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Induction of mutation in chrysanthemum (*Dendranthema grandiflorum* Tzvelev.) cultivar Bindiya through gamma irradiation

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ABSTRACT

The terminal rooted cuttings of chrysanthemum cv. Bindiya were exposed to 0, 10, 20 and 30 Gy doses of γ-rays and planted in earthen pots (8"). Each treatment consists of three replications with 25 treated cuttings each. LD_{50} dose was determined as 30 Gy dose. Among the irradiated population, the highest plant survival (82.03%) , No. of branches (4.18), minimum days to bud initiation (82.36 days) with early opening of lowering (120.36 days) was observed with 10 Gy dose. Morphological abnormalities such as fused leaves, with lower levels of chlorophyll were observed at higher dose (30 Gy). Marked variations were recorded between the mutated and control populations. The original flower colour of Bindiya is red and flower colour mutants were of nearest shades of red group 44 C, 46 C and red group 46 B as per RHS Colour Chart and isolated from plants irradiated with 10 and 20 Gy doses of gamma rays. The ray florets were normal in control whereas, ray florets were spoon shaped, tubular and irregular in induced variants. These mutants were multiplied on a large-scale and evaluated for their stability. This study developed a mutagenesis protocol that could be used to develop novel colour mutants in chrysanthemum.

Key words: Chrysanthemum, gamma irradiation, LD_{50}, mutants.

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflorum* Tzvelev.) is one of the most widely cultivated herbaceous perennial plant belongs to family Asteraceae and commonly known as "Autumn Queen" or "Queen of East" and reported to be native of northern hemisphere, mainly Europe and Asia (Bhattacharjee, 5). Chrysanthemum produces showy flowers with different flower colour and shape that can be used as pot plants for beautifying indoors and outdoors, as cut flowers for making bouquets and vase decoration. The modern day cultivars are result of different breeding techniques like selection, hybridization, mutagenesis and other biotechnological tools (Dilta *et al*., 8). Chrysanthemum, being a cross pollinated crop generally possess high degree of heterozygosity, which causes a complex inheritance of genetic factors, coupled with frequent polyploidy, which pose serious handicap in conventional breeding are taken to advantage of mutation breeding. Mutation breeding is one of the established methods by which one can induce variability in vegetatively propagated crops and it also offers advantage over conventional breeding for the improvement of one or more traits within a short span of time. Mutation derived varieties have had a significant impact on the array and choiced of genetic resources available in modern agriculture (Ahloowalia *et al*., 1). Spontaneous mutation or bud sports have played an important role in the evolution of many garden chrysanthemums. In addition to spontaneous mutation, another type of mutation that have a high potential for bringing about the further genetic improvement, is induced mutation through physical and chemical mutagens (Jain and Spencer, 12). The introduction of induced mutation has also attracted considerable attention in chrysanthemums due to the fact that any mutation in dominant genes is easily expressed in the first generation and thus the selection of mutations of directly perceptible characters like flower colour, shape, size *etc*. is generally very easy and directly be put in commercial use (Dilta *et al*., 8)

The objective of the present experiment was to find out the best dose of gamma rays for induction of variability in pot chrysanthemum cultivar Bindiya under *in vivo* conditions**.**

MATERIALS AND METHODS

The investigations were carried out at the Research Farm, Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana during the year 2011-2013. Four treatments comprising 0, 10, 20 and 30 Gy doses of γ-rays (^{60}Co radiation source) were given in gamma chamber at the Department of Fruit Science, PAU, Ludhiana. The terminal rooted cuttings of uniform size (5 cm) of chrysanthemum cv. Bindiya were selected as an experimental material. The terminal cuttings from mother plants were taken

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in 2nd week of July. Basal 2-3 leaves were clipped off and cuttings were planted in propagation trays. For rooting of terminal cuttings, burnt rice husk was used as rooting medium as it is porous, possesses good water holding capacity and facilitates growth of new roots. The rooted cuttings were exposed to different doses of gamma rays (0, 10, 20 and 30 Gy). Immediately after treatment, rooted cuttings along with control (untreated cuttings) were transplanted in earthen pots of uniform size (8") in 2nd week of August and total number of pots used in the experiment was 300.

The data were recorded on vegetative and flowering characters, which includes percent survival, plant height (cm) at the time of bud appearance, number of branches per plant, internodal length (cm), number of leaves per plant, leaf size (cm), leaf colour, leaf abnormalities, plant spread (cm), days to bud initiation, days to colour show stage, days to flower opening, number of flowers per plant, flower size (cm), flower weight (gm), number of ray florets, longevity of bloom (days), flower color variation, *i.e.* flower form variation and chimera if any. Flower and leaf colour of control (mother) and mutants were compared with Royal Horticulture Society Colour Chart. All the standard cultural operations were followed. The data was analyzed employing completely randomized design (CRD), replicated thrice and the percent data was subjected to Arc Sin √% transformation before ANOVA.

