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Effect of different doses of gamma rays to induce flower mutants in spray chrysanthemum (*Chrysanthemum morifolium Ramet.)*

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ABSTRACT: Three cultivars used for the study on effect of different doses of gamma rays exhibited their differential radio sensitivity. The survival percentage was decreased with increase in radiation dose from 0 to 20 Gy and no plants survived beyond 20 Gy. LD_{50} differed with the cultivars in which Bidhan Swapna is highly sensitive to gamma rays. Increase in radiation dose resulted in reduction of various growth parameters and delayed blooming. The doses of gamma rays (10, 15 and 20 Gy) used in the experiment are effective but the most efficient dose was 10 Gy for Bidhan Shova and BC-8-05 and 20 Gy for Bidhan Swapna because these doses generated maximum number of useful as well as viable mutants. Flower colour mutants in chimeric form was detected at 10 Gy in BC-8-05 and 20 Gy radiation dose gave solid colour mutants in Bidhan Swapna. Most of the floret shapes as well as changed flower colour and floret shape mutants were identified at 15 Gy and 20 Gy. The frequency of type V (floret shape) and type VI (flower colour) mutants was more in M_1V_1 and M_1V_2 generations respectively.

Keywords: Chrysanthemum, irradiation, mutant, survival percentage, flower colour, flower shape, solid mutant.

INTRODUCTION

Saying with flowers is an old practice in human civilization where different coloured flowers used for the expression. In flower industry, consumer's preference changes with time to time and always seeking for novel types hence there is a lot of demand for the development of new varieties in floriculture. Though, many varieties are available in the market but novelty in commercial traits like earliness, longer duration of flowering, adaptation to local conditions, flower colour, shape, size, growth habit, post-harvest life, fragrance etc., are always valued and generally preferred by consumers. Development of variants in flower colour and flower shape which is achieved through mutation breeding is one of the fastest approach in horticultural crops especially in flower crops by utilizing the advantage of vegetative propagation. Dose rate is one of the important factors of radiation treatment which affects somatic mutations in crop plants. Gamma irradiation results in the development of a large number of somatic flower colour and shape mutants in chrysanthemum. Overall the mutation frequency and spectrum in ornamentals concerned that 55% of the changes in flower colour and 15% in flower morphology (Schum and Preil 1998) even frequency of mutation increased with increase in exposure to gamma rays (Misra *et al*., 2006) to a certain dose range there after it decreased with increase in strength of gamma rays was well documented in many crops (Solanki and Sharma 1994; Muduli and Misra 2007; Ambavane *et al*., 2015). Many studies have been reported on irradiation effects in chrysanthemum and other ornamentals (Banerji and Datta 1992; Datta *et al.*, 2001; Lamseejan *et al*., 2000; Dilta *et al*., 2003; Mahure *et al*., 2010; Kapoor, 2012).

Chrysanthemum is one of the promising flower crops has the demand in various ways right from potted plant to a commercial crop and is most suitable for the mutation treatment. Before going for a mutation breeding programme various factors like choice of material, characters to be improved, type of mutagens and its dose to be used are to be studied which are highly needed. Keeping in view, the present has formulated to study the effect of different doses of gamma irradiation on growth and flowering of spray chrysanthemum.

MATERIAL AND METHODS

Terminal cuttings of 6-8 cm were taken from healthy mother stock of three varieties *viz.,* BC-8-05, Bidhan Swapna and Bidhan Shova due to the large scale cultivation of these varieties by the growers of the plains of West Bengal. The experiment was carried out at the Horticultural Research station, Mondouri farm under AICRP on Floriculture, BCKV, Nadia, West Bengal during the year 2013-14 and 2014-15. Twenty five rooted cuttings from each cultivar were treated with physical mutagen (gamma rays) with doses starting from 0 Gy (control), 10 Gy, 15 Gy, 20 Gy, 25 Gy and 30 Gy in Randomized Block Design in 3 cultivars with 3 replications (Gomez & Gomez 1984). Irradiation programme was conducted at UGC-DAE Consortium for Scientific Research, Kolkata Centre (South campus of Jadavpur University, Salt Lake, Kolkata). Immediately after irradiation the rooted cuttings were grown in the shade house for ten days thereafter planted in the main field with a spacing of 30 cm x 30 cm and followed standard cultural practices for the entire crop grown period.

