant and various leaf parameters depicted from data are elaborated in Table 1. The result revealed that the plant height was reduced with the increased gamma-ray doses. The plant height (15.69 cm) was the maximum in the control plants planted without gamma irradiation treatment. The least plant height (12.41 cm) was observed at the highest gamma irradiation dose, i.e., 20 Gy. The reduction in plant

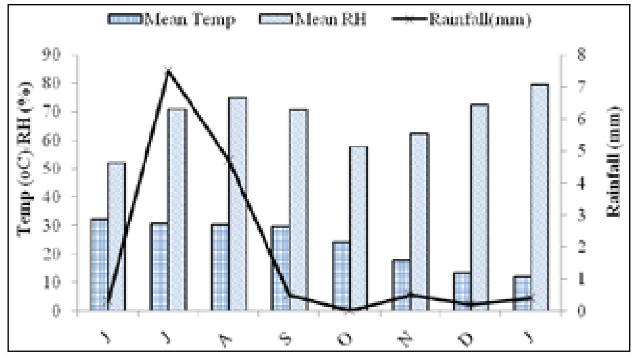

Fig. 1. Average agro meteorological data during the study period (June, 2010 to January, 2021).

height after gamma irradiations might be due to the damage caused to the cell elongation and cell division process (Walther, 21). The increased level of gamma irradiation may decrease or inactivates the level of auxins that may reduce the height of plants (Banerji and Datta, 4; Kapoor *et al.,* 9).

Plant spread also decreased significantly with the increasing gamma irradiation levels. Control plants exhibited the highest plant spread (31.83 cm), and the lowest plant spread of 22.75 cm was observed in plants treated with the higher 20 Gy dose. The significant decrease in plant spread of chrysanthemum cuttings treated with gamma rays at 0 kR and 2 kR doses has also been reported earlier (Dilta *et al*., 7). The percentage of plant survival recorded 15 days after transplanting was also found to be the highest, i.e. 92.00 % in control plants, and the least plant survival (62.00 %) was recorded with 20 Gy gamma rays dose. The LD_{50} (lethal dose) of gamma irradiations reduced the survivability one month after transplanting. The lethal dose based on survival percentage was estimated to be 20 Gy dose with a 47.00% survival rate, i.e. less than 50%. The results obtained earlier by different scientists

Table 1. Effect of gamma irradiation on vegetative growth and leaf characters of chrysanthemum variety 'Mother Teresa'.

γ-ray dose (Gy)	Plant height at bud initiation (cm)	Plant spread (cm)	Percent survival (15 DAT)	LD_{50} (30 DAT)	Branches per plant	Leaves per plant
Control	15.69[a]	31.83[a]	92.00[a]	87.00[a]	9.80[a]	130.90[a]
5	14.81[ab]	30.00[a]	85.00[b]	81.00[b]	8.67[b]	120.76[b]
10	14.15[ab]	26.71[b]	78.00[c]	73.00[c]	7.53[c]	110.76[c]
15	13.30[bc]	24.88[bc]	71.00[d]	64.00[d]	6.45[d]	98.61[d]
20	12.41[c]	22.75[c]	62.00[e]	47.00[e]	5.31[e]	86.32[e]
Mean	14.07	27.23	77.60	70.40	7.55	109.47
SEM (±)	0.23	0.17	1.02	0.69	0.02	1.03

Mean values in columns denoted with different letters differed significantly as per Tukey HSD[a] test ($p<0.05$).

have also evaluated the lethal dose of gamma rays based on the survivability of plants after gamma irradiation (Yamaguchi *et al.,* 22; Singh and Bala, 19). The decrease in per cent plant survival with higher gamma-ray doses might be due to genetic loss at the gene level and chromosomal aberrations (Tiwari and Kumar, 20).

The branches per plant (9.80) were also recorded as the highest in control (without irradiation treatment). The lowest number of branches per plant (5.31) was recorded at the highest γ-rays dose, i.e. 20 Gy. The significant reduction in the number of branches with increasing gamma-ray doses might be due to their inhibitory effect on plant growth. Banerji and Datta(4) also reported a significant decrease in branches per plant in chrysanthemum cv. 'Anupam' when treated with 1.5 kR to 2.5 kR doses of γ-rays. The increasing levels of gamma irradiation dose significantly affected the leaf number per plant. The maximum leaf number per plant (130.90) was observed in the control, and the minimum number of leaves per plant (86.32) was observed in plants exposed to 20 Gy dose. The reduction in leaf number after gamma irradiation might be due to the fewer branches formed per plant, as reported earlier (Mahure *et al.,* 13).

Changes in leaf colour were observed critically. The visual observations revealed that leaves were normal in control and plants irradiated at 5 Gy dose. Leaves were green at 10 Gy and slightly light green at 15 and 20 Gy γ-rays. The leaf abnormalities, such as serrated margins and small and fused leaves were also observed at 15 Gy and 20 Gy doses. Setia *et al.,*18; Singh and Bala, 19 also reported leaf colour variation at higher gamma dose. The adverse effect of irradiation on chlorophyll formation or reduced levels of substrates that affect the biochemical pathways might be the reasons for leaf colour variations. Variations in the leaf colour, shape form and texture after gamma-irradiation have also been reported earlier in chrysanthemum (Yamaguchi *et al.,* 22). Pale yellow or light-green, small, narrow, or lanceolate leaves with serrated margins and fused have been obtained earlier in roses when nodal explants were irradiated with gamma rays under *in vitro* conditions (Bala and Singh, 3).