RESULTS AND DISCUSSION

Data pertaining to the effects of gamma irradiation on vegetative characters of chrysanthemum are presented in Table 1. The LD_{50} value was estimated on the basis of percent plant survival. In the present investigation, the radio sensitivity was estimated to be 30 Gy (LD_{50}) based on the percentage of survival of irradiated rooted cuttings (Fig. 1). Percent plant survival was found to be decrease linearly with increasing exposure to gamma-rays.

Gamma ray sensitivity of chrysanthemum cv. 'Bongwhang' and an increase in lethality of the buds with the increasing gamma ray dose has been obtained by Dae Hoe (7). The reduction in survival rate at higher doses might attribute to genetic loss due chromosomal aberrations and gene mutation as reported by Tiwari and Kumar (15). The plant height at the time of first bud appearance decreased significantly inversely with increasing doses of gamma rays. Significant reduction in plant height has also been observed by Banerji and Datta (4) when they exposed the rooted cuttings of chrysanthemum cv. Surekha to 150, 200 and 250 Gy doses of gamma rays. The results are in conformity with the findings of Dilta *et al.* (9), and Boersen *et al*. (6).

The higher gamma irradiation doses significantly reduced the number of branches per plant. The less number of branches may be due to inhibitory effect of higher mutagenic doses of gamma rays. These results also justify the findings of Misra *et al.* (14) with reduction in number of branches. There was significant reduction in internodal length with increasing gamma irradiation dose. The reduction in internodal length may be due to decrease in plant height with increase in gamma ray doses as reported by Dilta *et al.* (9). The data pertaining to number of leaves per plant as influenced by different doses of gamma rays and irradiation had significant effect on number of leaves per shoot. In present study, it has been observed that all the treatments of gamma rays significantly reduced the number of leaves and the decrease in number of leaves increased with the increased doses. This decrease is mainly due the decrease in number of branches per plant as reported by Misra *et al.* (15). Similar results have been documented by Banerji and Datta (3), The

Table 1. Effect of gamma irradiation on vegetative characters of chrysanthemum cv. Bindiya.

Character	Dose of γ-rays (Gy)				CD at 5%
	Control	10	20	30	
Plant survival (%)	92.89 (74.57)	82.03 (64.89)	61.30 (51.51)	34.63 (36.03)	2.25
Plant height at first bud appearance stage	15.46	12.26	10.46	7.56	1.05
Plant spread (cm)	28.26	23.96	19.06	16.03	3.26
No. of branches per plant	4.40	4.18	3.86	3.20	0.73
Intermodal length (cm)	0.57	0.49	0.34	0.20	0.10
No. of leaves per plant	87.00	73.36	68.30	54.80	3.15
Leaf size (cm)	3.36	2.83	2.43	1.73	0.36
Leaf colour (RHS)	Dark Green	Dark Green	Light Green	Light Green	-
Leaf abnormalities	Normal	Normal	Small	Small & Fused	

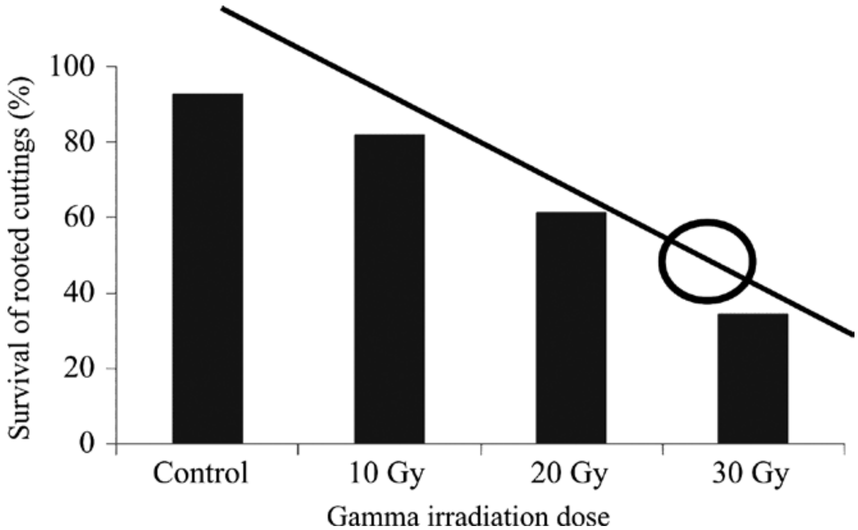

Fig .1. Percent survival to estimate LD_{50} value in irradiated rooted cuttings of chrysanthemum cv. Bindiya.

higher doses of gamma rays minimized the leaf size significantly. The reduction in leaf size of plants treated with higher doses of gamma rays may be due to inactivation or decrease in auxin content or disturbance in auxin synthesis. Significant reduction in leaf size was also observed by Banerji and Datta (4). Based on the visual observations dark green colour of leaves was recorded in all the plants under control as well as treated population with 10 Gy dose of gamma rays , whereas, on the higher doses (20 and 30 Gy) plants grew with light green leaves.