RESULTS AND DISCUSSION

1. Survival percentage: Gamma radiation had greatly influenced the survival percentage of rooted cuttings in which the percent plant survival of rooted cuttings was significantly reduced over control in M_1V_1 generation with increasing gamma radiation dose from 10-30 Gy with respect to cultivars which also differed significantly (Table 1). Among the different doses treated, untreated cuttings showed cent percent survival and it was minimum (22.10%) at 20 Gy. There was no plant survived beyond 20 Gy radiation dose indicates that a gamma ray radiation of 10-20 Gy is optimum for rooted cuttings of the cultivars used in this study and above 20 Gy is destructive.

The reduced survival percentage was observed with increased dose although all the cultivars were at par in M_1V_2 generation. In general percentage of survival was less in M_1V_1 generation as compared to M_1V_2 generation which might be due to the pronounced effect of ionizing radiation in M_1V_1 and diminishing effect in second year. The present findings are in line with the findings of Datta (Patil and Patil, 2009) in chrysanthemum.

Table 1: LD_{50} values of the cultivars of chrysanthemum.

Cultivar	LD $_{50}$ value	Regression equation
V_1- Bidhan Shova	13.46	Y= -24.4x+120.4
V_2- Bidhan Swapna	10.47	Y =-22.4x+107.2
V_3- BC-8-05	17.96	Y= -26.0x +137

2. Estimation of LD_{50} dose: LD_{50} dose for survival was estimated based on survival percentage and it was evident that LD_{50} differed with the cultivars (Table 3). Among those, Bidhan Swapna was highly sensitivity to gamma rays (LD_{50} 10.47 Gy) while BC-8-05 showed its resistance (LD_{50} 17.96). LD_{50} for different cultivars of chrysanthemum is ranged between 2.0 to 2.5 krad (Datta, 1985). The influence of genotype to radio sensitivity was clear in our experiment.

3. Plant height: Significant difference was observed with radiation dose and cultivar response in which increased dose of gamma rays reduced plant height upto two generations *i.e.* M_1V_1 and M_1V_2 generations. The interaction between the cultivars and gamma rays, an increased plant of 61.06 cm and 62.13 cm was recorded in BidhanShova at 10 Gy and the minimum (16.83 cm and 32.07 cm) was found in BC-8-05 at 20 Gy in M_1V_1 generation and M_1V_2 generation respectively. It was also observed that the reduction in plant height at higher doses in M_1V_1 generation and a slight increase in plant height M_1V_2 generation. This might be due to diminishing effect of gamma radiation in next generations and differential response of cultivars for plant height is attributed to the genotype also.

4. Number of primary branches per plant: High gamma irradiation dose reduced the number of primary branches per plant (Table 1). The interaction between the cultivars and gamma rays resulted that the maximum number of primary branches (3.33, 1.83, 3.33) was found in control of all the cultivars and minimum number of branches (1.00, 1.00, 1.16) was observed at 20 Gy gamma radiation dose in all the in M_1V_1 generation while in M_1V_2 generation the untreated plants of BC-8-05 recorded the highest number of primary branches (2.86) followed by BidhanShova at 15 Gy (2.40) and the lowest number of primary branches (1.20) were recorded in BidhanSwapna at 15 Gy. The same findings were also found in chrysanthemum cv. Himani (Datta and Banerji, 1986). Higher doses of gamma rays cause disturbance in auxin synthesis and renders its translocation which ultimately reduces the growth and number of branches might be the possible reason for reduced number of branches.