Gamma irradiations significantly affect days to first bud appearance, bud showing colour shown stage and opening of 50% flowers as presented in Fig. 2. The earliest first flower bud initiation was observed 84.45 days after transplanting in control plants, and the longest time taken for bud initiation (92.75 days) was observed in plants treated with 20 Gy dose of γ-rays. The number of days taken by plants to 50% buds showing colour (99.45 days) was recorded to be the lowest in control and highest, i.e. 111.75 days in

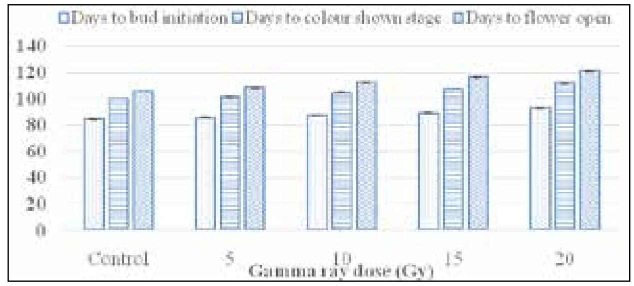

Fig. 2. Effect of gamma irradiation on days to bud initiation, colour shown and flower opening stage.

the plants exposed to the highest gamma irradiation dose (20 Gy). After irradiation, the least number of days to flower opening (105.45 days) was observed in control plants. In contrast, the highest gamma-ray dose of 20 Gy exhibited the highest days for flower opening (120.75 days). The delayed flower bud showing colour might be due to delayed first bud initiation that ultimately delayed colour break and late blooming in plants. Many biosynthesis pathways after gamma radiation are also altered within the plants, which might affect the flowers' physiology. The plants remain in the juvenile stage, thus unable to differentiate flower heads (Mahure *et al*., 13). Significant delay in bud initiation has also been reported earlier by (Neto and Latado,14).

The results on flowering duration, flowers per plant, flower size, ray florets per flower, flower colour and form variations are presented in Table 2. Flowering duration (32.80 days) was observed to be the highest in control, i.e. without treatment and flower duration (26.50 days) was lowest with 20 Gy dose of γ-rays. The reduction in flowering duration may be due to biochemical and physiological changes within the plants, a drop in the activities of cells that might have an ill effect on auxins level, chromosomal aberrations, DNA synthesis and disturbance in physiological processes (Santosh, 17). The present study observed a significant reduction in the number of flowers per plant by increasing gamma irradiation dose. The highest number of flowers per plant (70.67) was recorded in non-irradiated plants, and the lowest number of flowers per plant (54.75) was exhibited at 20 Gy. Similar to our findings, results have been quoted by (Patil *et al*., 16; Singh and Bala, 19) in chrysanthemum.

Flower size was significantly different with different doses of γ-rays in the present study. The results showed that flower size (3.61 cm) was reported to be the maximum in control, and flower size (3.16 cm) was noted to be the minimum in 20 Gy dose. The small size of the flower might be due to cytological, morphological and physiological change by γ-rays as

Table 2. Effect of gamma irradiation on flowering characters of chrysanthemum variety 'Mother Teresa'.

γ-ray dose (Gy)	Flowering duration (days)	Flowers per plant	Flower size (cm)	Number of ray florets	Flower colour variations (%)	Flower form variations (%)
Control	32.80[a]	70.67[a]	3.61[a]	41.79[a]	-	-
5	30.03[b]	69.85[b]	3.53[ab]	39.13[ab]	-	-
10	28.40[c]	66.40[c]	3.46[bc]	36.15[bc]	4.10	4.10
15	27.10[cd]	58.65[d]	3.36[c]	34.65[c]	1.56	4.68
20	26.50[d]	54.75[e]	3.16[d]	27.70[d]	4.25	6.38
Mean	29.16	64.04	3.42	35.88	-	-
SEM (±)	0.17	0.44	0.01	0.35	-	-

Mean values in columns denoted with different letters differed significantly as per Tukey HSD[a] test ($p<0.05$).

reported by (Gupta and Jugran, 8). The number of ray florets (27.70) was the lowest at 20 Gy gamma-ray doses, while in control plants, the highest ray florets per plant (41.79) were recorded. Chrysanthemum cultivar 'Argus' treated at 30 Gy, 40 Gy, and 50 Gy of gamma rays also decreased the number of ray florets, as reported earlier (Lee *et al.*, 12). The reduction in the number of ray florets might be due to a decrease in the vegetative growth of plants and disturbance in plant physiological processes (Pal, 15).