Leaf colour variation may be due to adverse effect of radiation on chlorophyll synthesis process. The higher doses of gamma rays resulted in variations in leaves such as leaf shape and serrated margins. The treated plants were normal at control and 10 Gy gamma ray dose. The maximum leaf variations was seen at 30 Gy gamma ray dose with small and fused leaves. Similarly, Banerji and Datta (3) also observed the increase in leaf abnormalities with increase in gamma ray doses. There was significant reduction in plant spread with increasing gamma irradiation dose. Similarly, Dilta *et al* (9) also observed the decrease in plant spread by treating rooted cuttings of various cultivars of chrysanthemum cultivars Ajay, Baggi, Bright Golden Anne, Ellen Van Langen, Glance, Mountaineer, Nanako, Shyamal and Snow Ball with gamma rays at 0 and 2 kR doses.

Data pertaining to the effects of gamma irradiation on flowering characters of chrysanthemum are presented in Table 2. Among the irradiated plants, the minimum time taken for bud initation was recorded at 10 Gy gamma ray dose and maximum dalay was recorded in 30 Gy dose. The delay in flower bud initation may be due to reduction in the rate of various physiological processes with gamma irradiation. Banerji and Datta (3) also reported a significant delay in flower bud due to increasing doses. Similarly, the higher gamma irradiation doses significantly increased the days to colour show and days to flower opening. The delay in bud initation ultimately resulted in late colour break, late blooming and late flower opening. These results corroborate the findings of Dilta *et al.* (9) chrysanthemum cv. 'Gulmohar' when exposed to 1.0, 1.5, 2.0, 2.5 and 3.0 kR doses of gamma rays. Irradiation altered many biosynthetic pathways, which are directly and indirectly associated with the flowering physiology (Mahure *et al.*, 13).

Varying response of gamma rays irradiated nodal explant to number of flower per plant was observed to be significant . The number of flowers got significantly reduced with increasing rate of gamma irradiation. The decrease in number of flowers per plant may be due to decrease in number of branches per plant. Significant reduction in number of flowers in cv. 'Gulmohar' when exposed to 1.0, 1.5, 2.0, 2.5 and 3.0 kR doses of gamma rays were recorded by Dilta *et al.* (10). The data shows that flower diameter varied with different doses of gamma rays. The maximum flower diameter was obtained in non-irradiated plants. Reduction in flower size could be due to physiological, morphological and cytological disturbance by gamma irradiation. The flower weight decreased inversely with increasing doses of gamma rays. Reduction in weight was recorded but was not overly affected by gamma rays. Reduction in flower weight may be due to reduced size of flower head and reduced number

Table 2. Effect of gamma irradiation on flowering characters of chrysanthemum cv. Bindiya mutants.

Character	Dose of γ-rays (Gy)				
	Control	10	20	30	CD at 5%
Days to bud initiation	75.26	82.36	89.86	102.70	2.09
Days to colour break stage	100.13	106.73	120.03	128.06	1.74
Days to flower opening	112.93	120.36	133.16	143.80	3.11
No. of flowers per plant	21.66	24.30	14.26	10.60	NS
Flower size (cm)	5.10	4.80	3.90	3.10	0.48
Flower weight (gm) per plant	23.86	18.60	11.23	7.20	2.10
No. of ray florets per flower	52.76	49.46	35.80	31.36	5.89
Longevity of bloom (days)	31.63	26.10	27.43	24.36	1.02
Flower colour (RHS)	Red Group (45C)	Red Group (44C) Red Group (46C)	Red Group (46B)	--	-
Flower form variation	Normal ray florets	Spoon shaped	Tubular ray florets	Irregular ray florets	