5. Number of secondary branches per plant: Number of secondary branches were differed significantly among the cultivars owing to gamma irradiation in both the generations (Table 1). The interaction between the cultivars and gamma rays indicated that untreated plants of BidhanShova showed the highest number of secondary branches (24.33) and it was least (6.33) in same cultivar at 20 Gy in M_1V_1 generation. However, in M_1V_2 generation BidhanShova at 15 Gy showed the maximum number of branches (22.33) and it was minimum (13.30) in BidhanSwapna at 20 Gy. The less number of branches might be due to inhibitory effect of higher doses of gamma rays.

6. Average spray length: At lower doses of 10 Gy and 15 Gy, increased spray length was observed in BidhanSwapna and BC-8-05 in M_1V_1 generation. Compared to M_1V_1 in M_1V_2 generation spray length was increased but it followed a decreased trend with increase in radiation dose in all the cultivars. In the present study differential cultivar response to irradiation was observed for increased or decreased spray length might be due to chromosomal aberrations in addition to genetic mutations which were quite common after mutagenic treatment which are in close conformity with the findings of Rather *et al.* (2002) in Dutch Iris, Patil and Dhaduk (2009) in gladiolus.

7. Days to flower bud initiation: Significant difference was found for days to flower bud initiation in all the cultivars with different doses of gamma irradiation and their interactions in both the generations (Table 3) untreated plants took less number of days to flower bud initiation (75.61days and 79.73 days) and it was delayed by 97.16 days at 20 Gy in M_1V_1 generation while in M_1V_2 generation flower bud initiation was delayed by 92.26 days at 15 Gy. The interaction between the cultivars and gamma rays shows less number of days to flower bud initiation (69.33 days) in BidhanShova of untreated plants and it was delayed (103 days) in cultivar BC-8-05 at 20 Gy in M_1V_1 generation. lthough in M_1V_2 generation the minimum days to flower bud initiation (63.86 days) was recorded in cultivar BidhanShova of untreated plants and BidhanSwapna at 15 Gy recorded delayed flower bud initiation by 94.46 days. These findings suggest that flower bud visibility was differed with cultivars and irradiation dose.

Table 2: Effect of gamma irradiation on survival percentage of rooted cuttings in M_1V_1 and M_1V_2 generations of chrysanthemum.

Treatments	Survival (%) in M_1V_1	Survival (%) in M_1V_2	Plant height (cm)		No. of primary branches/plant		No. of Secondary branches/plant		Average spray Length (cm)	
Cultivars (V)			M_1V_1	M_1V_2	M_1V_1	M_1V_2	M_1V_1	M_1V_2	M_1V_1	M_1V_2
V_1	58.40	99.37	47.95	59.35	1.83	1.95	13.87	19.23	30.17	39.67
V_2	46.60	99.03	46.72	56.90	1.29	1.35	10.29	16.64	21.27	29.66
V_3	71.73	99.85	25.24	34.37	2.04	2.06	12.87	15.33	13.97	23.07
SEm±	0.172	0.086	0.211	0.273	0.085	0.071	0.442	0.686	0.791	0.290
CD at 5 %	0.504	0.254	0.618	0.800	0.249	0.210	1.297	2.013	2.320	0.852
Radiation Dose (T)										
T_1	100	100	49.80	52.18	2.83	2.03	19.27	17.91	25.01	36.27
T_2	66.18	100	43.51	50.88	1.77	1.89	13.33	17.02	25.70	29.50
T_3	47.36	99.63	37.06	47.60	1.22	1.78	9.94	18.57	19.88	28.44
T_4	22.10	98.05	29.51	50.16	1.05	1.46	6.83	14.76	16.61	28.99
SEm±	0.198	0.099	0.243	0.315	0.098	0.083	0.511	0.793	0.913	0.335
CD at 5 %	0.581	0.293	0.714	0.923	0.288	0.242	1.497	2.325	2.679	0.983
(VxT)										
V_1T_1	100	100	61.06	62.13	3.33	1.73	24.33	21.86	37.68	46.28
V_1T_2	67.00	100	53.08	61.26	1.83	2.20	14.16	18.73	35.03	32.73
V_1T_3	43.33	99.23	42.50	55.60	1.16	2.40	10.66	22.33	27.41	36.46
V_1T_4	23.66	98.26	35.16	58.43	1.00	1.46	6.33	14.00	20.55	43.22
V_2T_1	100	100	53.60	58.52	1.83	1.50	13.83	17.80	24.80	36.38
V_2T_2	47.23	100	49.80	55.51	1.33	1.40	11.00	18.66	25.85	30.02
V_2T_3	23.66	99.73	46.95	53.59	1.00	1.20	9.00	16.80	17.30	26.13
V_2T_4	15.53	96.40	36.53	60.00	1.00	1.33	7.33	13.30	17.13	26.10
V_3T_1	100	100	34.75	35.90	3.33	2.86	19.66	14.06	12.16	26.16
V_3T_2	84.33	100	27.66	35.86	2.16	2.06	14.83	13.66	16.21	25.74
V_3T_3	75.10	99.63	21.73	33.63	1.50	1.73	10.16	16.60	14.94	22.72
V_3T_4	27.50	98.05	16.83	32.07	1.16	1.60	6.83	17.00	12.57	17.66
SEm±	0.343	0.172	0.421	0.545	0.170	0.143	0.884	1.373	1.582	0.581
CD at 5 %	1.007	0.507	1.236	1.599	0.498	0.419	2.594	4.027	4.640	1.703