Gamma irradiation resulted in altered flower colour and form variations, as presented in Tables 2, 3 and Fig. 3, 4. In this experiment, flower colour variations were observed on a visual basis and calculated in percentage. The highest percentage of flower colour variations (4.25%) were reordered in plants treated with the highest gamma irradiation dose, i.e. 20 Gy and 19 Gy dose with 4.10% variations in flower colour. The least variations of 1.56 % were reported with 15 Gy dose. Similarly, flower form variations (6.38%) were also found to be the highest at 20 Gy, and the least form variations (4.10%) were reported at 10 Gy dose of gamma irradiation. Normal flower colour and form were observed in non-irradiated plants and at lower dose, i.e. 5 Gy dose. The altered flower colours were matched with the Royal Horticultural Society colour chart. Bala and Singh (3) also confirmed the variations in ray florets' flower colour, form and shape after gamma irradiations. The higher levels of gamma ray may cause sensitivity to plants due to their impact on endogenous growth regulators that may cause separation or lack of chemical properties inside the plants (Ambavane *et al.,* 1).

Table 3. Effect of gamma irradiation on flower colour and form characters.

γ-ray dose (Gy)	Flower colour as per RHS colour chart	Flower colour variations	Flower form variations
Control	White (NN155D) ray florets, Yellow (2A) disc florets	Yellow disc with white ray florets	Normal disc and ray florets
5	White (NN155D) ray florets, Yellow (2A) disc florets	Yellow disc with white ray florets	Normal disc and ray florets
10	Yellow (5A) ray florets, Yellow (4C) disc florets	Dark yellow disc with light yellow outer florets	Large disc andfew ray florets.
	White (155D) ray florets, Yellow (2C) disc florets	Light pink ray florets	Large disc.
	Yellow (7A) ray florets, Yellow (4C) disc florets	Dark yellow disc with cream ray florets.	Small disc and more ray florets.
15	Yellow (17A) ray florets, Yellow (6A) disc florets	Dark yellow disc with yellow ray Florets	Small disc and compact ray florets. Scattered disc and irregular ray florets.
20	Yellow (7A) ray florets, Yellow (4C) disc florets	Dark yellow with light yellow ray florets	Small disc and compact ray florets.
	Yellow (2D) ray florets, Yellow (4D) disc florets	Light yellow disc with off white ray florets	Large disc and few ray florets. Small disc and more and compact ray florets.

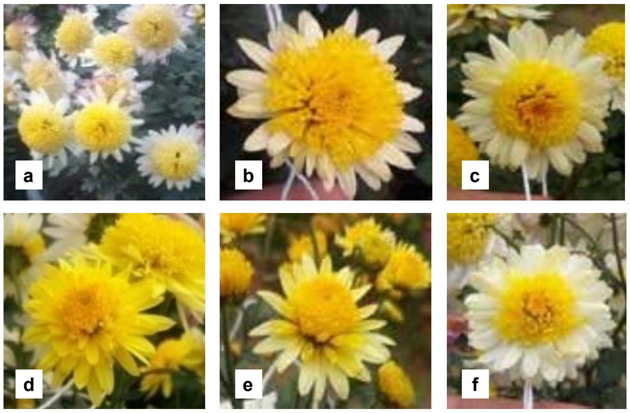

Fig. 3. Flower colour variations in chrysanthemum variety 'Mother Teresa'.

Gamma ray dose Flower colour variations

(a) Control : Yellow disc with white ray florets (Control)

(b) 10 Gy : Dark yellow disc with light yellow ray florets

(c) 10 Gy : Dark yellow disc with cream ray florets

(d) 15 Gy : Dark yellow disc with yellow ray florets

(e) 20 Gy : Dark yellow with light yellow ray florets

(f) 20 Gy : Light yellow disc with off white ray florets

Fig. 4. Flower form variation in chrysanthemum variety 'Mother Teresa'.

Gamma ray dose Flower form variations

(a) Control : Normal disc and ray florets (Control)

(b) 10 Gy : Large disc with few ray florets

(c) 10 Gy : Small disc with more ray florets

(d) 15 Gy : Scattered disc with irregular ray florets

(e) 20 Gy : Small disc with compact ray florets

(f) 20 Gy : Large disc with few ray florets

Gamma irradiations altered the flower form and colour, possibly due to chromosomal and genetic level mutation, changes in number or structure of chromosomes, or alteration of biochemical pathways (Singh and Bala,19). Present results closely conform with the several researchers (Lamseejan *et al.,* 11) as they also obtained altered flower colour stable variants in chrysanthemum. Similarly, results have been found by Setia *et al.* (18) and Patil *et al.* (16) in chrysanthemum.

The results revealed that γ-ray doses of 10 Gy and 15 Gy were the most effective doses for induction of flower colour and flower form variations in the chrysanthemum variety 'Mother Teresa'. The present study, 11 variants were detected with altered flower colour and form at 10 to 20 Gy doses of γ-rays. The study has developed an efficient protocol that can be exploited to create novel mutations in chrysanthemum to enrich the gene pool.

AUTHORS' CONTRIBUTION

Conceptualization of research (D.S., MB); Experimental Designing (M.B.); Experimental materials and execution of trial, data collection (D.S., MB); Data Analysis and interpretation (D.S., MB); Manuscript preparation (D.S., MB, AS).

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DECLARATION

The authors declared no conflict of interest.

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