of petals in flower head. Similarly, Geung-joo Lee *et al.* (11) also recorded reduction in number of ray florets in chrysanthemum cv. Argus upon irradiation with 30, 40 and 50 Gy doses of gamma rays. There was significant reduction in longevity of bloom with increasing gamma irradiation dose. Based on the visual observations in cv. Bindiya Red colour of flowers were recorded in all the plants under control while in treated population with 10 Gy dose of gamma rays colour of flowers changed to Red (44-C). Red (46-B) colour was observed on plants treated with 20 Gy dose of gamma rays, whereas, on the higher dose of (30 Gy) plants grew wih Red flower colour (46-B). Change in the flower colour might be due to the mutations at cellular level. Flower colour mutations after gamma irradiations induced in present study are in close conformity with the results obtained earlier for stability of flower colour mutants in chrysanthemum by various researchers Yamaguchi *et al.* (16). Based on the visual observations in cv. Bindiya normal ray florets were seen in all the plants under control, while in treated population with 10 Gy dose of gamma rays shape of ray florets changed to spoon and irregular. Tubular ray florets were observed on plants treated with 20 Gy dose of gamma rays, whereas, on the higher dose of 30 Gy, plants grew wih irregular ray florets (Fig. 2).

The flower weight decreased inversely with increasing doses of gamma rays. Reduction in weight was recorded but was not overly affected by gamma rays. Reduction in flower weight may be due to reduced size of flower head and reduced number of petals in flower head. Similar results were observed in rose by Bala and Singh (2). The higher doses, *i.e.*, 30 Gy and 20 Gy significantly reduced the number of ray florets per flower over the control and lower dose of 10 Gy. Similarly, Geung-joo Lee *et al.* (12) also recorded reduction in number of ray florets in chrysanthemum cv. Argus upon irradiation with 30, 40 and 50 Gy dose of gamma rays. There was significant reduction in longevity of bloom with increasing gamma irradiation dose. Based on the visual observations in cv. Bindiya Red (45-C) colour of flowers were recorded in all the plants under control while in treated population with 10 Gy dose of gamma rays colour of flowers changed to Red (44-C). Red (46-C) colour was observed on plants treated with 20 Gy dose of gamma rays, whereas, on the higher dose of (30 Gy) plants grew wih Red flower colour (46-B) (Fig. 2). Change in the flower colour might be due to the mutations at cellular level. Flower colour mutations after gamma irradiations induced in present study are in close conformity with the results obtained earlier for stability of flower colour mutants in chrysanthemum by various researchers Bala and Singh (2), and Yamaguchi *et al.* (16). Based on the visual observations in cv. Bindiya normal ray florets were seen in all the plants under control, while in treated population with 10 Gy dose of gamma rays shape of ray florets changed to spoon and irregular. Tubular ray florets were observed on plants treated with 20 Gy dose of gamma rays. Whereas, due the higher dose (30 Gy) plants grew wih irregular ray florets. The change in flower form was also recorded in chrysanthemum cv. Taihei with the chronic and acute gamma irradiation treatment. Misra *et al.* (14) developed one chrysanthemum mutant with tubular florets by gamma irradiation at 0.5 Gy, while the original florets were flat spoon shaped. Dwivedi *et al.* (10) treated

Fig. 2. Flower colour and form variations in chrysanthemum cv. Bindiya after gamma irradiation.

Flower colour		**Flower form**	
(a) Control	Red group (45 C)	(a) Control	Normal ray florets
(b) 10 Gy	Red group (44 C)	(b) 10 Gy	Spoon shaped ray florets
(c) 10 Gy	Red group (46 C)	(c) 10 Gy	Tubular ray florets
(d) 20 Gy	Red group (46 B)	(d) 20 Gy	Irregular ray florets

the rooted cuttings of chrysanthemum cv. Lilith with 1.0, 1.5 and 2.0 kR gamma rays doses and obtained some flower colour and leaf chlorophyll variegations as 'chimaeras'. Among the irradiated population, plants showing variations either in vegetative or floral characteristics such as plant height, leaf size, flower size, flower colour, number of petals *etc*. as compared to control were isolated and multiplied to check the stability.

Flower colour and form variants developed from chrysanthemum cv. Bindiya were coded as B-1, B-2 and B-3 (Fig. 2) and detailed as follows:

B-1 (Light red variant)

The flower colour of variant was the nearest shade of red (red 44-C) as compared original cv. Bindiya (control), which was derived at 10 Gy dose of gamma rays. Flowers were of medium size, with irregular ray florets and compact from the centre. Ray florets in variant were spoon shaped as compared to normal in original flower.

B-2 (Dark red variant)

This variant was induced by 10 Gy gamma irradiation dose. The flowers were irregular with dark red colour (red 46-C) as compared red in original cv. Bindiya. Ray florets were also different having tubular shape as compared to normal in original cultivar.

B-3 (Dark red variant)

This flower colour variant developed from treated rooted cuttings of chrysanthemum cultivar Bindiya induced by 20 Gy gamma irradiation dose. Flowers were large irregular with dark red coloured flowers (red 46-B) as compared to red in original cv. Bindiya (control). Ray florets were irregular in variants as compared to normal in original cultivar

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