8. Days to full bloom: The cultivar BidhanShova reached to full bloom stage early by 125.66 days in M_1V_1 generation and 123.08 days in M_1V_2 generation and it was delayed by 133.04 and 134.16 days in BidhanSwapna in both the generations respectively (Table 3). When different doses of gamma rays applied, the untreated plants took less number of days to full bloom (117.72 days and 120.97 days) and it was delayed by 139.11 days at 20 Gy in M_1V_1 generation. While in M_1V_2 generation, plants treated at 15 Gy took a maximum of 136.80 days to reach full bloom stage. Delayed blooming could be again due to reduction in the rate of various physiological processes as the plant height and number of leaves also decreased after irradiation. Consequently, physiological mechanism also played important role in radiation induced lateness in flowering.

9. Number of flowers per plant: Among the different doses tried, more number of flowers per plant (136.66 and 172.51) was recorded in untreated plants while it was less (75.72 and 124.86) at 20 Gy in M_1V_1 and M_1V_2 generations respectively which differed significantly. The untreated plants of all the cultivars recorded maximum number of flowers (144.16, 112.33 and 153.50) in M_1V_1 and (168.73, 178.20 and 170.60) in M_1V_2 generations over other interaction effects. This was followed by V_3T_2 (122.83) while a gamma radiation dose of 20 Gy in BidhanShova gradually reduced the number of flowers per plant (64.66 and 106.40) in M_1V_1 and M_1V_2 generations respectively.

The number of flowers per plant followed a decreasing tendency with increased radiation dose in both the generations. This reduction might be due to the decrease in plant physiological process and reduced vegetative growth, ultimately resulted in less number of flowers after irradiation. In M_1V_2 generation more number of flowers was recorded compared to M_1V_1 but the presence of lethal effect of gamma rays resulted in less number of flowers per plant (Misra and Datta 2004).

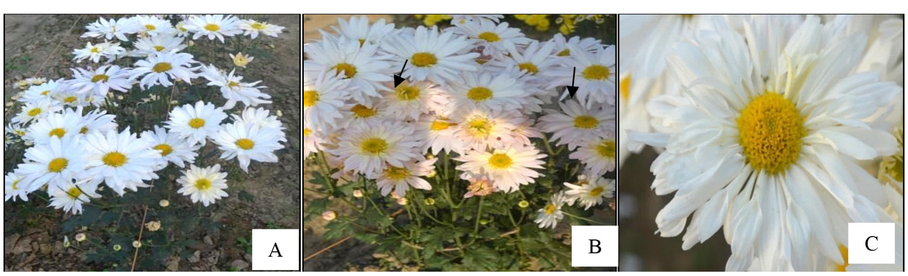
10. Mutation spectrum and frequency of macro mutations in M_1V_1 and M_1V_2 generations: Observations pertaining to variations in flower colour, flower shape were represented in Table 4 for M_1V_1 generation. Somatic mutations in flower colour/shape were detected after gamma irradiation in all the varieties (Fig. 1, 2 and 3).

The cultivar BidhanShova showed type V flowers in 1 plant at 20 Gy as increased floret length and 1/3rd of tip of the ray floret was revolute and basal part is flat. Type VI in 2, 1 plant each at 10 Gy and 15 Gy respectively in M_1V_1 generation in which number of rows of petals were increased to 3-4 or 5 and in some flowers more than five rows of petals were observed. In M_1V_2 generation, type V flowers at 20 Gy in 1 plant which showed the variation in ray florets as tubular and flat type. Similarly, 3 plants from BidhanSwapna showed type V flowers at 10 Gy and 1 plant at 20 Gy respectively in M_1V_1 generation. The shape of the florets changed from flat to tubular and spoon types.

In cultivar BC-8-05 type I, type II, type III and type IV chimeric flowers in 3, 2, 3 and 1 plant respectively was noticed at 10 Gy in M_1V_1 generation. 7 plants from 15 Gy and 1 plant from 20 Gy treatment exhibited type V flowers. Type VI macromutation was produced 1 plant at gamma ray dose of 20 Gy.

Table 3: Effect of gamma irradiation on in M_1V_1 and M_1V_2 generations of chrysanthemum.

Treatments	Days to flower bud initiation		Days to full bloom		No. of flowers per plant	
Cultivars (V)	**M_1V_1**	**M_1V_2**	**M_1V_1**	**M_1V_2**	**M_1V_1**	**M_1V_2**
V_1	84.50	79.88	125.66	123.08	101.54	139.10
V_2	87.66	89.89	133.04	134.16	99.16	140.36
V_3	89.70	91.43	127.04	131.56	115.54	158.26
SEm±	1.164	0.596	1.417	1.014	0.580	0.367
CD at 5 %	2.414	1.747	2.939	2.975	1.701	1.078
Radiation Dose (T)						
T_1	75.61	79.73	117.72	120.97	136.66	172.51
T_2	87.77	88.20	129.72	129.62	111.88	148.53
T_3	88.61	92.26	127.77	136.80	97.38	137.74
T_4	97.16	88.08	139.11	131.02	75.72	124.86
SEm±	1.344	0.688	1.636	1.171	0.670	0.424
CD at 5 %	2.788	2.017	3.393	3.436	1.965	1.244
(VxT)						
V_1T_1	69.33	63.86	111.83	102.73	144.16	168.73
V_1T_2	84.66	85.60	129.33	126.00	109.66	141.73
V_1T_3	89.83	84.26	127.83	131.26	87.66	139.56
V_1T_4	94.16	85.80	133.66	132.33	64.66	106.40
V_2T_1	80.66	87.20	127.33	132.13	112.33	178.20
V_2T_2	87.00	88.46	132.16	135.06	103.16	145.40
V_2T_3	88.66	96.46	134.50	143.46	100.83	111.53
V_2T_4	94.33	87.44	138.16	126.00	80.33	126.33
V_3T_1	76.83	88.13	114.00	128.06	153.50	170.60
V_3T_2	91.66	90.53	127.66	127.80	122.83	158.46
V_3T_3	87.33	96.06	121.00	135.66	103.66	162.13
V_3T_4	103.00	91.00	145.50	134.73	82.16	141.86
SEm±	2.328	1.191	2.834	2.029	1.160	0.735
CD at 5 %	4.828	3.494	5.877	5.950	3.403	2.155



(A) Untreated plants (B) florets with incurl (C) inner row florets showing spoon type and outer row florets are flat type at 20 Gy in M_1V_2 generation.

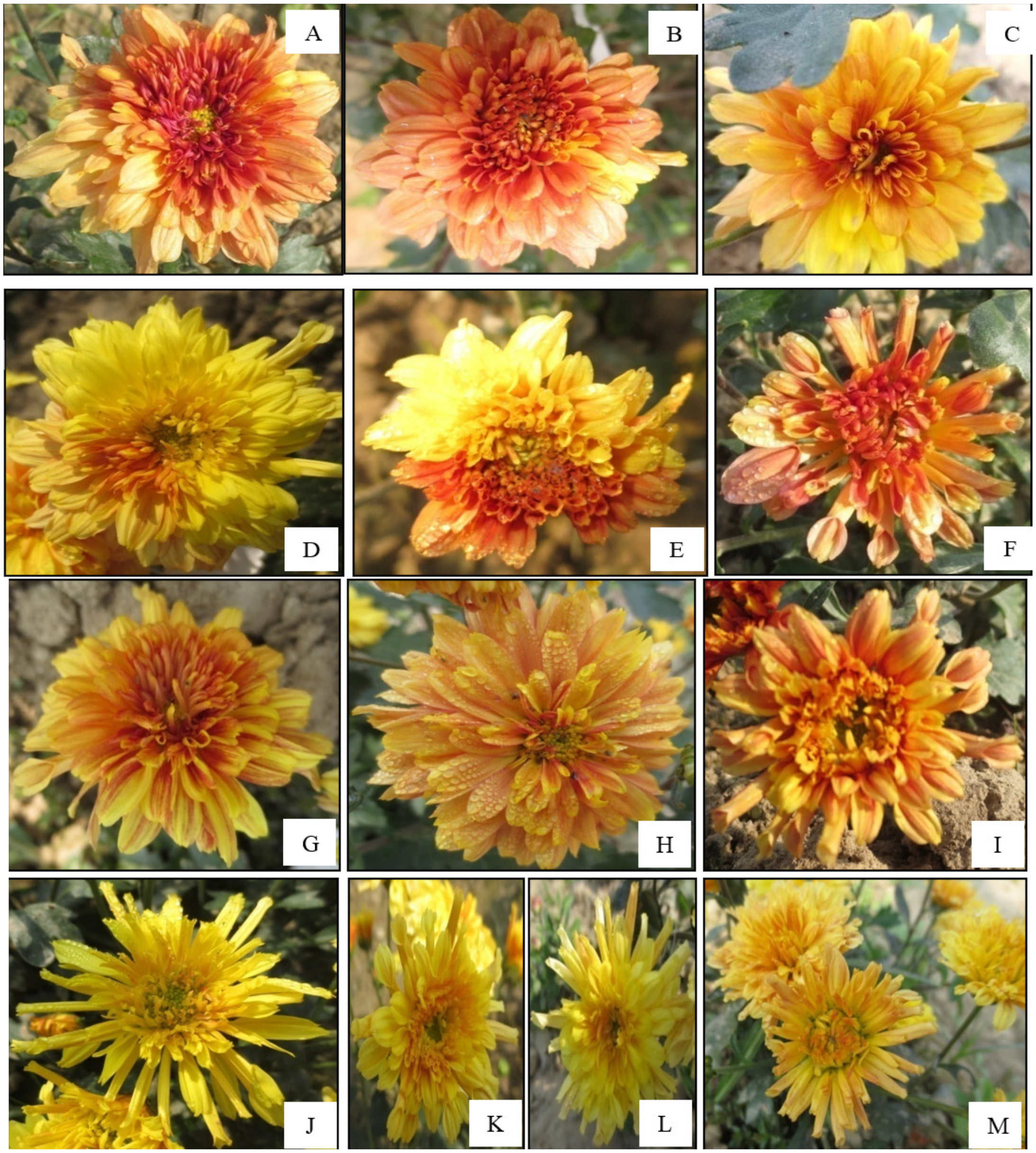
Fig. 1. Flower mutants observed in Bidhan Shova.

Table 4: Overall mutation spectrum and frequency of macro mutations in M_1V_1 generation.

Cultivar	**Original colour and flower head type**	**Radiation Dose (Gy)**	**Type of macro mutations**							
			Plants with Chimeric flowers				Plants with changed flower shape			
			Type I (≥50 %	Type II (<50 %	Type III (stripes on the florets)	Type IV (solid colour+floret shape)	Type V (floret shape- I)	Type VI (others)	Total plants with somatic mutations	Percentage of plants with somatic mutations
BidhanShova	White , single korean (2 rows of petals)	10 Gy	-		-	-	-	2 (3-4 rows of petals)	2	11.76
		15 Gy						1 (5 rows of petals)	1	9.09
		20 Gy	-	-	-	-	1(revolute ray florets)		1	16.66
BidhanSwapna	Pink, decorative	10 Gy	-	-	-	-	1 (Spoon)+ 2(Tubular + spoon)		3	25.00
		15 Gy	-	-	-	-	-	-	-	
		20 Gy	-	-	-	-	1 (Tubular to spoon)		1	14.28
BC-8-05	Yellowish brown, decorative	10 Gy	3	2	3	1	-	-	9	42.85
		15 Gy	-	-	-	-	2 (tubular+ spatual opening+flat,2(tubular)+3 tubular+flat type)		7	36.84
		20 Gy				-	1(tubular, spatual opening)	1 (changed colour and shape)	2	50.00
		Total	3	2	3	1	13	4		

(A) Untreated flower (B), (C) Outer row florets as tubular and inner rows as spoon type at 20 Gy (D) Tubular and spoon type florets at 10 Gy (E)Spoon type ray florets at 10 Gy and disc florets are compact and incurved (F) flower with tubular ray florets at 20 Gy.

Fig. 2. Flower mutants observed in Bidhan Swapnain M_1V_1 generation.



(A) Untreated flower (B), (C), (D) &(E) chimeric flowers at 10 Gy (F) tubular with open tips as fimbriate and spoon florets at 15 Gy (G) brown coloured stripes on yellow florets at 10 Gy (H) colour mutant at 10 Gy (I) tubular &spoon type flower at 20 Gy (J) tubular + yellow coloured florets at 20 Gy (K)colour and shape (tubular and flat) mutant at 20 Gy (L) changed colour, spoon, tubular florets at 15 Gy (M) tubular florets with open tips mutant at 15 Gy

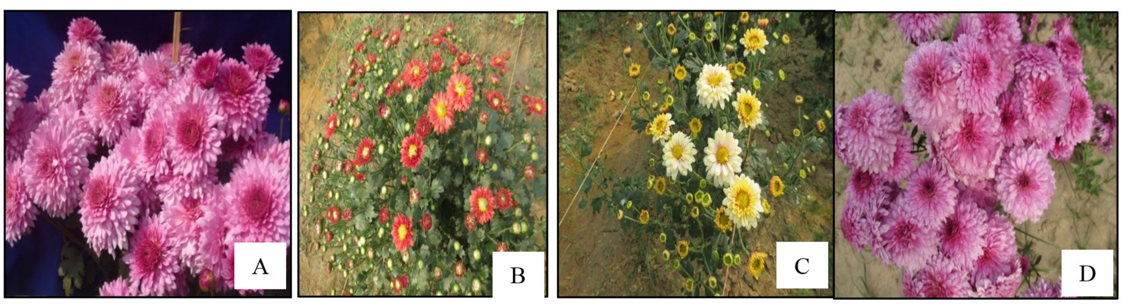
Fig. 3. Flower mutants observed in BC-8-05.

Among the various classes of macromutations type V is the most frequent (13) followed by Type VI (4) and Type I and type III (3). The percentage of plants with high somatic mutation rate was observed in cultivar BC-8-05 (50%) at 20 Gy followed by 42.85 % at 10 Gy. Whereas the percentage of flowers with mutation were high (18.33%) in BC-8-05 at 20 Gy followed by 16% in BidhanShova at 10 Gy (Fig. 3). Therefore BC-8-05 is highly sensitive for producing chimeric flowers. Plants from 15 Gy treatments in BidhanShova showed a less percentage of plants with macro mutations which indicate that it is resistance to gamma irradiation.

Mutation frequency was more at 10 Gy and 15 Gy as compared to other doses of gamma rays. A reduction in mutation frequency at higher doses might be due to the fact that increased doses cause more disturbances in physiological processes as well as genetic material. The macro mutations observed in M_1V_2 generation were given in Table 5 and Fig. (4&5). Gamma ray treatment at 20 Gy in BidhanSwapna produced solid flower colour (type VI) macro mutations in 4 plants. Two of them were creamish white coloured flowers (RHS 155 C) with single korean type of flower heads and other two were dark purple brown (RHS 181 A, RHS 185 B) with single korean type of flower heads. In case of BC-8-05, type VI macro mutation was produced at 10 Gy treatments in 3 plants as brown colour florets, outer row florets were tubular and rest of them were flat type. Overall, in M_1V_2 production of type VI macro mutations are more. Comparatively in both the generations, cultivars BC-8-05 and BidhanShova resulted in more number of viable mutants at 10, 15 and 20 Gy treatments. From these observations it is clear that, flower colour mutation frequency is genotype and dose dependent phenomenon as different cultivars had different mutation frequencies at different doses. The similar findings were also reported by Datta (2009) in rose.

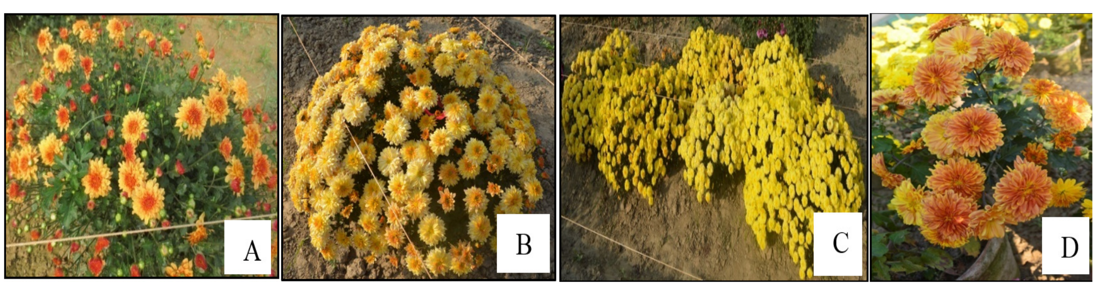
Table 5: Overall mutation spectrum and frequency of macro mutations in M_1V_2 generation.

Cultivars	Original colour and flower head type	Radiation Dose (Gy)	Type IV (Solid colour+ floret shape)	Type V (Floret shape)	Type VI (Flower colour)	Plants with somatic mutation (%)
BidhanShova	White, single korean	20	-	1(Spoon+ flat type	-	6.66
BidhanSwapna	Pink, decorative	20			2 (White colour), 2 (Brown-red)-	66.66
BC-8-05	Bronze, decorative	10	3 (Brown with yellow tinge, tubular+flat type)			75



(A) Mother plant of Bidhan Swapna (B) Solid flower colour mutant at 20 Gy (C) Solid flower colour mutant at 20 Gy (D) Plant with compact flowers at 10 Gy

Fig. 4. Flower mutants of Bidhan Swapna in M_1V_2 generation.



(A) Untreated plants of BC-8-05 (Spreading habit)(B) Plant growth habit as hemispherical and change in flower colour at early and (C) at full bloom stage at 20 Gy (D) Solid brown colour with yellow tinge and outer row tubular and inner row flat type florets

Fig. 5. Flower mutants of BC-8-05 in M_1V_2 generation.

CONCLUSIONS

In the present experiment 2-3 types of flower form mutants were isolated as solid mutants and some chimeric mutants which shows that the changes for flower colour and flower form are due to independent events and the pleiotropic effect is ruled out. Like mutation frequency, the spectrum of mutation also varied with cultivars and dose of gamma rays where pink coloured varieties have a tendency to produce solid mutants and yellow –bronze coloured varieties give raise to flower shape sports and chimeras.

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